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Polyglutamine Disorders

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Preface

On Polyglutamine Diseases

Polyglutamine (polyQ) diseases are a group of rare neurodegenerative disorders that share a common genetic cause: they arise as a result of abnormal expansions of CAG trinucleotide sequences occurring at particular genome loci. In contrast with other repeat-related disorders, the repeat-bearing tracts associated with polyQ diseases are present at the codifying region of genes, being translated as expanded polyQ tracts in their respective protein products. Although these genes and proteins are otherwise unrelated and share no significant homology outside the CAG/polyQ tract, proteins carrying an abnormally expanded polyQ tract tend to aggregate, forming insoluble protein aggregates that constitute a key neuropathological feature of polyQ disorders. The group currently includes nine disorders: Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA), spinal and bulbar muscular atrophy (SBMA), and six different types of spinocerebellar ataxia: SCA 1, 2, 3, 6, 7, and 17. PolyQ diseases are highly incapacitating and, to this date, no therapy able to modify disease progression is available for any of them.

PolyQ disorders share several features. These include (a) the existence of a positive correlation between the variable CAG repeat number and both the severity and precocity of symptoms; (b) the generational instability in CAG repeat number transmission; and (c) the aforementioned propensity for the protein products to aggregate and to constitute large intracellular multiprotein inclusions that are detected in patients' neuronal tissue. Despite the genetic cause of polyQ disorders being clearly identified, the molecular mechanisms involved in their pathogenesis are not fully understood. Though it is frequently accepted that these disorders share common pathogenic mechanisms, the symptoms and the regional patterns of neurodegeneration are not shared among disorders. Taken together, the similarities and differences existing in this group of disorders suggest that the search for disease mechanisms and putative therapeutic strategies will benefit from an integrative view that conjugates common factors and motifs with the particularities of each polyQ disease.

For this book entitled *Polyglutamine Disorders*, we have gathered many of the main experts around the world in the field, whom we would like to acknowledge for their work and collaboration, providing a state-of-the-art revision about several aspects of the different polyQ diseases. The book addresses the molecular mechanisms described to underlie disease pathogenesis, the animal models developed to study these diseases and, importantly, the advanced therapeutic strategies being investigated for these disorders.

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Chapter 1
Clinical Features of Huntington's Disease

Rhia Ghosh and Sarah J. Tabrizi

Abstract Huntington's disease (HD) is the most common monogenic neurodegenerative disease and the commonest genetic dementia in the developed world. With autosomal dominant inheritance, typically mid-life onset, and unrelenting progressive motor, cognitive and psychiatric symptoms over 15–20 years, its impact on patients and their families is devastating. The causative genetic mutation is an expanded CAG trinucleotide repeat in the gene encoding the Huntingtin protein, which leads to a prolonged polyglutamine stretch at the N-terminus of the protein. Since the discovery of the gene over 20 years ago much progress has been made in HD research, and although there are currently no disease-modifying treatments available, there are a number of exciting potential therapeutic developments in the pipeline. In this chapter we discuss the epidemiology, genetics and pathogenesis of HD as well as the clinical presentation and management of HD, which is currently focused on symptomatic treatment. The principles of genetic testing for HD are also explained. Recent developments in therapeutics research, including gene silencing and targeted small molecule approaches are also discussed, as well as the search for HD biomarkers that will assist the validation of these potentially new treatments.

Keywords Huntington's disease · Genetics · Symptoms · Management
Therapeutics · Biomarkers

1.1 Introduction

Huntington's disease (HD) is one of the most common causes of dominantly inherited neurodegenerative disease [1]. There is typically adult onset, with irreversible progression of motor, psychiatric and cognitive symptoms over 15–20 years, followed

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by death [2]. The condition was first described in the USA by George Huntingtin in 1872, himself a newly qualified doctor at the time, and his original descriptions of the disease remain true today.

As yet there are still no disease modifying treatments available, but an intensive international research effort is underway with many clinical trials currently on-going. In this chapter we will first cover the epidemiology, genetics, and pathogenesis of HD and then discuss clinical aspects of the disease and the latest developments in HD therapeutics research.

1.2 Epidemiology

The prevalence of HD had been thought to be 4–10/100,000 in Western populations [3], though a more recent study in the UK suggests a figure of 12.3/100,000 [4]. The true prevalence of HD may have been underestimated in the past due to the stigma that has traditionally been attached to the diagnosis; in recent centuries, sufferers have even been accused of witchcraft, pressuring families into hiding the condition [5]. The wider availability of genetic testing may also have contributed to an apparent increase in disease prevalence [6].

The disease is thought to have migrated from North-West Europe to other parts of the world, and there is global variation in prevalence, with naturally low rates in Japan, Hong Kong and Taiwan [7]. One of the highest rates of HD occurs in Venezuela where communities living near the edge of Lake Maracaibo see a prevalence of 700/100,000 people. It was in this population that genetic linkage studies led to the discovery of the causative genetic mutation in HD [8, 9].

1.3 Genetics of Huntington's Disease

HD is a single-gene disorder with autosomal dominant transmission and so the presence of the mutation on either allele leads to the disease. Therefore an affected parent has a 50% chance of passing it on to their child. The mutation is an expanded CAG triplet repeat near the start of exon 1 of the Huntingtin gene (*HTT*), which lies on the short arm of chromosome 4. On translation, this leads to the presence of a polyglutamine (polyQ) stretch at the N-terminus of Huntingtin protein (HTT).

The wild-type gene carries 10–35 CAG repeats, with a mean value of 18 CAG repeats across the population (although this mean value is greater in populations with higher disease prevalence) [7, 10]. The mutation is fully penetrant at 40 or more repeats. Between 36 and 39 repeats there is reduced penetrance; carriers may develop HD symptoms in later life or not at all [11]. *HTT* with 27–35 CAG repeats is referred to as an "intermediate allele".

Intermediate alleles in the general population are thought to arise from stepwise expansion of the CAG repeat over many generations. People who inherit

intermediate length alleles have long been thought to be unaffected, but a behavioural phenotype has now been identified in this group [12]. During meiosis it is possible for intermediate alleles to expand further, leading to offspring who may carry a disease gene with 36 or more repeats. The risk of this is as high as 21% for those with 35 CAG repeats [13].

This intrinsic instability of the CAG repeat during meiosis especially affects the disease gene, leading to expansions (and sometimes contractions) in repeat length inherited by successive generations [14]. As longer CAG repeat length correlates with earlier age of onset, symptoms may develop at progressively younger ages as the condition is passed down the family tree. This is known as *anticipation*. Due to differences between spermatogenesis and oogenesis this is more likely to occur when transmission occurs through the paternal bloodline [15]. In rare cases large increases in CAG repeat length above 55 can occur, leading to Juvenile HD (JHD), when the age of onset is less than 20 years old. 90% of JHD cases result from paternal inheritance [16, 17].

As with all genetic conditions a detailed family history is essential to help make a correct diagnosis. However 6–8% of patients with newly diagnosed HD have no family history [18]. As mentioned above, de novo mutations may arise from intermediate length alleles, leading to sporadic cases. *Seemingly* sporadic cases may occur following non-paternity, misdiagnosis in prior generations, or when deaths of family members occur from other causes before development of neurological symptoms thus masking the presence of the HD gene.

1.3.1 Effect of CAG Repeat Length on Disease Phenotype

Disease onset is defined clinically as the presence of unequivocal extrapyramidal motor signs suggestive of HD. In typical mid-life onset HD with CAG repeat 40–55, the length of the CAG repeat accounts for ~56% of the variability in age at motor onset [19]. A recent genome-wide association (GWA) study has identified genes involved in DNA handling and repair mechanisms, including *MLH1*, as contributing to further variation in the age of disease onset [20]. Remaining variation is likely determined by other genetic and environmental modifiers [21].

Taking into account the CAG repeat length as well as the number of disease free years already lived, a conditional probability model was developed by Langbehn et al. which is able to estimate the chance of on-going disease free survival over a number of years [22, 23]. However, models based on population data cannot be applied in a one-to-one clinical setting and it is not possible to accurately predict an individual's age of onset of disease from their CAG repeat length.

Patients often experience psychiatric and cognitive symptoms, as well as very subtle motor disturbances for many years before their official disease onset. CAG repeat length correlates much less strongly with age of onset of psychiatric symptoms, but does show some correlation with rate of disease progression [24, 25]. The duration of disease from diagnosis to death is independent of CAG repeat length.

1.4 Pathogenesis of Huntington's Disease

Huntingtin is a large 350 kDa protein comprised of multiple repeated units of 50 amino acids, termed HEAT repeats, which assemble into a superhelical structure with a hydrophobic core. Compared to the wild-type protein, the mutant protein contains an expanded polyglutamine stretch starting at residue 18 [26]. This triggers a pathogenic cascade, with both cell autonomous and non-cell autonomous mechanisms involved which are summarised in Table 1.1.

Table 1.1 Pathogenic mechanisms in Huntington's disease

Mechanism	Role of mutant Huntingtin
Proteolysis and generation of mHTT fragments	Multiple lines of evidence support the "toxic fragment hypothesis" that proteolytic cleavage of mutant HTT liberates toxic N-terminal fragments containing the expanded polyQ tract, which contribute to cell death through accumulation and additional activation of further proteolytic caspases [27]. HTT is cleaved by multiple proteases including caspases, calpains, cathepsins and matrix metalloproteases (MMPs), and mutant HTT fragments have been detected in many animal models [28]. Recent evidence also raises the possibility that these short N-terminal fragments may arise from alternative splicing of exon 1 of HTT [29], and mHTT exon 1 fragments cause neurodegeneration in HD model systems [30, 31]
Inclusion formation	Intranuclear inclusions of mutant HTT are a pathognomic feature of HD. Inclusions also form in the cytoplasm, dendrites and axon terminals, though to a lesser extent. Inclusions are heterogeneous population comprising different forms of mHTT [32], and their definitive role (whether toxic or protective) has not yet been established [33]
Post-translational modification of mHTT	HTT undergoes extensive post-translational modification at multiple sites through phosphorylation, SUMOylation, acetylation, ubiquitination and palmitoylation. All these modifications can exert an effect on mHTT pathogenicity, but their full significance, interdependence and exact role in any pathogenic mechanism remains largely unknown [28]
Loss of neurotrophin (BDNF) support	There is loss of brain-derived neurotrophic factor (BDNF) support from cortico-striatal projections, as mHTT is known to interfere with both the expression and trafficking of BDNF that promotes survival of striatal neurons [34]
Transcriptional dysregulation	Transcriptional dysregulation occurs before symptom onset in HD, and affects a large number of different transcription factors and regulatory DNA target sequences (e.g. the CREB-binding protein (CBP) [35] and repressor element 1 transcription factor (REST) [36]). This has been confirmed by DNA microarray studies [37]. Mutant HTT has also been shown to inhibit histone acetyltransferase activity, thus leading to the condensation of chromatin and a downregulation of gene transcription. This paves the way for histone deacetylase (HDAC) inhibitors as a potential therapy [38]

(continued)

Table 1.1 (continued)

Mechanism	Role of mutant Huntingtin
Inflammation	Microglial activation has been demonstrated in HD patients, both in manifest [39] and premanifest disease [40]. Recent evidence suggests that this is at least in part a cell-autonomous effect, as microglia that express mHTT have been shown to have enhanced transcription of pro-inflammatory factors [41]. A peripheral inflammatory response has also been shown with raised levels of pro-inflammatory cytokines found in blood samples of manifest and premanifest HD patients [42]
Trans-neuronal spread	Recently evidence for the non-cell autonomous, trans-neuronal spread of mHTT has come to light. mHTT was shown to spread to wild-type human neurons within an HD mouse model. This was blocked by botulinum neurotoxins, suggesting the involvement of synaptic machinery [43]. Furthermore, post-mortem studies of HD patients who have received experimental treatment with neural stem cell transplantation have revealed the presence of huntingtin inclusions in the grafted donor tissue [44]
Cytoskeleton signalling and vesicular trafficking	Through its interactions with huntingtin-associated protein 1 (HAP1), huntingtin-associated protein of 40 kDa (HAP40) and dynein, HTT regulates vesicle transport and recycling [45]. Expression of mHTT disrupts this transport, including vesicular trafficking of BDNF [46, 47]. mHTT aggregates can also physically obstruct subcellular transport due to their large size [48]
Mitochondrial dysfunction	Mutant HTT affects the axonal transport of mitochondria and also has a direct effect on mitochondrial function. Mitochondrial calcium handling is impaired, and transcription of nuclear genes responsible for the proper functioning of this organelle is affected, leading to impaired respiration [49]. An increase in mitochondrial DNA mutations has also been found in neurons from the cortex of HD patients [50]
Excitotoxicity	Injection of quinolinic acid (an NMDA receptor agonist) into the striatum of mouse models recapitulates many aspects of the HD phenotype [51]. In addition it has now been shown that alterations in glutamatergic signalling occur through changes in glutamate release [52], overactivity of glutamate receptors [53], decreased levels of glutamate transporters and reduced glutamate uptake [54]. Increased MSN excitability has also been shown to result from decreased potassium channel expression in mHTT expressing astrocytes [55]
Clearance of mHTT	Two major cellular pathways for degradation of misfolded proteins are the ubiquitin-proteasome system (UPS) and autophagy. Impairment of the UPS has been noted in HD mouse models and human post-mortem brain tissue [56]. In addition, mHTT can interfere with target recognition and impair autophagic clearance [57, 58]
Somatic instability of CAG repeat	CAG repeat expansion in germline cells has already been described, and can result in cases of juvenile HD. Somatic expansion has also been shown to occur in the human striatum [59] and is associated with younger onset, thus may contribute to disease pathogenesis [60]. Impairment of DNA repair pathways has been implicated in this [61]

Ultimately there is massive striatal degeneration in the patient brain, as revealed by post-mortem studies. Up to 95% of the GABAergic medium spiny neurons (MSNs) that project to the globus pallidus and substantia nigra are lost, and there is atrophy (though less so) of the cerebral cortex, subcortical white matter, thalamus and hypothalamic nuclei [27].

1.5 Clinical Aspects of Huntington's Disease

Huntington's disease is characterized by a triad of progressive motor, cognitive and psychiatric symptoms. The average age of onset is 45 years and the disease is fatal after 15–20 years [62].

1.5.1 Natural History and Disease Progression

As mentioned above, onset of "manifest" HD is said to occur when patients develop definitive motor signs suggestive of HD, which have no other explanation. However prior to this, patients may have a "prodromal" phase of HD for many years, during which subtle motor signs, psychiatric or cognitive symptoms may be present. The TRACK-HD study found differences in speeded tapping (a fine motor task) over 36 months between control and premanifest patients who were predicated to be over 10.8 years from disease onset. During this time a corresponding change in neurobiology, with loss of corticostriatal connectivity and striatal atrophy, is seen (Fig. 1.1).

Prior to development of manifest HD, patients who carry the HD gene mutation are referred to as "premanifest". Around 10–15 years before disease onset, premanifest individuals are indistinguishable from controls. However as disease onset approaches, patients are no longer completely asymptomatic and so the term "perimanifest" disease is used by clinicians to describe patients who are suffering from *prodromal* HD symptoms, and are thought to likely develop the concrete motor signs of manifest HD in the near future (Fig. 1.2).

1.5.1.1 Rating Scales and Disease Progression

The Unified HD Rating Scale (UHDRS) is comprised of motor, cognitive, behavioural, emotional and functional components and is the most common rating scale for HD [65]. It is widely utilised in HD research, and some of its component parts are also useful in a clinical setting. The total motor score (TMS) subscale is helpful in diagnosing disease onset; based on the score and their overall clinical impression, the clinician assigns a diagnostic confidence score (DCS) between 0 and 4, which reflects their belief that the motor signs are representative of HD.

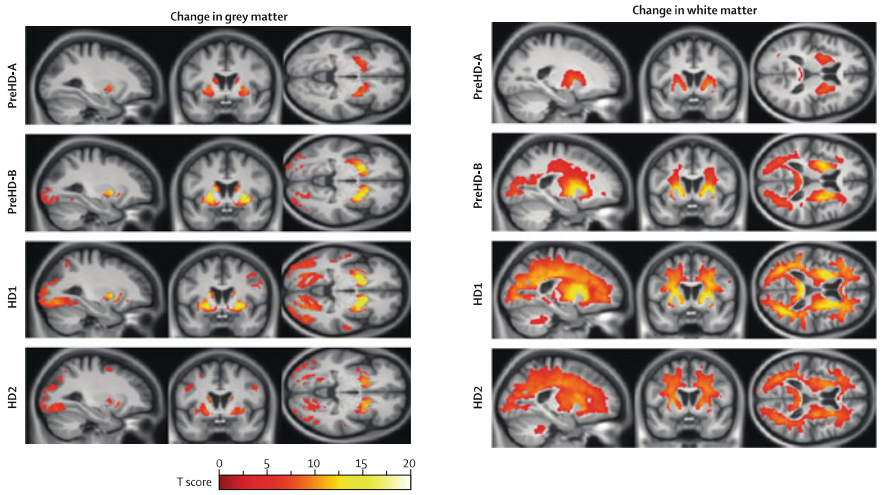

Fig. 1.1 Parametric maps showing regions with statistically significant atrophy in grey and white matter at baseline, 12- and 24 months (from left to right in each frame). PreHD-A and preHD-B are premanifest Huntington's disease gene carriers with estimated time to clinical disease onset greater than and less than 10.8 years, respectively. HD1 and HD2 are patients with early manifest disease who have no functional impairment and mild functional impairment, respectively. The striatum is affected early on, with more widespread atrophy at later stages. Adapted from Tabrizi et al. [63] from Elsevier publishing group

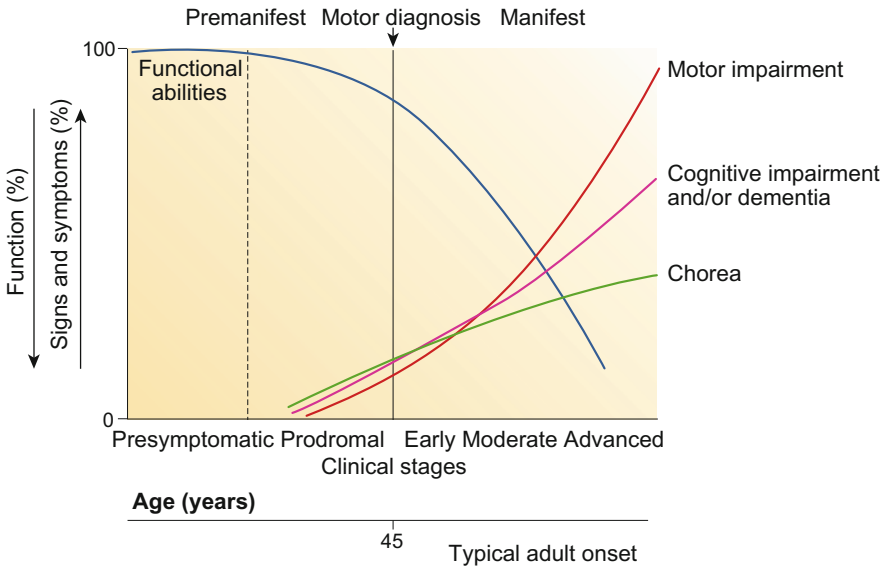

Fig. 1.2 Natural history of clinical Huntington's disease. Adapted from Ross et al. [64] from Nature Publishing Group

Table 1.2 The Shoulson-Fahn Staging system

Stage of disease	Engagement in occupation	Capacity to handle financial affairs	Capacity to manage domestic responsibilities	Capacity to perform activities of daily living	Care can be provided at…
I	Usual level	Full	Full	Full	Home
II	Lower level	Requires slight assistance	Full	Full	Home
III	Marginal	Requires major assistance	Impaired	Mildly impaired	Home
IV	Unable	Unable	Unable	Moderately impaired	Home or extended care facility
V	Unable	Unable	Unable	Severely impaired	Total care facility

Diagnosis of disease onset can be a difficult and emotional time for patients and their families, as they are faced with the prospect of inevitable decline, and will have often witnessed other family members suffering the same fate. There are also implications for patients in terms of their employment, insurance and driving, the exact rules of which will vary between countries.

Following disease onset, there is progression through five disease stages, originally described by Shoulson and Fahn (Table 1.2). These stages also correspond to Total Functional Capacity (TFC) subscale score of the UHDRS, which is based on the same functional domains. In a clinical setting, the more general terms of early, moderate and advanced disease are used (Table 1.3).

1.5.2 Clinical Presentation

HD displays much clinical heterogeneity, even within families, in terms of the balance of motor, cognitive and psychiatric features that predominate.

Motor symptoms are comprised of both involuntary movements, seen in early-moderate stage typical adult onset disease, and impaired voluntary movement seen in more advanced stages. Chorea is one of the most striking features, and is

Table 1.3 The Total Functional Capacity (TFC) score and its relationship to Shoulson-Fahn stages and clinical descriptors

	Total Functional Capacity (TFC)	Stage
Early	11–13	I
	7–10	II
Moderate or mid	4–6	III
Advanced or late	1–3	IV
	0	V

defined as short-lived, involuntary, excessive movements, which are semi-purposeful. Chorea progresses from occasional, low amplitude twitches of the face and extremities to constant, large amplitude movements of the entire body. Head turning, eye closure and tongue protrusion occur following activation of facial and neck muscles and back extension results from involvement of axial muscle groups. Chorea may not always bother the patient, even when quite severe. It can however cause problems with writing, eating and maintaining balance.

Other motor features include dystonia, myoclonic jerks and tics. Dystonia is the most common of these and is caused by inappropriate, sustained muscle contractions that lead to abnormal postures such as torticollis (neck turning) and opisthotonos (back arching).

As disease progresses, bradykinesia, akinesia, rigidity and impaired postural reflexes dominate. This can cause particular problems with gait and falls, especially on uneven terrain. Motor symptoms can also rapidly deteriorate in the event of intercurrent infections, stress and anxiety, but with appropriate treatment, this is usually temporary.

Cognitive symptoms are universal in HD. Subtle cognitive impairments may occur years before disease onset [66], and can progress to frank subcortical and frontal dementia in advanced disease. The TRACK-HD study found early deficits in visual attention, psychomotor speed, and visuomotor and spatial integration [25], though patients themselves are often unaware of any problems. Executive dysfunction, with development of concrete thinking is very common. Other cognitive problems include general slowing and impaired short-term memory.

Psychiatric symptoms affect a large proportion of patients; depression is the most common feature, which has a prevalence of 40% in HD patients [67]. Anxiety is the second most common psychiatric condition. Neither depression nor anxiety relate to disease stage and can occur in premanifest HD [68]. Apathy, which is characterised by a general loss of interest, difficulty with initiating activities and passive behaviour, is a common and disabling symptom that is related to disease stage and worsens over time. It has been shown to be a significant predictor of functional decline [25], and is resistant to medication.

Other psychiatric symptoms include irritability and aggression, obsessive, compulsive thoughts and behaviours, and more rarely, psychosis in later stages. Hyper- and hyposexuality can be a problem in early and late HD respectively [69].

Suicide is the second most common cause of death in HD [70]. Risk of this is highest when premanifest patients start to develop manifest disease, and again when loss of independence starts to occur in advanced HD. In a study of 4171 HD patients, 10% had made a previous suicide attempt, and 17.5% had been affected by suicidal thoughts [71]. Risk factors include depression and impulsivity [72].

1.5.2.1 Other Neurological Symptoms

In addition to the triad of characteristic motor, cognitive and psychiatric symptoms described above, patients may have problems with their speech, swallowing and

sleep. Speech difficulties arise from a combination of dysarthria (from incoordination of the tongue and orofacial muscles) and word finding difficulties. In advanced disease, some patients may develop complete anarthria. Swallowing problems also result from incoordination of oral and pharyngeal muscles, and can lead to choking episodes and, in severe cases, aspiration pneumonia.

Sleep disturbance may result from primary dysfunction of circadian rhythms, leading to reversal of the sleep-wake cycle [73]. Low mood, anxiety and night-time chorea also contribute to insomnia and can significantly impair quality of life.

1.5.2.2 Peripheral Symptoms

HD is primarily a disease of the central nervous system (CNS) but cells throughout the body express Huntingtin protein, and consequently, a range of systemic symptoms are seen in HD [74]. Severe weight loss, secondary to an underlying catabolic state, is common and may lead to cachexia. This may begin during prodromal HD, and HD patients usually have very high calorie requirement in order to simply maintain their weight. There is an association between higher body mass index at disease onset and slower rate of disease progression [75].

Other peripheral symptoms include osteoporosis and skeletal muscle atrophy. Heart failure occurs in 30% of patients (compared to 2% of age-matched controls) [70]. Endocrine dysfunction in the form of impaired glucose tolerance, hypothyroidism and low male testosterone levels, also occurs. Testicular atrophy with abnormal seminiferous tubules and reduced numbers of germ cells is noted, but does not affect fertility.

1.5.2.3 Juvenile Huntington's Disease (JHD)

As mentioned previously, JHD is defined by an age of onset less than 20 years old, and generally arises in those who have inherited >55 CAG repeats [76]. The clinical presentation differs from adult onset disease in that rigidity, bradykinesia and akinesia are present from the outset, with fewer hyperkinetic movements. Seizures occur in 30–50% of patients and there may be learning difficulties and behavioural issues whilst at school [76]. This rigid phenotype of HD is also known as the Westphal or akinetic-rigid variant (on rare occasions, adult onset disease may present with this form).

1.5.3 Diagnosis and Investigations

Clinical findings including definite motor signs, combined with a family history suggestive of autosomal dominant transmission are suggestive of the diagnosis, which is confirmed through genetic testing to determine CAG repeat length [77]. As

mentioned above, in some cases a family history is absent, so a high index of suspicion is required if the clinical picture is otherwise consistent with HD. MRI and CSF testing do not currently play a role in the diagnosis of HD, other than to rule out alternative diagnoses.

Around 1% of patients who are thought to have HD, subsequently test negative for the HD gene mutation. These patients may have different underlying genetic mutations that are collectively known as "HD phenocopies" [78]. The most common cause is the *C9orf72* hexanucleotide repeat expansion mutation, which is also a major cause of sporadic and familial frontotemporal lobar degeneration and amyotrophic lateral sclerosis [79]. Known causes of HD phenocopies are shown in Table 1.4. In the majority of cases however, the diagnosis remains unknown.

Table 1.4 Diseases that can manifest as HD phenocopies, and their corresponding genetic cause

Disease	Mutation
Familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)	C9orf72—hexanucleotide repeat expansion in chromosome 9 open reading frame 72 protein
Huntington's disease like syndrome (HDL) 1	PRNP—octapeptide insertion in gene encoding prion protein
HDL2	JPH3—triplet repeat expansion in gene encoding junctophilin-3
HDL3	Causative mutation as yet unknown
Spinocerebellar ataxia (SCA) 17 (HDL4)	TBP—triplet repeat expansion in gene encoding TATA-box binding protein
SCA1/2/3	ATXN 1/2/3 0 triplet repeat expansion in gene encoding Ataxin-1/2/3 respectively
Dentatorubral-pallidoluysian atrophy (DRPLA)	ATN1—triplet repeat expansion in gene encoding atrophin-1
Chorea-acanthocytosis	VPS13A—mutation in gene encoding chorein
McLeod Syndrome	XK—mutation in XK gene on X-chromosome, encoding a supporting protein for Kell antigen on surface of red blood cell
Neuroferritinopathy (NBIA2)	PLA2G6—mutation in gene encoding ferritin light chain
Neurodegeneration with brain iron accumulation (NBIA1) or Pantothenate-kinase associated Neurodegeneration (PKAN)	PANK2—mutation in gene encoding pantothenate kinase 2
Inherited prion disease	PRNP—mutations in gene encoding prion protein
Friedreich's ataxia	FXN—triplet repeat expansion in gene encoding frataxin

1.5.4 Management of HD

A multidisciplinary approach involving neurologists, psychiatrists, general practitioners, physiotherapists, occupational therapists, speech and language therapists, dieticians and nurses, is required for the management of HD [80]. This is best achieved through specialist HD clinics, where representatives from local HD support groups can also attend to give help with practical, emotional and social matters. Such clinics are also useful for recruiting patients into research studies or clinical trials if appropriate; taking part in research can be of great psychological benefit to patients and their carers.

1.5.4.1 Drug Treatments

Although there are no disease-modifying treatments available currently, there are medications to alleviate symptoms [81, 82].

Chorea may not always require treatment as it often does not bother patients, but when function is impaired as a result then medication should be considered. Tetrabenazine (TBZ) is a dopamine-depleting agent that has proven efficacy at reducing chorea. It can trigger or worsen depression and should be avoided in those with a history of severe depression or current low mood [83]. Patients must be warned of this side effect and monitored. TBZ must not be prescribed with cytochrome P450 2D6 inhibitors (such as fluoxetine and paroxetine) as this is required for its clearance [84].

Although a Cochrane review concluded that only TBZ showed clear benefit for chorea, in practice other medications are also used [85]. Atypical neuroleptics such as olanzapine, risperidone and quetiapine are very useful, especially if there is coexistent irritability, agitation or anxiety. Older typical neuroleptics such as sulpiride and haloperidol are also used, but there are greater side effects associated. All neuroleptic use warrants an ECG review prior to starting the prescription, and regular monitoring of blood glucose and cholesterol whilst on treatment. Clonazepam is useful if chorea is combined with dystonia, rigidity or myoclonus. Sodium valproate or levetiracetam can be useful if there is significant myoclonus alone.

In later disease, anti-chorea medications may need to be weaned and stopped as rigidity and spasticity develop. Tizanidine and baclofen may be helpful at this stage and botulinum toxin injections are used for targeted muscle spasm. In JHD, a trial of levodopa may be helpful.

In terms of psychiatric symptoms, depression may be treated with selective serotonin reuptake inhibitors (SSRIs) such as citalopram. Mirtazapine, a sedating antidepressant, is useful when insomnia is also a problem. A specific risk assessment with respect to suicide should be carried out when evaluating psychiatric symptoms.

SSRIs may also be useful for the management of anxiety. Irritability and aggression may be treated with atypical neuroleptics (as for chorea). Higher doses may occasionally be needed for the treatment of psychosis. For sleep disturbances, a short-term course of zopiclone could be tried, or melatonin can also be useful to address the reversal of the sleep-wake cycle.

1.5.4.2 Non-Drug Treatments

Physiotherapy is useful to optimise gait and balance for as long as possible, and in later stages physiotherapists can offer walking aids if needed. Occupational therapists adapt the home environment in order to prolong independent functioning and increase safety.

Speech and language therapists can improve communication, both verbally up to a point, and later with communication aids. They can also assess swallowing and advise on appropriate diet. In advanced HD, enteral nutrition via a gastrostomy may be needed to maintain adequate nutrition. The feeding tube may be inserted as a percutaneous endoscopic gastrostomy (PEG) or through radiological guidance (RIG) if endoscopy cannot be tolerated. Dieticians can then set up enteral feeding regimens, and also give more general advice on calorie supplementation.

It is essential to liaise with local community mental health teams that provide psychiatric care and regular follow up or monitoring close to home. Cognitive behavioural therapy (CBT) can be administered by psychologists to appropriately selected patients, and can help with anxiety, depression and OCD symptoms.

Social workers are able to arrange home care when patients are no longer fully able to care for themselves, and if necessary to co-ordinate moving into a residential or nursing home. HD support organisations, e.g. the Huntington's disease association (HDA) in the UK, are invaluable with helping patients and carers with a range of issues from legal matters, employment issues, and claiming any entitlements, to organising local discussion/support groups.

One important issue that it is essential to address with patients is that of driving. By law, patients must notify the driving authorities once they have a diagnosis of manifest HD, and it is inevitable that at some point during the disease course, their driving licence will be revoked. It can cause patients a great deal of distress when this happens, particularly if they do not recognise that their driving ability is impaired.

1.5.4.3 End of Life Care

Inevitably as HD is a progressive condition, end of life care is important for all patients. Though discussion around this subject can be difficult, it is worth addressing these issues whilst patients retain capacity to make decisions, and before significant cognitive or psychiatric deterioration takes place.

As swallowing difficulties progress, oral feeding may become very risky. Whilst some patients are keen to establish enteral feeding under these circumstances, others do not want this under any circumstances. In the event of potentially terminal medical deterioration, patients also differ in the levels of care they would want in terms of antibiotics, hospital admission, resuscitation etc.

In the UK patients can draw up an advanced directive, which outlines the care they would want under particular circumstances should they lose capacity in the future. Alternatively patients may nominate an individual to have lasting power of attorney (LPA), who can make decisions on their behalf if it becomes necessary. In either case, these decisions must be made whilst the patient still has capacity to do so.

In advanced disease, home care may no longer be possible and a residential or nursing home may be necessary. At the very end of life, some patients may not want to go to hospital, preferring instead to be cared for at home or in a hospice setting. Community palliative care teams, district nurses and GPs can help provide symptomatic relief in these circumstances.

1.5.5 Genetic Testing

There is a readily available genetic test that determines the CAG repeat length in each allele of the *HTT* gene, which can be carried out on a standard blood sample. The relative ease of performing the actual test belies the complexity of the issues surrounding the genetic testing process [77].

1.5.5.1 Diagnostic Testing

Diagnostic genetic tests are requested by neurologists in order to confirm the suspected diagnosis in those patients who are already displaying motor signs of HD. Informed consent from the patient must be obtained prior to testing. The implications of a positive result for the patient and their family must be clearly outlined. Both the nature of HD itself and its autosomal dominant inheritance must be explained. It should be made clear that if they test positive for HD, any of their children would have a 50% chance of inheriting it. Their siblings would also be at risk with a 50% chance of having inherited the mutation themselves (and some may already be developing signs of HD).

Test results must be given in person at a follow up appointment, and in the case of a positive result, referral to a specialist HD clinic should be offered. Details of HD support groups should be given, and family members should be offered referral for genetic counselling if they wish.

It is worth noting that patients who have psychiatric or cognitive symptoms in the context of a positive family history of HD, but who do not have motor signs, must be referred to a clinical geneticist for testing as this would qualify as predictive rather than diagnostic testing.

1.5.5.2 Predictive Testing

Predictive testing refers to genetic testing that is carried out in asymptomatic individuals, who are at known to be at risk of inheriting the mutation due to a positive family history. There are international guidelines for predictive testing in HD [86–88]; testing must take place in specialist centres by a clinical geneticist. There must be at least one session of pre-test counselling, followed by a period of reflection, and a second counselling session. Written consent must be given, and as always, strict confidentiality observed. Results must be given face-to-face with further post-test counselling available.

As with diagnostic testing, it is essential to explain the dominant inheritance of HD and its potential impact on the whole family. A positive test result means that an individual will get HD at some point in the future, but it is not possible to predict their specific age of onset or rate of progression [22]. Patients who have CAG repeat lengths of reduced penetrance (36–40) or intermediate repeat lengths (27–35) should have a full explanation of the implications of this [89] (see Sect. 5.3. Genetics of Huntington's disease).

The personal consequences of either a positive or a negative result should be explored with individuals prior to testing. Following a positive result, patients will know that they are going to develop an incurable neurodegenerative disease in the future, and they may have already witnessed first-hand the devastating impact of this on their own relatives. A negative test result can also cause enormous feelings of guilt when other family members are affected, and lead to strain within family relationships. Difficult situations can also arise if certain family members do not wish to know their gene status. For example the grandchild of an affected individual may wish to undergo predictive testing, when their own parent does not. In this case a positive test result in the grandchild would necessarily mean that their at-risk parent was in fact carrying the mutation also.

It is not legal to carry out predictive testing in children under the age of 18. Children cannot give informed consent, and no one can consent on their behalf because they have a right *not* to know their gene status as an adult. Growing up with the knowledge of a positive test result would in any case be very damaging for the psychological development of most children.

5–20% of at-risk patients proceed with predictive genetic testing [90]. Common reasons for proceeding include planning for the future, especially in terms of careers or deciding to have children.

1.5.5.3 Reproductive Options

Pre-implantation genetic diagnosis (PGD) is available for HD gene carriers in many countries, including the UK. A number of embryos are created using in vitro fertilisation (IVF) techniques, and then screened for the mutation. Negative embryos are then selected for implantation into the uterus. Multiple cycles of IVF are sometimes required before a successful pregnancy results, and the process can

therefore be emotionally and physically demanding. There are also high costs associated with PGD, although in the UK a certain number of cycles are usually funded by the national healthcare system.

It is possible for at-risk patients to undergo PGD without finding out their own gene status. Linkage analysis to look for genetic markers linked to the *HTT* genes of the parents of the at-risk patient, can identify embryos that have a 50% chance or <1% chance of carrying the mutation. Some private clinics also offer non-disclosure testing when the at-risk prospective parent is tested for HD in order to make a decision on embryo selection, but then the same IVF procedure is followed and the genetic test results are not disclosed to the patient.

Another option is antenatal testing during pregnancy. Chorionic villous sampling (CVS) can be carried out at 11–13 weeks of gestation, or amniocentesis after 16 weeks, to obtain foetal tissue for genetic testing. Detailed counselling must be given prior to this, as parents need to be certain that they would terminate a pregnancy in which the foetus was positive for the mutation. If the pregnancy is continued in the face of a positive test result than the resulting child would effectively have had a positive predictive test to which they never consented. Other reproductive options include using a sperm or egg donor, or adoption.

It is worth discussing with patients who wish to become parents that although they may well be symptom free or many years (or lifelong if they are in fact gene negative), there is a chance that they may develop symptoms whilst their children are still young, or even during pregnancy. Patients may wish to see a neurologist for examination before making their decision to proceed with having children, which is of course a highly personal decision. Indeed some patients who have had positive predictive testing in the past, or who know they are at risk, may simply choose to start a family without seeking further medical help or any intervention at all.

1.6 Huntington's Disease Therapeutics Research

1.6.1 Disease Modifying Therapies

There is an urgent and as yet unmet need for a treatment which can slow, prevent or even reverse the symptoms of HD in mutation carriers. A number of promising approaches, including the reduction of Huntingtin expression and targeted small molecule therapeutics are currently under investigation in research studies and clinical trials (Fig. 1.3).

1.6.1.1 "Gene Suppression" or Reduction of Huntingtin Expression

Mutant HTT primarily exerts a dominant toxic effect, and so a reduction in its expression should reduce any downstream pathology. Strategies to lower HTT levels include both post-transcriptional inhibition such as RNA interference (RNAi) and

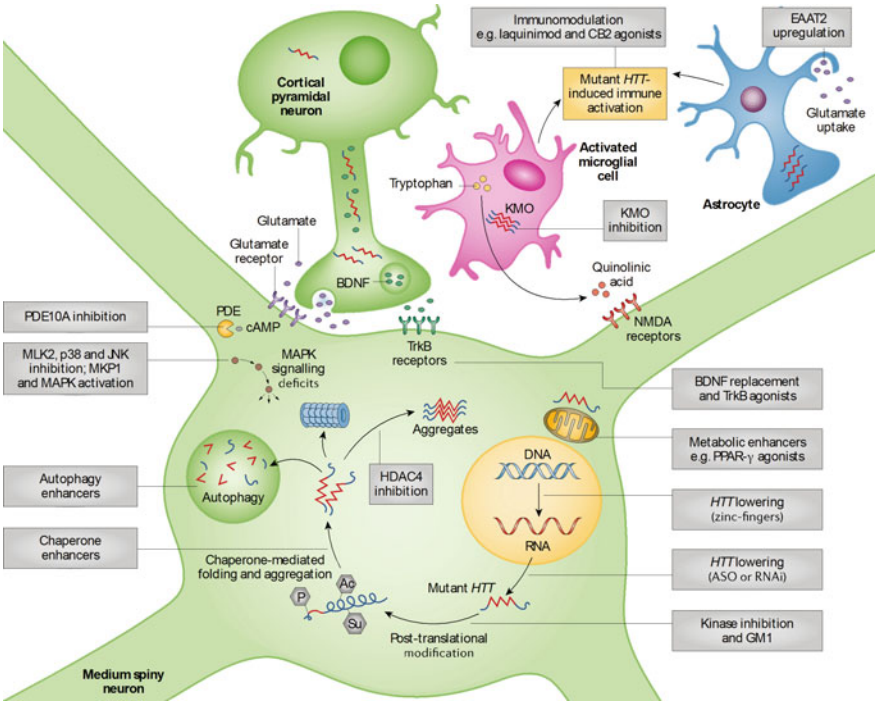

Fig. 1.3 Therapeutic targets under investigation in Huntington's disease. Reproduced from Ross et al. [64], Nature Publishing Group

anti-sense oligonucleotides (ASOs), and genome editing techniques such as zinc finger proteins (ZFPs) and CRISPR/Cas9.

Administration of complementary small interfering RNA molecules (siRNAs) results in Argonaut-2 mediated cleavage and degradation of mature, spliced HTT mRNA in the cytosol. ASOs have a more upstream site of action and recruit RNaseH1, an endogenous enzyme that recognises RNA/DNA duplexes and degrades HTT pre-mRNA [91].

siRNAs [92, 93] and ASOs [94] designed to lower total HTT levels have both been shown to improve disease symptoms in rodents. In humans there is potential concern with regards to the long-term effect of reducing wild-type HTT, although reduction of endogenous HTT has been shown to be well tolerated in non-human primates [95, 96].

It has been challenging to achieve allele-specific lowering of mutant HTT. However ASOs complementary to the expanded CAG have recently been produced which have selectivity for mHTT over wild-type HTT, and have led to phenotypic improvement in rodent models [97]. siRNAs designed to target single nucleotide polymorphisms (SNPs) that reside only on the mutant HTT allele could also be used to achieve allele specific suppression [98, 99]. ASOs targeting mutant-allele

linked SNPs have also been designed that lead to even greater (up to 50-fold) selectivity of the mHTT allele over the normal allele [100, 101]. Such compounds will soon proceed to clinical trials, and involve screening potential trial subjects for the presence of specific SNPs prior to drug administration.

Reduction of Huntingtin expression can also be achieved at the transcriptional level, using zinc finger proteins (ZFPs). These contain a zinc finger domain that can be synthetically manipulated to bind *HTT* DNA, fused to a functional domain such as a nuclease. Drug delivery is through the use of viral vectors, and this approach will also be proceeding into clinical trials in the near future.

Another recently discovered genome editing technique is through manipulation of the endogenous clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas9 system which recognises and destroys foreign DNA in prokaryotic cells. The mutant allele is inactivated by a synthetic guide RNA (gRNA) strand that targets a particular DNA location for cutting, followed by the insertion of a desired DNA sequence (for example stop codons) [102]. The technique has been successfully demonstrated in HD patient-derived fibroblasts, leading to the dramatic reduction of mutant HTT RNA and mHTT protein [103].

A seminal Phase 1/2a clinical trial (IONIS-HTTRx) is currently being carried out by Ionis Pharmaceuticals to test the safety and tolerability of an ASO targeting human HTT, delivered by lumbar intrathecal bolus administration to early stage HD patients [104]. Encouragingly, a recent clinical trial of intrathecal infusion of an ASO targeting SOD1 mRNA in patients with amyotrophic lateral sclerosis (ALS) has demonstrated the safety and tolerability of this general approach [105]. Furthermore, patients with spinal muscular atrophy (SMA) Type 1 (infantile onset) have been treated with lumbar intrathecal bolus injections of an ASO (Nusinersen) that alters mRNA splicing, and have shown dramatic improvement [106].

1.6.1.2 Targeted Small Molecule Approaches

Mutant Huntingtin has been shown to exert its toxic effect through many different pathological pathways. Therefore a number of potential targets for disease modification exist that have been or are currently being tested in animal models and in some cases, in clinical trials.

One approach is to target the post-translational modifications of mHTT, for example by increasing phosphorylation at certain neuroprotective residues. Administration of the ganglioside GM1 causes reversal of disease phenotype in HD mice, which is thought to occur by increasing beneficial phosphorylation [107]. Acetylation of mHTT promotes its clearance by autophagy, and the inhibition of the deacetylase sirtuin 1 by selisistat has been shown to have benefit in various HD models and more recently, has been found to be safe and tolerable to human patients [108].

Inhibition of phosphodiesterase (PDE) 10A (which is a major modulator of striatal synaptic biology regulating cAMP and cGMP signalling and synaptic plasticity) led to multiple phenotypic improvements in HD mice [109]. However,

the recently completed Pfizer "Amaryllis" trial of PDE10A inhibition in patients has not found any beneficial effect on motor function, or other HD symptoms that were tested [110].

Potentiation of the neurotrophin BDNF (which is depleted in HD), through agonism of the tyrosine receptor kinase B (TrkB), was initially thought to be a promising approach [111]. Agonism by monocloncal antibodies is currently under investigation [112]. However cysteamine, which is also thought to act through increasing BDNF levels, has not demonstrated efficacy in a recent clinical trial (CYST-HD) [113].

Inhibition of kynurenine 3-monooxygenase (KMO) improves the balance of neuroprotective over excitotoxic tryptophan metabolites produced by microglia. The peripherally administered KMO inhibitor JM6 has shown benefit in HD mouse models [114]. However more recently this has not been replicated using the novel KMO inhibitor CHDI-340246, although electrophysiological alterations were restored [115].

There is hyperactivity of the innate immune system in HD. The immune modulator laquinimod, which has been shown to reduce NFkB activation in astrocytes [116] and has demonstrated potential in the treatment of multiple sclerosis [117], is currently being tested in LEGATO-HD [118]. Its effect on motor function and brain imaging in early HD is being assessed.

Cellular metabolism is known to be affected in HD; the PPARγ agonist rosiglitazone has been shown to reduce mHTT-induced toxicity in striatal cells and improve motor function in HD mice [119]. The mitogen-activated protein kinase (MAPK) signalling pathway is deranged in HD and presents many therapeutic targets for potential amelioration, however this area is complex and not yet fully understood [120]. Counteracting excitotoxicity caused by mHTT has been tested using ceftriaxone, which upregulates the expression of the EAAT2 glutamate transporter, and has been shown to be beneficial in mouse models [121].

Other therapeutic targets that have shown potential success include the upregulation of chaperone proteins to reduce mHTT misfolding and aggregation [122, 123]. Inhibition of histone deacetylases (HDACs) in an effort to reduce the transcriptional dysregulation caused by mHTT has also been shown to be of benefit in HD models, although the mechanism by which it exerts its effects were different to that expected [124].

1.6.1.3 Stem Cell Therapy

There have been a number of small trials of foetal striatal transplantation in HD patients over the last 15 years in the UK [124−126], France [127, 128], Germany [129], Italy [130] and the USA [131], but the outcomes form these trials have been mixed. Improvement or stabilisation of motor, functional and neuropsychiatric symptoms has been reported by some [128], but not all the groups. Variability in results may be accounted for by the small sample size of patients at each centre and the heterogeneity between studies in terms of patient selection. There were also

differences with respect to the type of tissue transplanted (cells from whole ganglionic eminence versus lateral ganglionic eminence, and the foetal gestation), as well as the preparation of the donor tissue and numbers of cells transplanted [125].

Post-mortem studies have shown poor long-term graft survival, possibly due to allograft immunoreactivity, excitotoxicity or microglial responses directed against donor tissue [132]. More recently, aggregates of mHTT have been found in the grafted donor cells, implying the spread of mutant protein between neurons, and calling into question the feasibility of foetal striatal transplantation therapy as a strategy to treat HD [44].

1.6.1.4 Other Current and Recent HD Clinical Trials

Thus far there have been no completed Phase 3 clinical trials of potentially disease modifying therapies with a successful outcome. Trials of vitamin E, idebenone, baclofen, lamotrigine, creatine, coenzyme Q10 + remacemide, ethyl-eicosapentanoic acid and riluzole have all had negative results [133].

Trials aimed at improving overall function for people with HD include 2CARE and CREST-E, but both were recently terminated prematurely on the grounds of futility [134, 135]. 2CARE evaluated coenzyme Q10 at a dose of 2400 mg/day for a planned duration of 5 years, and CREST-E evaluated creatine at doses of up to 40 g/day for a planned duration of 3 years. Assessment of overall function was based on the Total Functional Capacity (TFC) score, but this may not have been a sensitive enough tool to detect any potential impact on disease progression [62].

Pridopidine, which is thought to act as a dopamine stabiliser in the CNS, failed to reach its primary endpoint in two clinical trials: HART [136] and MermaiHD [137]. However, both trials showed an improvement in motor symptoms as evaluated by the Total Motor Score (TMS) and so this drug was re-evaluated for its effect on TMS in the PRIDE-HD trial. Unfortunately, once again the drug did not differentiate from placebo, although there was some beneficial effect on TFC at lower doses [138]. This highlights the need for sensitive markers of clinical progression when designing clinical trials.

Deutetrabenazine (SD-809), designed for the treatment of chorea, has been shown in the FIRST-HD trial to result in improved motor signs over a period of 12 weeks when compared to placebo [139]. The ongoing, open-label extension of this study (ARC-HD) has shown that overnight conversion from tetrabenazine to deutetrabenazine is safe and effectively maintained chorea control [140]. A trial of deep brain stimulation for treatment of motor symptoms, HD-DBS [141], is also underway.

Non-motor features of HD have also been a target of clinical trials. The Reach2HD trial tested the drug PBT2 (thought to reduce metal-induced aggregation of mutant Huntingtin) in patients with early and moderate HD. The drug was found to be safe and well tolerated in patients, and a potential benefit on cognition (executive function) was observed, which needs to be followed up with a larger study [142]. Other trials targeting non-motor features of HD are Action-HD, which

has recently reported no significant effect of the drug bupropion on apathy (though there was a large placebo/participation effect in this study), and the ETON-Study [143], which is looking at the effect of epigallocatechin gallate on cognitive function. Finally, NEUROHD [144] is comparing the effect of olanzapine, tetrabenazine and tiapiride on overall function as assessed by the independence scale of the UHDRS.

1.6.2 Biomarkers

Objective biomarkers that accurately track disease progression are essential in order to detect any beneficial effects of potential therapies in a trial setting. Commonly used clinical scales such as the UHDRS might not be sensitive enough to detect subtle changes, and are susceptible to inter- and intra-rater variability. Furthermore, once effective therapies exist, preventing or delaying neurodegeneration in premanifest patients would be the goal; biomarkers are key in helping to decide when to time such interventions.

Longitudinal observational studies, including TRACK-HD [25] and PREDICT-HD [66] led to a more detailed understanding of the natural progression of HD from premanifest through to manifest disease. Structural MRI has demonstrated significantly faster rates of decline in striatal volume in premanifest and manifest individuals compared with age-matched controls, even in those estimated to be >15 years from estimated disease onset [23].

Other imaging modalities that have potential as biomarkers include diffusion tensor imaging (DTI) which has shown abnormalities in neuronal fibre orientation and integrity in white matter and subcortical grey matter structures in both premanifest and manifest HD, functional MRI techniques, and [18] F-fluorodeoxyglucose (FDG)-PET [145]. Magnetic resonance spectroscopy (MRS) also demonstrates abnormal metabolic activity, indicative of neuronal health and could be used to assess response to a therapeutic intervention [146].

Cognitive biomarkers are often limited by floor and ceiling effects, and confounded by educational level and mood. One potential measure is the Huntington's disease cognitive assessment battery (HD-CAB) [147]. Deterioration in emotion recognition is found in premanifest HD, and in early HD, performance on the Stroop test and indirect circle tracing also tracks clinical decline. Quantitative motor tasks such as the speeding tapping task (which is affected in premanifest HD), grip force variability and tongue force measures may also be of use in HD drug trials [25].

An ultrasensitive single-molecule counting (SMC) mHTT immunoassay has been developed that can quantify very low levels (in the femtomolar range) of mHTT in the cerebrospinal fluid (CSF). The level of mHTT detected was associated with proximity to disease onset and diminished cognitive and motor function, and is a new biomarker being taken forward into clinical trials [148]. Levels of tau in the CSF also show promise as a biomarker in HD [149]. Recently the level of

neurofilament light protein (NfL) in plasma, has demonstrated potential as a prognostic blood biomarker of disease onset and progression in Huntington's disease [150].

1.7 Conclusion

Thanks to intensive, collaborative, international research efforts spanning the last few decades, we now know a great deal about the genetics, pathogenesis and natural history of HD. Clinical presentation is with progressive motor, cognitive and psychiatric features for which there are currently only symptomatic treatments. Management is best achieved through specialist multidisciplinary clinics, which are linked to a wider research team that patients can access if they wish to. There are currently many exciting therapeutic developments in the pipeline, giving hope for a potentially disease modifying treatment in the near future.

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References

1. Fisher ER, Hayden MR (2014) Multisource ascertainment of Huntington disease in Canada: prevalence and population at risk. Mov Disord 29:105–114
2. Ross CA, Tabrizi SJ (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 10:83–98
3. Harper P (2002) The epidemiology of Huntington's disease. Huntington's disease. Oxford Medical Publications, Oxford
4. Evans SJ, Douglas I, Rawlins MD, Wexler NS, Tabrizi SJ, Smeeth L (2013) Prevalence of adult Huntington's disease in the UK based on diagnoses recorded in general practice records. J Neurol Neurosurg Psychiatry
5. Rawlins M (2010) Huntington's disease out of the closet? Lancet 376:1372–1373
6. Morrison PJ (2012) Prevalence estimates of Huntington disease in Caucasian populations are gross underestimates. Mov Disord 27:1707–1708. (author reply 8–9)
7. Kay C, Fisher E, Michael H (2014) Epidemiology. In: Tabrizi SJ, Jones L (eds) Bates G. Oxford University Press, Huntington's disease, pp 131–164
8. Gusella JF, Wexler NS, Conneally PM et al (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306:234–238
9. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, Barnes G et al (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983
10. Snell RG, MacMillan JC, Cheadle JP et al (1993) Relationship between trinucleotide repeat expansion and phenotypic variation in Huntington's disease. Nat Genet 4:393–397

11. Rubinsztein DC, Leggo J, Coles R et al (1996) Phenotypic characterization of individuals with 30-40 CAG repeats in the Huntington disease (HD) gene reveals HD cases with 36 repeats and apparently normal elderly individuals with 36-39 repeats. Am J Hum Genet 59:16–22
12. Killoran A, Biglan KM, Jankovic J, et al (2013) Characterization of the Huntington intermediate CAG repeat expansion phenotype in PHAROS. Neurology
13. Semaka A, Kay C, Doty C et al (2013) CAG size-specific risk estimates for intermediate allele repeat instability in Huntington disease. J Med Genet 50:696–703
14. Zühlke C, Riess O, Bockel B, Lange H, Thies U (1993) Mitotic stability and meiotic variability of the (CAG)n repeat in the Huntington disease gene. Hum Mol Genet 2:2063–2067
15. Kremer B, Almqvist E, Theilmann J et al (1995) Sex-dependent mechanisms for expansions and contractions of the CAG repeat on affected Huntington disease chromosomes. Am J Hum Genet 57:343–350
16. Barbeau A (1970) Parental ascent in the juvenile form of Huntington's chorea. Lancet 2:937
17. Kremer B (2002) Clinical Neurology of Huntington's disease. In: Harper P, (ed) Huntington's disease. Oxford Medical Publications, Oxford
18. Siesling S, Vegter-van de Vlis M, Losekoot M et al (2000) Family history and DNA analysis in patients with suspected Huntington's disease. J Neurol Neurosurg Psychiatry 69:54–59
19. Gusella JF, MacDonald ME, Lee JM (2014) Genetic modifiers of Huntington's disease. Mov Disord 29:1359–1365
20. Consortium GMoHsDG-H (2015) Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease. Cell 162:516–26
21. Wexler NS, Lorimer J, Porter J et al (2004) Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. Proc Natl Acad Sci U S A 101:3498–3503
22. Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR (2004) Group IHsDC. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. Clin Genet 65:267–277
23. Aylward EH, Nopoulos PC, Ross CA et al (2011) Longitudinal change in regional brain volumes in prodromal Huntington disease. J Neurol Neurosurg Psychiatry 82:405–410
24. Rosenblatt A, Kumar BV, Mo A, Welsh CS, Margolis RL, Ross CA (2012) Age, CAG repeat length, and clinical progression in Huntington's disease. Mov Disord 27:272–276
25. Tabrizi SJ, Scahill RI, Owen G, et al (2013) Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. Lancet Neurol
26. Zuccato C, Valenza M, Cattaneo E (2010) Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiol Rev 90:905–981
27. Halliday GM, McRitchie DA, Macdonald V, Double KL, Trent RJ, McCusker E (1998) Regional specificity of brain atrophy in Huntington's disease. Exp Neurol 154:663–672
28. Hughes A, Jones L (2014) Pathogenic mechanisms in Huntington's disease. In: Bates GP, Tabrizi SJ, Jones L (eds) Huntington's Disease, 4th edn. Oxford University Press
29. Sathasivam K, Neueder A, Gipson TA et al (2013) Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. Proc Natl Acad Sci U S A 110:2366–2370
30. Mangiarini L, Sathasivam K, Seller M et al (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506
31. Barbaro BA, Lukacsovich T, Agrawal N et al (2015) Comparative study of naturally occurring huntingtin fragments in Drosophila points to exon 1 as the most pathogenic species in Huntington's disease. Hum Mol Genet 24:913–925
32. Sun CS, Lee CC, Li YN et al (2015) Conformational switch of polyglutamine-expanded huntingtin into benign aggregates leads to neuroprotective effect. Sci Rep 5:14992
33. Arrasate M, Finkbeiner S (2012) Protein aggregates in Huntington's disease. Exp Neurol 238:1–11
34. Zuccato C, Marullo M, Conforti P, MacDonald ME, Tartari M, Cattaneo E (2008) Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. Brain Pathol 18:225–238

35. Steffan JS, Kazantsev A, Spasic-Boskovic O et al (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci U S A 97:6763–6768
36. Zuccato C, Tartari M, Crotti A et al (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 35:76–83
37. Hodges A, Strand AD, Aragaki AK et al (2006) Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet 15:965–977
38. Butler R, Bates GP (2006) Histone deacetylase inhibitors as therapeutics for polyglutamine disorders. Nat Rev Neurosci 7:784–796
39. Pavese N, Gerhard A, Tai YF et al (2006) Microglial activation correlates with severity in Huntington disease: a clinical and PET study. Neurology 66:1638–1643
40. Tai YF, Pavese N, Gerhard A et al (2007) Microglial activation in presymptomatic Huntington's disease gene carriers. Brain 130:1759–1766
41. Crotti A, Benner C, Kerman BE et al (2014) Mutant Huntingtin promotes autonomous microglia activation via myeloid lineage-determining factors. Nat Neurosci 17:513–521
42. Björkqvist M, Wild EJ, Thiele J et al (2008) A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. J Exp Med 205:1869–1877
43. Pecho-Vrieseling E, Rieker C, Fuchs S et al (2014) Transneuronal propagation of mutant huntingtin contributes to non-cell autonomous pathology in neurons. Nat Neurosci 17:1064–1072
44. Cicchetti F, Lacroix S, Cisbani G et al (2014) Mutant huntingtin is present in neuronal grafts in huntington disease patients. Ann Neurol 76:31–42
45. Pardo R, Molina-Calavita M, Poizat G, Keryer G, Humbert S, Saudou F (2010) pARIS-htt: an optimised expression platform to study huntingtin reveals functional domains required for vesicular trafficking. Mol Brain 3:17
46. Gauthier LR, Charrin BC, Borrell-Pagès M et al (2004) Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 118:127–138
47. Roux JC, Zala D, Panayotis N, Borges-Correia A, Saudou F, Villard L (2012) Modification of Mecp2 dosage alters axonal transport through the Huntingtin/Hap1 pathway. Neurobiol Dis 45:786–795
48. Li H, Wyman T, Yu ZX, Li SH, Li XJ (2003) Abnormal association of mutant huntingtin with synaptic vesicles inhibits glutamate release. Hum Mol Genet 12:2021–2030
49. Jin YN, Johnson GV (2010) The interrelationship between mitochondrial dysfunction and transcriptional dysregulation in Huntington disease. J Bioenerg Biomembr 42:199–205
50. Siddiqui A, Rivera-Sánchez S, MeR Castro et al (2012) Mitochondrial DNA damage is associated with reduced mitochondrial bioenergetics in Huntington's disease. Free Radic Biol Med 53:1478–1488
51. Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB (1986) Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature 321:168–171
52. Joshi PR, Wu NP, André VM et al (2009) Age-dependent alterations of corticostriatal activity in the YAC128 mouse model of Huntington disease. J Neurosci 29:2414–2427
53. Benn CL, Slow EJ, Farrell LA et al (2007) Glutamate receptor abnormalities in the YAC128 transgenic mouse model of Huntington's disease. Neuroscience 147:354–372
54. Huang SS, He J, Zhao DM, Xu XY, Tan HP, Li H (2010) Effects of mutant huntingtin on mGluR5-mediated dual signaling pathways: implications for therapeutic interventions. Cell Mol Neurobiol 30:1107–1115
55. Tong X, Ao Y, Faas GC et al (2014) Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nat Neurosci 17:694–703
56. Bennett EJ, Shaler TA, Woodman B et al (2007) Global changes to the ubiquitin system in Huntington's disease. Nature 448:704–708
57. Martinez-Vicente M, Talloczy Z, Wong E et al (2010) Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nat Neurosci 13:567–576
58. Qi L, Zhang XD, Wu JC et al (2012) The role of chaperone-mediated autophagy in huntingtin degradation. PLoS ONE 7:e46834

59. Kennedy L, Evans E, Chen CM et al (2003) Dramatic tissue-specific mutation length increases are an early molecular event in Huntington disease pathogenesis. Hum Mol Genet 12:3359–3367
60. Swami M, Hendricks AE, Gillis T et al (2009) Somatic expansion of the Huntington's disease CAG repeat in the brain is associated with an earlier age of disease onset. Hum Mol Genet 18:3039–3047
61. Dragileva E, Hendricks A, Teed A et al (2009) Intergenerational and striatal CAG repeat instability in Huntington's disease knock-in mice involve different DNA repair genes. Neurobiol Dis 33:37–47
62. Bates GP, Dorsey R, Gusella JF et al (2015) Huntington disease. Nat Rev Dis Primers 1:15005
63. Tabrizi SJ, Reilmann R, Roos RA et al (2012) Potential endpoints for clinical trials in premanifest and early Huntington's disease in the TRACK-HD study: analysis of 24 month observational data. Lancet Neurol 11:42–53
64. Ross CA, Aylward EH, Wild EJ et al (2014) Huntington disease: natural history, biomarkers and prospects for therapeutics. Nat Rev Neurol 10:204–216
65. Kieburtz K, Penney JB, Corno P, Ranen N, Shoulson I, Feigin A, Abwender D et al (2001) Unified Huntington's disease rating scale: reliability and consistency. Huntington Study Group. Mov Disord 11:136–142
66. Paulsen JS, Langbehn DR, Stout JC et al (2008) Detection of Huntington's disease decades before diagnosis: the Predict-HD study. J Neurol Neurosurg Psychiatry 79:874–880
67. van Duijn E, Kingma EM, van der Mast RC (2007) Psychopathology in verified Huntington's disease gene carriers. J Neuropsychiatry Clin Neurosci 19:441–448
68. Craufurd D, Snowden J (2002) Neuropsychological and neuropsychiatric aspects of Huntington's disease, in Huntington's disease. In: PS H (ed) Huntington's disease. Oxford Medical Publications, Oxford
69. Novak MJ, Tabrizi SJ (2010) Huntington's disease. BMJ 340:c3109
70. Lanska DJ, Lanska MJ, Lavine L, Schoenberg BS (1988) Conditions associated with Huntington's disease at death. A case-control study. Arch Neurol 45:878–880
71. Paulsen JS, Hoth KF, Nehl C, Stierman L (2005) Critical periods of suicide risk in Huntington's disease. Am J Psychiatry 162:725–731
72. Lipe H, Schultz A, Bird TD (1993) Risk factors for suicide in Huntingtons disease: a retrospective case controlled study. Am J Med Genet 48:231–233
73. Videnovic A, Leurgans S, Fan W, Jaglin J, Shannon KM (2009) Daytime somnolence and nocturnal sleep disturbances in Huntington disease. Parkinsonism. Relat Disord 15:471–474
74. van der Burg JM, Björkqvist M, Brundin P (2009) Beyond the brain: widespread pathology in Huntington's disease. Lancet Neurol 8:765–774
75. Myers RH, Sax DS, Koroshetz WJ et al (1991) Factors associated with slow progression in Huntington's disease. Arch Neurol 48:800–804
76. Andrew SE, Goldberg YP, Kremer B et al (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. Nat Genet 4:398–403
77. Craufurd D, MacLeod R, Frontali M, et al (2014) Diagnostic genetic testing for Huntington's disease. Pract Neurol
78. Wild EJ, Mudanohwo EE, Sweeney MG et al (2008) Huntington's disease phenocopies are clinically and genetically heterogeneous. Mov Disord 23:716–720
79. Hensman Moss DJ, Poulter M, Beck J et al (2014) C9orf72 expansions are the most common genetic cause of Huntington disease phenocopies. Neurology 82:292–299
80. Novak MJ, Tabrizi SJ (2011) Huntington's disease: clinical presentation and treatment. Int Rev Neurobiol 98:297–323
81. Bonelli RM, Hofmann P (2007) A systematic review of the treatment studies in Huntington's disease since 1990. Expert Opin Pharmacother 8:141–153
82. Frank S (2014) Treatment of Huntington's disease. Neurotherapeutics 11:153–160
83. Group HS (2006) Tetrabenazine as antichorea therapy in Huntington disease: a randomized controlled trial. Neurology 66:366–372

84. Guay DR (2010) Tetrabenazine, a monoamine-depleting drug used in the treatment of hyperkinetic movement disorders. Am J Geriatr Pharmacother 8:331–373
85. Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C (2009) Therapeutic interventions for symptomatic treatment in Huntington's disease. Cochrane Database Syst Rev CD006456
86. Craufurd D, Tyler A (1992) Predictive testing for Huntington's disease: protocol of the UK Huntington's Prediction Consortium. J Med Genet 29:915–918
87. Went L (1990) Ethical issues policy statement on Huntington's disease molecular genetics predictive test. International Huntington Association. World Federation of Neurology. J Med Genet 27:34–38
88. Guidelines for the molecular genetics predictive test in Huntington's disease. International Huntington Association (IHA) and the World Federation of Neurology (WFN) Research Group on Huntington's Chorea. Neurology 1994;44:1533–6
89. Semaka A, Hayden MR (2014) Evidence-based genetic counselling implications for Huntington disease intermediate allele predictive test results. Clin Genet 85:303–311
90. Harper PS, Lim C, Craufurd D (2000) Ten years of presymptomatic testing for Huntington's disease: the experience of the UK Huntington's Disease Prediction Consortium. J Med Genet 37:567–571
91. Keiser MS, Kordasiewicz HB, McBride JL (2016) Gene suppression strategies for dominantly inherited neurodegenerative diseases: lessons from Huntington's disease and spinocerebellar ataxia. Hum Mol Genet 25:53–64
92. Harper SQ, Staber PD, He X et al (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proc Natl Acad Sci U S A 102:5820–5825
93. Stanek LM, Sardi SP, Mastis B et al (2014) Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. Hum Gene Ther 25:461–474
94. Kordasiewicz HB, Stanek LM, Wancewicz EV et al (2012) Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron 74:1031–1044
95. McBride JL, Pitzer MR, Boudreau RL et al (2011) Preclinical safety of RNAi-mediated HTT suppression in the rhesus macaque as a potential therapy for Huntington's disease. Mol Ther 19:2152–2162
96. Grondin R, Kaytor MD, Ai Y et al (2012) Six-month partial suppression of Huntingtin is well tolerated in the adult rhesus striatum. Brain 135:1197–1209
97. Datson NA, González-Barriga A, Kourkouta E et al (2017) The expanded CAG repeat in the huntingtin gene as target for therapeutic RNA modulation throughout the HD mouse brain. PLoS ONE 12:e0171127
98. Pfister EL, Kennington L, Straubhaar J et al (2009) Five siRNAs targeting three SNPs may provide therapy for three-quarters of Huntington's disease patients. Curr Biol 19:774–778
99. Miller JRC, Pfister EL, Liu W et al (2017) Allele-selective suppression of mutant Huntingtin in primary human blood cells. Sci Rep 7:46740
100. Southwell AL, Skotte NH, Kordasiewicz HB et al (2014) In vivo evaluation of candidate allele-specific mutant huntingtin gene silencing antisense oligonucleotides. Mol Ther 22:2093–2106
101. Carroll JB, Warby SC, Southwell AL et al (2011) Potent and selective antisense oligonucleotides targeting single-nucleotide polymorphisms in the Huntington disease gene/allele-specific silencing of mutant huntingtin. Mol Ther 19:2178–2185
102. Cox DB, Platt RJ, Zhang F (2015) Therapeutic genome editing: prospects and challenges. Nat Med 21:121–131
103. Shin JW, Kim KH, Chao MJ et al (2016) Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. Hum Mol Genet 25:4566–4576
104. Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of IONIS-HTTRx in Patients With Early Manifest Huntington's Disease. NCT02519036. Aug 1st 2015
105. Miller TM, Pestronk A, David W et al (2013) An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. Lancet Neurol 12:435–442

106. Finkel RS, Chiriboga CA, Vajsar J et al (2016) Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. Lancet 388:3017–3026
107. Di Pardo A, Maglione V, Alpaugh M et al (2012) Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proc Natl Acad Sci U S A 109:3528–3533
108. Süssmuth SD, Haider S, Landwehrmeyer GB et al (2015) An exploratory double-blind, randomized clinical trial with selisistat, a SirT1 inhibitor, in patients with Huntington's disease. Br J Clin Pharmacol 79:465–476
109. Giampà C, Laurenti D, Anzilotti S, Bernardi G, Menniti FS, Fusco FR (2010) Inhibition of the striatal specific phosphodiesterase PDE10A ameliorates striatal and cortical pathology in R6/2 mouse model of Huntington's disease. PLoS ONE 5:e13417
110. Health NIo. A Phase 2, Double-Blind Randomized, Sequential Treatment Group, Placebo-Controlled Study To Evaluate The Safety, Tolerability And Brain Cortico-Striatal Function Of 2 Doses Of PF-02545920 In Subjects With Early Huntington's Disease. Pfizer; 2014
111. Simmons DA, Belichenko NP, Yang T et al (2013) A small molecule TrkB ligand reduces motor impairment and neuropathology in R6/2 and BACHD mouse models of Huntington's disease. J Neurosci 33:18712–18727
112. Todd D, Gowers I, Dowler SJ et al (2014) A monoclonal antibody TrkB receptor agonist as a potential therapeutic for Huntington's disease. PLoS ONE 9:e87923
113. Verny C, Bachoud-Lévi AC, Durr A et al (2017) A randomized, double-blind, placebo-controlled trial evaluating cysteamine in Huntington's disease. Mov Disord 32:932–936
114. Zwilling D, Huang SY, Sathyasaikumar KV et al (2011) Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. Cell 145:863–874
115. Beaumont V, Mrzljak L, Dijkman U et al (2016) The novel KMO inhibitor CHDI-340246 leads to a restoration of electrophysiological alterations in mouse models of Huntington's disease. Exp Neurol 282:99–118
116. Brück W, Pförtner R, Pham T et al (2012) Reduced astrocytic NF-κB activation by laquinimod protects from cuprizone-induced demyelination. Acta Neuropathol 124:411–424
117. Comi G, Jeffery D, Kappos L et al (2012) Placebo-controlled trial of oral laquinimod for multiple sclerosis. N Engl J Med 366:1000–1009
118. A Clinical Study in Subjects With Huntington's Disease to Assess the Efficacy and Safety of Three Oral Doses of Laquinimod (LEGATO-HD). Teva Branded Pharmaceutical Products, R&D Inc. 2016 ongoing
119. Jin J, Albertz J, Guo Z et al (2013) Neuroprotective effects of PPAR-γ agonist rosiglitazone in N171-82Q mouse model of Huntington's disease. J Neurochem 125:410–419
120. Wild EJ, Tabrizi SJ (2014) Targets for future clinical trials in Huntington's disease: What's in the pipeline? Mov Disord 29:1434–1445
121. Miller BR, Dorner JL, Shou M et al (2008) Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse. Neuroscience 153:329–337
122. Sontag EM, Joachimiak LA, Tan Z et al (2013) Exogenous delivery of chaperonin subunit fragment ApiCCT1 modulates mutant Huntingtin cellular phenotypes. Proc Natl Acad Sci U S A 110:3077–3082
123. Labbadia J, Novoselov SS, Bett JS et al (2012) Suppression of protein aggregation by chaperone modification of high molecular weight complexes. Brain 135:1180–1196
124. Mielcarek M, Landles C, Weiss A et al (2013) HDAC4 reduction: a novel therapeutic strategy to target cytoplasmic huntingtin and ameliorate neurodegeneration. PLoS Biol 11:e1001717
125. Barker RA, Mason SL, Harrower TP et al (2013) The long-term safety and efficacy of bilateral transplantation of human fetal striatal tissue in patients with mild to moderate Huntington's disease. J Neurol Neurosurg Psychiatry 84:657–665
126. Reuter I, Tai YF, Pavese N et al (2008) Long-term clinical and positron emission tomography outcome of fetal striatal transplantation in Huntington's disease. J Neurol Neurosurg Psychiatry 79:948–951
127. Bachoud-Lévi AC, Rémy P, Nguyen JP et al (2000) Motor and cognitive improvements in patients with Huntington's disease after neural transplantation. Lancet 356:1975–1979

128. Bachoud-Lévi AC, Gaura V, Brugières P et al (2006) Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term follow-up study. Lancet Neurol 5:303–309
129. Capetian P, Knoth R, Maciaczyk J et al (2009) Histological findings on fetal striatal grafts in a Huntington's disease patient early after transplantation. Neuroscience 160:661–675
130. Gallina P, Paganini M, Lombardini L et al (2010) Human striatal neuroblasts develop and build a striatal-like structure into the brain of Huntington's disease patients after transplantation. Exp Neurol 222:30–41
131. Hauser RA, Furtado S, Cimino CR et al (2002) Bilateral human fetal striatal transplantation in Huntington's disease. Neurology 58:687–695
132. Wijeyekoon R, Barker RA (2011) The current status of neural grafting in the treatment of Huntington's disease. A Review. Front Integr Neurosci 5:78
133. Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C (2009) Therapeutic interventions for disease progression in Huntington's disease. Cochrane Database Syst Rev CD006455
134. Announcement of 2CARE Early Study Closure. http://huntingtonstudygroup.org/tag/2care/2014
135. Group HS nnouncement of CREST-E Early Study Closure. http://huntingtonstudygroup.org/tag/crest-e/2014
136. Investigators HSGH (2013) A randomized, double-blind, placebo-controlled trial of pridopidine in Huntington's disease. Mov Disord 28:1407–1415
137. de Yebenes JG, Landwehrmeyer B, Squitieri F et al (2011) Pridopidine for the treatment of motor function in patients with Huntington's disease (MermaiHD): a phase 3, randomised, double-blind, placebo-controlled trial. Lancet Neurol 10:1049–1057
138. Reilmann R, AM, Landwehrmeyer G, Kieburtz K, Grachev I, Eyal E, Savola J, Borowsky B, Papapetropoulos S, Hayden M (2017) Efficacy, safety, and tolerability of pridopidine in Huntington disease (HD): results from the phase ii dose-ranging study, pride-HD. In: 21st international congress of parkinson's disease and movement disorders, 2017, Vancouver, BC, Mov Disord. 32(suppl 2), http://www.mdsabstracts.org/abstract/efficacy-safety-and-tolerability-of-pridopidine-in-huntington-disease-hd-results-from-the-phase-ii-dose-ranging-study-pride-hd/
139. Frank S, Testa CM, Stamler D et al (2016) Effect of deutetrabenazine on chorea among patients with Huntington disease: a randomized clinical trial. JAMA 316:40–50
140. Frank S, Stamler D, Kayson E et al (2017) Safety of converting from tetrabenazine to deutetrabenazine for the treatment of chorea. JAMA Neurol 74:977–982
141. Deep Brain Stimulation (DBS) of the Globus Pallidus (GP) in Huntington's Disease (HD) (HD-DBS). 2015, ongoing
142. Investigators HSGRH. Safety, tolerability, and efficacy of PBT2 in Huntington's disease: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet Neurol 2015;14:39–47
143. Effects of EGCG (Epigallocatechin Gallate) in Huntington's Disease (ETON-Study) (ETON)
144. Neuroleptic and Huntington Disease Comparison of: Olanzapine, la Tetrabenazine and Tiapride (NEUROHD
145. Tang CC, Feigin A, Ma Y et al (2013) Metabolic network as a progression biomarker of premanifest Huntington's disease. J Clin Invest 123:4076–4088
146. Sturrock A, Laule C, Decolongon J et al (2010) Magnetic resonance spectroscopy biomarkers in premanifest and early Huntington disease. Neurology 75:1702–1710
147. Stout JC, Queller S, Baker KN et al (2014) HD-CAB: a cognitive assessment battery for clinical trials in Huntington's disease 1,2,3. Mov Disord 29:1281–1288
148. Wild EJ, Boggio R, Langbehn D et al (2015) Quantification of mutant huntingtin protein in cerebrospinal fluid from Huntington's disease patients. J Clin Invest 125:1979–1986
149. Rodrigues FB, Byrne L, McColgan P et al (2016) Cerebrospinal fluid total tau concentration predicts clinical phenotype in Huntington's disease. J Neurochem 139:22–25
150. Byrne LM, Rodrigues FB, Blennow K et al (2017) Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. Lancet Neurol 16:601–609

Chapter 2
Genetic Rodent Models of Huntington Disease

J. Stricker-Shaver, A. Novati, L. Yu-Taeger and H. P. Nguyen

Abstract The monogenic nature of Huntington disease (HD) has led to the development of a spectrum of useful genetically modified models. In particular, rodents have pioneered as the first HD model being generated and have since been the most widely used animal model for HD in both basic research and preclinical therapeutic studies. Based on the generation strategies, these rodent models can be classified into 3 major groups, the transgenic fragment models, the transgenic full-length models and the knock-in models. These models display a range of HD-like characteristics which resemble the clinical symptoms of HD patients. Their applications in research are thus regarded as an invaluable approach to speeding up the unraveling of the underlying pathological mechanisms of HD and for finding a disease-modifying treatment for this devastating disease. In this chapter, the similarities and differences of the most commonly used rodent HD models and their relevance to human HD will be compared and discussed. This also serves to guide the selection of an appropriate rodent HD model according to the nature of investigation.

Keywords Huntington disease · Transgenic · Knock-in · Mouse · Rat

2.1 Introduction

The discovery of the sole genetic cause of Huntington disease (HD)—the expansion of the CAG repeats in the huntingtin gene (*HTT*) [1]—allowed the generation of animal models to different extents that mimic the genetic aspects of the disease [2]. These HD animal models have made core contributions to understanding HD pathogenesis [3], identifying potential therapeutic approaches [4–6] and developing

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new diagnostic methods for assessing the disease stage in patients. Among all HD animal models, rodents are the most frequently used species. At present, these genetically modified HD rodent models can be classified into 3 groups based on their generation strategies. The first group of animal models, the transgenic fragment models, carries only the N-terminal portion of the human *HTT* gene where the CAG expansion is located. The second group is the transgenic full-length models that carry the entire mutant huntingtin (Htt) protein. While these 2 groups involve the insertion of the human *HTT* gene into the rodent genomes, the third group of animal models are generated by modifying the length of the CAG repeats of the endogenous rodent *HTT* gene and they are known as the knock-in models. The constructs used for generating these models commonly differ in the number of CAG repeats, type of transcription promoter, strain and genetic background (i.e. homozygosity and heterozygosity). In some cases, genetic tools and strategies (such as the Cre/LoxP system) are used to generate constructs with conditional and reversible expression of mutant huntingtin (mHtt). As a result, in addition to the acute lesion models, where HD-like features are generated by direct injection of neurotoxic chemicals (e.g. quinolinic acid and 3-NP) to the striatum, we have currently a variety of rodent models available that displays a diversity of HD-like properties. Hence, in this chapter, we aim to provide a comprehensive description of the commonly used models, their constructs, phenotypes and predictability of various HD-related pathologies and their relevance to basic research and preclinical therapeutic studies in HD.

2.2 Mouse Models

The mouse represents an excellent model organism for studying genetic disorders and identifying therapeutic targets due to their well-established and extensive use within various fields of genetics research [7]; reviewed in [8]. As a result, mouse was chosen as the first transgenic mammalian model for HD [9]. A range of HD mouse models have since then been established. In this chapter, we will highlight the important characteristics relevant to human HD and details will be summarized in Table 2.1.

2.2.1 Transgenic Fragment Models

This group represents the first type of rodent model generated for HD. In 1996, 2 transgenic lines, R6/1 and R6/2, were established with a 1.9 kb human genomic fragment containing the human huntingtin (human *HTT*) promoter, exon 1 of the human *HTT* gene carrying expanded CAG repeats and the first 262 bp of intron 1 [9]. The R6/1 model carries 115 CAG repeats while the R6/2 model has 145 CAG repeats [10, 9] although variability of repeat expansions have been reported due to

Table 2.1 Mouse models of Huntington disease

Transgenic fragment mouse models					Transgenic full-length mouse models	
Mouse models	R6/1	R6/2	N171-82Q	N586-82Q	YAC128	BACHD
Promoter	Human *HTT*	Human *HTT*	Murine prion	Murine prion	Human *HTT* locus	Human *HTT* locus
PolyQ size	115 CAG	145 CAG	82 CAG	82 CAG	128 CAG	97 CAG/CAA
Strain	CBA C57BL/6 mixed background	CBA C57BL/6 mixed background	C3H/HEJ C57BL/6JF1 mixed background	C57BL/6 C3H	FVB/N	FVB/NJ
Gene/protein context	Human Htt Amino acids 1–82	Human Htt Amino acids 1–82	Human Htt Amino acids 1–171	Human Htt Amino acids 1–586	Human full-length mHtt	Human full-length mHtt
Protein expression level (relative to endogenous Htt) (%)	31	75	20	Approx. 100	75	150–200
Repeat stability	Unstable	Unstable	Unstable			Stable
Body weight	Weight loss	Weight loss (10 wks)	Weight loss (8 wks)	Weight loss	Weight gain	Weight gain
Brain atrophy	Yes	Yes	Yes	Yes	Yes	Yes
Life span	Reduced life span (32–40 wks)	Reduced life span (10–13 wks)	Reduced life span (Line 77–2.5 months, lines 81 and 100–5–6 months, Line 6–8–11 months)	Reduced life span (8–9 months for N586-82Q-63C)	Reduced life span in male	Normal
Motor performance	Progressive decline—rotarod (8 wks), tail suspension (16–20 wks) Reduced activity—open field (23 wks) Mild tremor and intermittent movement disorders as in R6/2 (24–28 wks)	Progressive decline from 5 wks—rotarod (5–6 wks), swimming test, beam walking, footprint test and home cage behaviour (8–9 wks), grip strength and limbs weakness (9–11 wks), severe impairments (12 wks onwards)	Progressive decline—gait abnormalities (2 months), rotarod (3 months), hypoactivity (5 months), tail suspension (end-stage)	Progressive decline—rotarod (3 months), open-field (4 months) Dyskinesia with ataxia-like movements (4 months onwards)	Progressive decline—rotarod (4 months) Gait abnormalities—beam—walking and footprint tests (8 months) Hyperactivity (3 months) followed by hypoactivity (12 months)	Progressive decline—rotarod (2 months) Gait deficits—Catwalk analysis (9–10 months) Decreased activity in the open field (from 6 months)

(continued)

Table 2.1 (continued)

Transgenic fragment mouse models					Transgenic full-length mouse models	
Psychiatric phenotypes	Anxiety-like behaviour—open field Anxio-depressive-like phenotype—novelty suppressed feeding test Depressive-like phenotype—sucrose preference test (female, 8 wks), forced swim test (female, 8 wks), tail suspension (female, 12 wks)	Anxiety-like behaviour—open field (8 wks), light/dark box (12–14 wks)	Depressive-like behaviour—forced swim test (14 wks)		Depressive-like phenotype (3 months)—sucrose consumption, forced swim and tail suspension tests Anxiety-like phenotype—light/dark box (12 months), elevated plus maze (2 months), zero maze and open field tests (6 months)	Depressive-like phenotype (2 months)—sucrose consumption and forced swim tests Anxiety-like phenotype (2 months)—light/dark box, elevated plus maze, zero maze and open field tests Emotional alteration—fear conditioning test (9–10 months)
Cognitive phenotypes	Impairments in acoustic startle and prepulse inhibition (PPI) (8 wks) Social behaviour and social interaction (12 wks) Spatial learning deficits—Morris water maze, food reinforcement in open maze, Barnes maze (12 wks)	Progressive learning and memory deficits Spatial learning—Morris water maze (3.5 wks), T-maze (5 wks) Reversal learning—Two-choice swim tank (6.5 wks) Exploratory and fear conditioning (5–6 wks) Avoidance—visual cliff avoidance (7 wks)	Working and reference memory deficits—radial arm water maze (14 wks) Motor learning deficit—rotarod (14 wks)	Contextual and cue dependent memory impairments—fear conditioning (8 months)	Progressive motor learning deficit—rotarod (2 months) Novel recognition memory deficit—novel object location and preference tests (6–7 months) Procedural learning impairment—simple swimming test (8 months) Spatial learning deficit—3 stage water maze test (4 months) Reversal learning impairments—swimming T maze (2 months), 3 stage water maze and novel water	Progressive motor learning deficit—rotarod (2 months) Novel object recognition memory deficit (6 months) Reversal learning and strategy shift deficits—water T maze and cross maze (9–10 months) Sensorimotor gating disturbances—startle response (9 months) and

(continued)

Table 2.1 (continued)

Transgenic fragment mouse models					Transgenic full-length mouse models	
					T-maze set-shifting setup (post 2 months) Extra-dimensional shift deficits—17 months Sensorimotor gating disturbances—startle response and prepulse inhibition (12 months)	prepulse inhibition (7 months)
Other/ peripheral phenotypes	Mild alterations in circadian activity patterns (12 wks) Sleep disturbance (16 wks)	Cardiac dysfunction (8–12 wks) Sleep abnormalities (9 wks) Muscle atrophy (9 wks) Testicular atrophy (5 wks)			Abnormal morphology of the testis and decreased number of developing sperm (9 months) Lower testis weight (12 months)	
NeuropathologyNeuropathology	Neuronal intranuclear Inclusions Neurotransmitter receptors level alternation	Neuronal intranuclear inclusions (first observed at 4 wks in cerebral cortex) Reduced brain volume but no white matter degeneration (12–13 wks) Decreased mRNA expression of mGluR1, mGluR2, mGluR3, D1 and D2 receptors	Neuronal intranuclear inclusionsInclusions No neuronal loss Reduced brain volume Neuron apoptosis Neuritic aggregates Diffuse nuclear localization of mutant huntingtin Dopamine and serotonin levels unchanged	Astrogliosis in cerebellum, striatum and cortex Large inclusions in all brain regions Progressive degeneration of granule cells in cerebellum No diffuse accumulation of huntingtin Reduced total brain volume Cerebellar and hippocampal atrophy	Intranuclear inclusions in striatum, nucleus accumbens, cortex and cerebellum (15 months) Neuronal loss in cortex and striatum (12 months) Forebrain weight reduction (9 months) Progressive decrease of total brain, striatal, cortical and white matter volume (3 months)	mHtt inclusions in cortex and striatum (12 months) No neuronal loss Cortical and striatal volume reduction (12 months)

(continued)

Table 2.1 (continued)

Knock-in nouse models						
Mouse models	HdhQ111	CAG140	zQ175	HdhQ150 (HdH(CAG)150 or CHL2)	HdhQ200	HdhQ250
Promoter	Murine *HTT*	Murine *HTT*	Murine *HTT*	Murine *HTT*	Murine *HTT*	Murine *HTT*
PolyQ size	111 CAG	140 CAG	175 CAG	150 CAG	200 CAG	250 CAG
Strain	129/CD1 mixed background	129/Sv C57BL/6 mixed background	129/Sv C57BL/6 mixed background	129/Ola C57BL/6J mixed background (when generated in 2001) CBA C57BL/6 mixed background (when generated in 2007)	129/Ola C57BL/6J mixed background	129/Ola C57BL/6J mixed background
Gene/ Protein context	Endogenous murine *HTT* gene, chimeric human/ mouse exon 1	Endogenous murine *HTT* gene, chimeric human/mouse exon 1	Endogenous murine *HTT* gene, chimeric human/mouse exon 1	Endogenous murine *HTT* gene	Endogenous murine *HTT* gene	Endogenous murine *HTT* gene
Protein expression level (relative to endogenous Htt) (%)	50 or 100	50 or 100	Approx. 75	Approx. 100	Approx. 100	
Repeat stability	Unstable					
Body weight	No observable changes until 12.5 months	Decreased body weight from 12 months in males and 25 months in females	Weight loss	Weight loss starts at 14 months of age in both genders	Weight loss from 3 months (homozygous) or 10 months of age (heterozygous)	Weight loss from 5 months of age
Brain atrophy	No	Yes	Yes	Yes	Yes	Yes
Life span	Normal life span	Normal life span	Reduced life span(19 months)	Normal life span		
Motor performance	Gait abnormalities—tunnel walk (24 months), Catwalk and vertical pole No detectable changes in spontaneous locomotor	Hyperactivity (1 month) and hypoactivity (4 months) Sensorimotor performance impairment—vertical	Progressive decline—open field (2 months), grip strength (homozygous, 1 month), Phenotube (homozygous, 4 months), climbing activity (homozygous, 8 months), cylinder test (heterozygous, 1 month), nesting (heterozygous, 16 months)	Impairments (earliest 4 months)—clasping, grip strength, rotarod, beam walking, activity cages	Impaired performance—balance beam (homozygous, 4 months, heterozygous, 12.5 months) Decreased muscle strength—grip strength test (20 months)	Impaired performance—open field, beam walking (12 months)

(continued)

Table 2.1 (continued)

Knock-in nouse models						
	activity in an automated cage	pole, non-accelerating rotarod, running wheels (4 months) Gait abnormalities (12 months)		(homozygous,25 months)	Gait abnormalities (15 months) No impairment observed on rotarod	
Psychiatric phenotypes	Depressive-like phenotype (female)—splash test and forced swim test Anxiety-like phenotype (male)—open field test Anxio-depressive-like phenotype—novelty suppressed feeding test Olfactory discrimination deficit Impaired social discrimination (males)	Anxiety-like phenotype—light/dark box (1.5 months) No detectable depressive-like behaviour—forced swim and tail suspension tests				Depressive-like phenotype—forced swim test (3 months)
Cognitive phenotypes	Altered motor learning—rotarod Long-term object recognition memory impairment (4 months) Spatial memory impairment (8 months) Reversal learning and working memory deficits—delayed matching and non-matching to position tasks (8 months)	Long term recognition memory impairment (4 months)	Procedural learning deficit—two choice swim test (homozygous, 10 months) Working memory deficit—Y maze (heterozygous, 16 months) Executive functioning impairment—go/no go test (7 months) Cognitive flexibility—two choice visual discrimination test (7 months)	Impaired spatial and reversal learning—3 stage water maze (4 and 8 months respectively) Extra-dimensional shift performance impairments (6 months) Decreased reactivity to startle stimuli (homozygous, 6 months)		
			Declined circadian rhythm			

(continued)

Table 2.1 (continued)

Knock-in nouse models						
Other/ Peripheral phenotypes		Altered leptin and adiponectin levels (7 months) 40–60% decrease in female gonadal and subcutaneous fat mass (22 months)				
Neuropathology	mHtt inclusions (12 months) Neuronal loss (24 months) No striatal atrophy	Nuclear and neurophil aggregates in striatum, nucleus accumbens and olfactory tubercle (2–4 months) Gliosis in cortex (12 months) and striatum (23 months) Neuronal loss (23 months) Reduced corpus callosum volume (20–26 months)	Nuclear inclusions (heterozygous) Striatal atrophy Cortical thinning Dopamine and BDNF levels reduction	Intranuclear inclusions (13 months) Gliosis (heterozygous, 14 months) Neuronal loss (12.5 months) Striatal atrophy (12.5 months) Decreased D1 and D2 (only homozygous) receptor binding	Intranuclear inclusions in striatum and cortex (13 months) Striatal and cortical astrogliosis (20 months) 50% reduction in striatal dopamine receptor binding (20 months) Cerebellar abnormalities—reduced mRNA and protein levels of Purkinje cell markers, Purkinje cell number and firing rate (11.5 months)	mHtt aggregates in striatum (6 months) Striatal and cortical atrophy (6 months) Disturbed myelination (postnatal day 14) Neuronal loss Reduced BDNF levels in striatum and cortex

The time points in brackets indicate the earliest assessed/detectable ages for the respective changes or the time points when the tests were performed
wks, weeks

germ line instability [10, 11]. The resulting phenotypes observed in the R6 models highly resemble the clinical symptoms in HD patients with the R6/2 model displaying a more aggressive phenotype than the R6/1 model. In 1999, Schilling et al. generated another HD fragment mouse model, N171-82Q [12]. These transgenic mice express steady-state level of N-terminally truncated huntingtin cDNA that encodes the first 171 amino acids of human huntingtin with 82 CAG repeats driven by the mouse prion promoter. Based on the findings that a fragment terminating at residue 586 of human Htt protein can be generated by caspase-6 is critical in mediating the HD phenotypes in YAC128 mice [13], Tebbenkamp et al. generated the N586-82Q model that expresses the N-terminal 586 amino acids of human mHtt with 82 CAG repeats, which corresponds to the fragments generated by caspase-6 [14]. 2 lines (C62 and C63) have been established with the N586-82Q-C63 model showing a more robust phenotypic profile and relatively stable phenotypes over generations. In addition to these models, many fragment models have been generated over time due to their robust phenotypes and similarities to human HD. However, we will focus on these fragment models in this chapter for their popularity and relevance to preclinical trials.

2.2.1.1 Life Span, General Health Status and Body Weight

Consistent with the clinical observation in HD patients, R6, N171-82Q, and N586-82Q mice show progressive weight loss and shortened life span (Table 2.1) [10, 12, 14, 15, 16]. For R6/2 and N171-82Q models, weight loss occurs despite normal food intake when the disease progresses. Autopsy of the R6/2 mice shows the weight loss of muscle tissues with no signs of myopathy as indicated by muscle fibre regeneration [9, 17], whereas N171-82Q mice show no gross abnormalities in visceral organs and blood glucose levels [12].

2.2.1.2 Behavioural Abnormalities

Progressive motor dysfunction such as tremors, stereotypical grooming, hypokinesis, abnormal gait and dyskinesia of the hind limbs have been observed in R6 and N171-82Q mice [9, 12, 15, 18, 19], whereas profound dyskinesia with ataxia-like movements in addition to motor impairments has been observed in the N586-82Q mice [14, 20]. While the abnormal gait sets in at 4 months of age (Line C63), the dyskinesia continues to increase in severity until the mice are 8 months old. At this time point, these mice are heavily affected by the movement disorder and they are not capable of feeding and drinking [14]. In some colonies of R6/2, it has been reported that they develop handling-induced seizures [21].

In HD patients, cognitive decline has been documented and most often appears prior to the motor deficits [22, 23]. As in humans, the R6/2 mice have been reported to have learning and memory deficits [24]. R6/2 mice begin to show spatial learning deficits in the Morris water maze (3.5 weeks), T-maze (5 weeks) and exploratory

and fear conditioning abnormalities [25] before the onset of motor symptoms [15]. In 2006, Morton et al. introduced an automated touchscreen-based cognitive-testing system to evaluate the cognitive decline of motor impaired R6/2 mice and reported learning deficits in the R6/2 model from 9 to 16 weeks of age [26]. The N171-82Q mice show deficits in the radial arm water maze test of working and reference memory [27] and motor learning at 14 weeks [28]. In HD patients, cognitive decline has been suggested to be the result of impaired fronto-striatal circuitry. However, as in previously described studies, this cognitive deficit in these mouse models may be due to the numerous Htt inclusions in the hippocampus rather than the fronto-striatal circuitry [27]. In addition, the N586-82Q model also shows contextual and cue dependent memory deficits in the fear conditioning test [20].

Neuropsychiatric disturbances in HD patients can appear already in the prodromal phase of the disease and do not worsen with time. Major psychiatric symptoms are depression, anxiety, impulsivity and irritability [29]. In HD mouse models, neuropsychiatric-like phenotypes have mostly been studied in terms of depressive- and anxious-like behaviours. Depressive-like phenotype has been demonstrated in N171-82Q [28] and R6 models [30] by, for instance, their decreased consumption of sucrose in the sucrose preference test, their increased immobility duration in the forced swim and tail suspension tests. Anxiety-related behaviour of R6/2 has been demonstrated by the open field test [28], and R6/1 mice are shown to exhibit progressive anxiety-like behaviour at a later stage (peak at 24 weeks of age) [30]. Using the light/dark box test, R6/2 mice are shown to have anxiety-like behaviour with gender differences (males start earlier than females). Anxio-depressive-like behaviour assessed by novelty suppressed feeding test was also reported in R6/1 mice [30].

2.2.1.3 Neuropathology and Neurochemical Alterations

All the fragment models described here show neuropathological features that recapitulate the human HD condition. Both R6/2 and end-stage N171-82Q mice develop neuronal intranuclear inclusions, containing the proteins huntingtin and ubiquitin, which are highly similar to nuclear abnormalities observed in biopsy material from HD patients [10]. In both N586-82Q lines (C62 and C63), large mHtt inclusions are also found in all brain structures but are predominantly cytoplasmic [14]. These inclusions are composed of a mixture of full-length N586-82Q protein and Cp-A/1-sized fragments, the latter of which have been described as principal components of intranuclear inclusions [14, 31, 32]. Unlike the R6/2 and N171-82Q models [10, 12], no diffuse nuclear localization of mHtt has been observed in N586-82Q mice [14].

As in human HD [33], brain volume reduction has been observed in N586-82Q, R6 and N171-82Q models. Brains from the R6/2 mice are 20% smaller than those of their wild-type littermates by 12–13 weeks of age [9, 10] although there is no difference in brain weight during weaning [16]. Note that the reduction in brain weight is not a consequence of the reduction in total body weight, as the loss in

brain weight precedes the loss in body weight as shown by a longitudinal study [10]. In the basal ganglia of R6/2 mice, while the white matter of the corpus callosum and the fascicles of fibres forming the internal capsule show no difference from those of the wild-type littermates [9], the reduction in size was constant throughout all central nervous system (CNS) structures with normal neuronal density [10]. In N586-82Q-C63, the brain weight is approximately 50% of their wild-type littermates attributed to hippocampal and cerebellar atrophy [14]. The obvious loss of cerebellar granule cells is the main reason for the ataxia-like abnormalities [14]. Unlike the R6 and N586-82Q models, N171-82Q mice have slightly smaller brains than their control littermates with no signs of abnormal development [12]. No severe neuronal loss has been observed [12].

It has been reported that the neurotransmitter systems of the fragment models have been disrupted. Using receptor-binding autoradiography, R6/2 mice are found to have marked decreases in mRNA expression for mGluR1, mGluR2 and mGluR3 metabotropic receptors, and D1 and D2 dopamine receptors. The decreases in mGluR1, D1 and D2 receptor mRNA levels in the striatum are significant by 4 weeks of age, and the decrease in mGluR2 mRNA expression in the cortex is significant by 8 weeks of age. In N171-82Q, their dopamine and serotonin levels are unchanged at 4 weeks and 6 months of age although alterations of motor performance are associated with abnormalities of the dopaminergic neurotransmitter systems [34]. Hence it has been speculated that their respective receptors have been disrupted resulting in the motor impairments [34].

2.2.2 *Transgenic Full-Length Models*

The most studied transgenic HD mouse models in this category are yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC) models carrying the human *HTT* transgene in yeast and bacterial chromosome respectively [35, 36]. The first Huntington YAC mouse models to be generated are YAC18 expressing normal Htt with 18 glutamines and YAC46 and YAC72 mice which express mHtt with 46 and 72 CAG repeats respectively [37]. YAC46 and YAC72 were to a certain extent representative of the human disease and showed early electrophysiological alterations followed by selective degeneration of medium spiny neurons along with some alterations in motor behaviour and activity [37]. Even though these models have been useful for understanding pathological aspects in HD, they were not optimal for quantitative measurements in preclinical studies partly due to the high sample size required. In order to improve the expression of the phenotype, a model with 128 glutamines, named YAC128 was created [35]. By having a higher number of glutamines, YAC128 mice develop an HD-associated phenotype earlier and stronger when compared to the first YAC models. More recently, 2 BAC models of HD have been generated. The first one is the BACHD model that expresses full-length human Htt with 97 CAG/CAA repeats [36] and the second one is BAC-225Q. The latter is a recently generated model which carries the full-length mouse Htt with 225 CAG repeats driven by the mouse Htt promoter [38].

In this chapter only YAC128 and BACHD models will be described as their pattern and selectivity of neuropathological alterations resemble the neuropathology in human HD brains. The progressive development of motor abnormalities, cognitive and neuropsychiatric disturbances in these models mimics HD symptoms in humans and hence are regarded as popular models for HD research.

2.2.2.1 Life Span, General Health Status and Body Weight

BACHD mice have a normal life span while survival is decreased in male YAC128 mice and this effect is aggravated if wild-type huntingtin is lost [39]. Unlike HD patients that experience body weight loss [40], in HD full-length models body weight is increased (Table 2.1) [36, 41]. BACHD female mice have been reported to have more body fat when compared to non-transgenic female [42]. In HD models, altered body weight is a meaningful parameter pertinent to the physiological alterations of the disease and could be a confounding factor for behavioural measures. In YAC128, a 20–30% body weight gain can be detected at 2–6 months and is maintained till 12 months [43]. This body weight increase is attributed to increased total fat mass, but is independent of food and water consumption [43]. Interestingly, most organs in this model show weight gain except the brain and testis that are atrophic and display the highest mHtt toxicity [43–45]. Unlike YAC128 mice, altered body weight in BACHD mice has been suggested to be related to increased food intake [46]. With an early obese phenotype (2 months of age), BACHD mice show endocrine abnormalities including increased basal glucose and insulin levels, impaired glucose tolerance and insulin resistance and altered levels of central and peripheral factors regulating food intake, appetite and adipose tissue metabolism [42, 46]. Some of these neuroendocrine aberrations like insulin resistance are also known in HD patients [47]. In these full-length models, one important factor related to body weight increase is the dosage of full-length Htt [48, 43]. Accordingly, BACHD mice have both higher mHtt levels and body weight when compared to YAC128 mice [41]. The opposite direction of change in body weight in these full-length models and HD patients should be taken into account when considering their use in therapeutic studies.

Abnormalities in HD are not limited to the brain [45, 49] and one of the affected systems is the hypothalamic-pituitary-gonadal axis. Male HD patients have decreased luteinizing hormone and testosterone levels [50] as well as decreased number of germ cells and anomalous morphology of the seminiferous tubules [45]. Similarly, YAC128 mice show abnormal morphology of the testis at 9 months and decreased number of developing sperm as well as lower testis weight by 12 months [44, 45]. Such peripheral physiological changes have been suggested to be a direct effect of the highly expressed Htt in the testis as the number of hypothalamic gonadotropin-releasing hormone neurons and the testosterone level are normal [45].

2.2.2.2 Behavioural Abnormalities

Both BACHD and YAC128 models show progressive motor deterioration [36, 39, 51]. They have shorter fall latency on the rotarod and at later ages they develop balance and gait abnormalities [35, 39, 52, 51]. YAC128 mice display hyperactivity at 3 months but hypoactivity at 12 months of age [35] and motor impairments begin before the hypoactive phase. The early hyperactive phenotype observed in YAC128 mice has not been shown in young BACHD mice that display decreased activity in the open field from 6 months onwards [53]. In YAC128, motor deficits on the rotarod appear before neuronal loss [35]. As motor performance at 6 months correlates with neuronal loss at later time points, it has been suggested that some neuronal dysfunction may be already present at the onset of the motor impairment.

In HD, cognitive decline precedes motor symptoms and is associated to cortical and hippocampal neuronal dysfunctionality [54, 55]. Both BACHD and YAC128 mice display learning deficits of different types that resemble clinical symptoms, including deficits in motor learning [36], novel object recognition memory [56, 57], sensorimotor gating [41, 51, 58], reversal learning and strategy shift [58–62]. Interestingly, cognitive impairment in YAC128 mice has been shown with tests mostly sensitive to corticostriatal and hippocampal dysfunction and deficits can manifest before the appearance of the first neuropathological signs [58].

For neuropsychiatric phenotypes, similarities have been observed in the YAC128 and BACHD mice, and these phenotypes have been shown to appear early and not to be progressive [63, 64]. Both models display increased immobility in the forced swim test which is indicative of behavioural despair [41, 42, 60, 63, 64] and show decreased preference/consumption for a sweet solution in the sucrose preference test [63, 64]. These observations have been suggested to be independent of motor inabilities [63, 64]. The anxious-like phenotype is evident in both models by open field [28, 60], zero maze [28, 60] and light dark choice [51], and in BACHD mice also by elevated plus maze [63]. An additional emotional alteration in BACHD mice is an increased freezing response in the fear conditioning test which has been shown in 9–10 month-old animals [59].

2.2.2.3 Neuropathology and Neurochemical Alterations

Neuropathology in YAC128 mice is characterized by typical HD features including development of aggregates, striatal and cortical atrophy and striatal neuronal loss [65, 35], whereas BACHD mice display a late onset neuropathology with cortical and striatal atrophy by 12 months of age with detectable mHtt inclusions but not neuronal loss [36]. In contrast, the number of striatal neurons in YAC128 mice is significantly reduced by 12 months and such neuronal loss is paralleled by decreased neuronal size [35]. Revealed by magnetic resonance imaging (MRI), YAC128 shows a progressive decrease in total brain, striatal, cortical and white matter volumes [35, 66, 67]. White matter changes have also been observed in other animal models of HD like YFP(*J16*)-R6/2, HdhQ250 and BACHD rats [68, 69, 67] and in

human HD brains [70]. At the neurochemical level, the expression of a series of striatal mRNA transcripts (e.g. DARPP-32, enkephalin, cannabinoid receptor 1 and dopamine receptors D1 and D2) is altered in YAC128, but not in BACHD mice [41].

2.2.3 *Knock-in Models*

Knock-in (KI) mouse models can carry 1 or 2 copies of the *mHTT* gene and are regarded as genetically precise because they are genetically representative of the human condition. They have overcome the random gene insertion problem as in transgenic models, which lead to gene copy number and expression variations over generations. As they exhibit HD-like features and late onset phenotype, they are suitable for investigating early neurophysiological changes that are casual to the observed behavioural alterations. In most of these models, behavioural and neuropathological alterations are milder and progress more slowly when compared to transgenic models [71].

Until now, 2 strategies have been used to generate the KI models. The first knock-in lines, HdhQ20, HdhQ50, HdhQ92 and HdhQ111, were generated by inserting a chimeric murine Huntington disease gene homolog (Hdh)/human mHtt exon 1 into the endogenous murine Hdh locus under the control of the endogenous mouse Htt promoter [72]. The same strategy was later used to generate the CAG140 [73] and the zQ175 models, the latter of which was a result from a spontaneous expansion of the CAG repeat number in the CAG140 model [74]. The second subtype of KI models was generated by replacing the short CAG repeat of the mouse *HTT* exon 1 with a repeat containing 50-365 CAG repeats. Amongst these models, HdhQ150 [75] and the HdhQ200 [7] and HdhQ250 [69] models derived from its selective breedings are more well-characterized and hence will be described here.

2.2.3.1 Life Span, General Health Status and Body Weight

Unlike the transgenic full-length models, the KI models show a reduction in body weight that resembles the clinical observation of HD patients [7, 69, 74, 75, 76, 77], though the onset of weight loss differs in each model. The lifespan of zQ175 [74] have been reported to be decreased while HdhQ150 [75], HdhQ111 [78] and CAG140 [79] have normal life span. Interestingly, as in the BACHD rat model (will be described later in this chapter) that exhibits metabolic alterations, the leptin and adiponectin levels and the fat mass in the CAG140 model have also been reported to be altered [77].

2.2.3.2 Behavioural Abnormalities

All the KI mouse models described here (Table 2.1) exhibit varying degrees of motor impairments. Their performance deteriorates with age as shown by decreased activity, rotarod impairments and gait abnormalities [7, 69, 73, 74, 75, 76, 79, 80].

As in the transgenic mouse models, cognitive disturbances have been investigated and described in the KI models (HdhQ111, CAG140, zQ175 and HdhQ150). In line with findings in HD subjects [80], they exhibit learning and memory impairments [55, 79, 81, 82, 83] in both homozygous and heterozygous animals. In particular, learning deficits of Hdh150 have been shown in homozygous mice prior to major motor impairment [84]. The early appearance of cognitive deficits relative to motor alterations is an important characteristic of Hdh150 since in human HD cognitive disturbances are among the earliest symptoms and often appear before motor deficits [54].

Neuropsychiatric phenotypes have only been reported in some of the KI models (HdhQ111, CAG140 and HdhQ250). The neuropsychiatric phenotype in HdhQ111 mice is characterized by alterations of different nature in male and female animals at around 3–4 months [85]. An anxious-like phenotype is evident in male HdhQ111 mice in the open field test with a shorter time and a decreased number of entries in the central area of the field. Females instead show a depressive-like phenotype suggested by increased grooming time in the splash test and increased immobility in the forced swim test. Both genders of HdhQ111 take a longer time to feed in the novelty suppressed feeding test which suggests an anxio-depressive-like phenotype [85]. For CAG140, they are impaired in long term recognition memory at 4 months and take longer time to enter the light compartment in the light/dark box at 1.5 but not at 8 months. The latter could be interpreted as an early non-persisting anxious-like phenotype [79]. Note that CAG140 mice do not display depressive-like behaviour in the forced swim and tail suspension tests [79]. On the contrary, HdhQ250 displays an increased floating score at 3 months and a decreased score at 12 months when tested in the forced swim test [86]. This indicates an early but non-progressive depressive-like phenotype as in HD patients [29, 86].

2.2.3.3 Neuropathology and Neurochemical Alterations

The KI models have been reported to display a spectrum of HD-distinctive neuropathological characteristics with each model exhibiting unique features resembling human HD. HdhQ111 lacks striatal atrophy but shows nuclear localization of mHtt especially in medium spiny neurons and formation of N-terminal inclusions and insoluble aggregates [87]. Nuclear EM48 reactivity in HdhQ111 is first shown at the age of 1.5 months and EM48 puncta are visible by 5 months while nuclear inclusions are evident only starting from 12 months [87, 78]. Neurodegeneration shown by the presence of toluidine blue stained neurons take place only at later phases, 24 months, and seems not to involve apoptosis [78]. Neuropathological alterations in the CAG140 model follow behavioural changes and have been reported to appear between 2 and 4 months of age [79, 73]. Both nuclear and neuropil aggregates have been observed starting from dorsal striatum, nucleus accumbens and olfactory tubercle [73]. By 12 months of age, CAG140 mice show reduced levels of DARPP32 in striatum and gliosis in cortex. By 23 months, gliosis

can also be detected in the striatum [79]. At this stage, striatal atrophy and loss of mature and immature neuronal spines as well as decreased dendritic complexity become evident [88]. Electrophysiological studies have also shown impaired corticostriatal circuitry function and altered synaptic transmission of medium spiny neurons [89]. Late stage neuropathological features include reduced corpus callosum volume and loss of tyrosine hydroxylase immunostaining in 20–26 months old mice [88]. zQ175 brains are characterized by striatal atrophy, development of mHtt inclusions, decreased striatal dopamine and brain-derived neurotrophic factor (BDNF) levels as well as cortical thinning [83]. Analyses in heterozygous mice show degeneration of medium spiny neurons while studies in both homozygous and heterozygous mice demonstrated medium spiny neuron electrophysiological alterations [82, 83]. Both homozygous and heterozygous mice show changes in the expression of different mRNA transcripts including DARPP-32, cannabinoid receptor 1 and phosphodiesterase which start at 3 months and progress faster in homozygous than in heterozygous mice [74]. Nuclear inclusions in heterozygous mice have first been observed in the dorsal striatum and cortex and later in other brain regions including nucleus accumbens, hippocampus, amygdala, hypothalamus and thalamus [83].

Neuropathology in HdhQ150 mice include reactive gliosis, development of mHtt nuclear inclusions, degeneration of cytoplasmic organelles in both neuronal axons and cell bodies, neuronal and volume loss in striatum as well as decrease in striatal dopamine D1 and D2 receptor binding potential [80, 75, 90]. Besides nuclear inclusions, EM48 positive neuropil aggregates have also been detected [90]. Recent research using a time-resolved fluorescence resonance energy transfer (TR-FRET)-based immunoassays demonstrated that the levels of soluble mHtt are inversely correlated with the load of aggregated mHtt in the aging HdhQ150 mouse brain [91]. Size exclusion chromatography coupled to TR-FRET showed that mHtt fragments and not full-length mHtt form a soluble pool of oligomers that is visible already in the first month of life and declines with age [92]. Importantly, a comparable oligomer pool has also been shown in a human brain [92]. While the pool-size of soluble mHtt oligomers is comparable in heterozygous and homozygous HdhQ150 mice, the formation of insoluble aggregate is faster in the homozygous and pathological alterations have been shown to be dependent on the mHtt dosage in HdhQ150 mice [92, 18]. Similar to HD patients, 2 copies of the m*HTT* gene correlate with a more severe clinical disease course [93]. Stereological measurements of HdhQ150 brains show decreased striatal volume and neuronal number in homozygous and heterozygous mice by about 25 months and the volume loss is stronger in the homozygous population [80]. The related model, HdhQ200, displays early brain pathology characterized by accumulation of Htt aggregates in cytoplasmic foci by 9 weeks of age and neuronal intranuclear inclusions that are first observable at 20 weeks and reach a massive distribution at 40 weeks [7]. HdhQ200 intranuclear inclusions are limited to striatum and cortex with higher density in the striatal area [7]. Their shape and size (3–5 μm) is comparable to that in HD human brains [65]. Interestingly, intranuclear inclusions at 40 weeks have a perinuclear distribution and are associated to ubiquitin and autophagosome marker

LC3 [94]. By 80 weeks heterozygous HdhQ200 brains show striatal and cortical astrogliosis [7] that is also reported in the HdhQ150 model [75], which is a neuropathological hallmark of human HD [33]. Astrogliosis in HdhQ200 mice is paralleled by a 50% reduction of striatal dopamine receptor binding that was not observed in HdhQ150 mice [7]. Besides striatal dysfunction, cerebellar abnormalities have also been described in this model at the age of 50 weeks. These consist of reduced mRNA and protein levels of Purkinje cell markers as well as decreased Purkinje cell number and firing rate [95].

Myelination deficiency has been reported in early postnatal development in HdhQ250 mice [69]. Comparing to wild-type mice, HdhQ250 mouse striatum has lower levels of all isoforms of myelin basic protein and myelin oligodendrocyte glycoprotein and displays fewer myelinated axons in the corpus callosum. Importantly, these white matter abnormalities persist in adulthood as shown by the high number of small hypomyelinated axons in the corpus callosum of 12-month-old mice, and are paralleled by altered proliferation of oligodendrocyte precursor cells in both corpus callosum and striatum [69]. At 6 months of age, mHtt aggregates are observable in striatum and atrophy occurs selectively in cortex and striatum. Cortical and striatal levels of BDNF and DARPP32 in medium spiny neurons are also reduced [69]. Most of these alterations are consistent with changes in HD human brains where white matter morphology and integrity are disrupted [70], medium spiny neurons undergo selective degeneration [96] and BDNF levels and transport are altered [97].

2.3 Rat Models

Although the mouse represents an excellent model organism for HD due to its established use in genetics research, it has certain shortcomings. Because mice have a small body size, it limits the resolution and quality of in vivo imaging methods such as MRI and positron emission tomography (PET), which are currently being developed as diagnostics for HD [98]. Another reason is that mice generally require more training in psychiatric and/or cognitive tests (e.g. operant conditioning tasks) that are of interest when evaluating these aspects of HD. To overcome these problems, a small number of genetically modified rat models have been developed for HD.

2.3.1 Transgenic Fragment Models

At present, only 1 transgenic rat fragment model of HD has been generated. These rats, known as the TgHD rats, carry a transgenic construct that expresses a human/rat mixed fragment of the *HTT* gene. The fragment contains 51 CAG repeats and is governed by the rat endogenous promoter, which is included in the construct [99].

So far, the TgHD rats have only been kept on a Sprague-Dawley background. Published studies have focused on either male or female hemi- or homozygote TgHD rats, although the following text will focus on the general consensus.

In contrast to the frequently used mouse fragment models (e.g. R6/2), the TgHD rats show a late and slowly progressing disease phenotype [99]. This is primarily thought to be due to the more limited number of CAG repeats and the longer fragment used in the TgHD rats' construct. Thus, the TgHD rat is generally considered to be a good model for the adult onset HD that is commonly seen in patients.

The TgHD rat's transgenic fragment is expressed at a lower level than the endogenous rat Htt. The translated protein is detectable in most of the CNS, although the expression is low in the cerebellum and spinal cord [99]. Like other transgenic animal models of HD, the expression of the mutated protein results in gradual development of Htt-containing protein aggregates [100, 101, 102, 99]. This is first apparent at around 6 months of age, with the nucleus accumbens being particularly affected [101, 99]. As the animals age, additional brain regions are also affected. In the caudate-putamen, aggregate formation is primarily in its dorsomedial parts, which becomes apparent at around 9 months of age [101, 99].

Volumetric analyses, using either MRI or stereology, have yielded conflicting results concerning HD-related neuropathology in the TgHD rat [103, 101, 102, 99, 104]. Some studies have found enlarged ventricles and reduced striatal volume [103, 101, 102] already at 8 months of age [99], while others have failed to detect these phenotypes [105, 100] even in 18 months old rats [104]. A smaller number of studies that specifically investigated neuronal loss have detected the presence of darkly stained degenerated neurons in both striatum and cortex and a reduced number of striatal neurons at 12 months of age [103, 102]. In addition, a range of other neuropathological features has been reported, including shrinkage of striatal neurons [106], a drop in striatal D2R density [100], reduced proliferation of neuronal stem cells [107] and changes in brain metabolism [106, 99].

There has also been extensive characterization of behavioural phenotypes in the TgHD rats. Accordingly, the TgHD rat has been found to display a head movement phenotype reminiscent of the chorea found in HD patients [108, 105, 99], with an onset at around 15 months of age [108, 105]. In addition, impaired performance in specific tests of motor function has been found [100, 101, 109, 99], although the exact onset of these phenotypes is uncertain. TgHD rats have been found to be less anxious than wild-type rats in several behavioural tests [101, 109, 99], a phenotype which appears to be present already before 5 months of age [101, 99]. TgHD rats have also been assessed in quite a wide range of tests for cognitive function. Initial studies found indications that TgHD rats had impaired spatial working memory starting at 6 months of age [101, 99], while spatial reference memory was impaired at 12 months of age [101]. TgHD rats have also shown impaired recall memory [110] and indications of learning deficits in tests of spatial navigation [100]. These phenotypes appear to emerge when animals are around 6–10 months old. Investigation in more complicated operant conditioning protocols have indicated an attentional impairment among TgHD rats, which is present at 9–15 months of age, depending on the respective protocol [108, 103]. Although a range of other studies

have also investigated the rats' performance using other cognitive tests, most of which have not been repeated or have shown conflicting results [105, 109]. Regarding the life span of this model, they have been reported to show increased mortality at 24 months of age [99], although the phenotype has not been further investigated (Table 2.2).

Table 2.2 Rat models of Huntington disease

Model name	TgHD rat	BACHD rat
Host animal	Rat	Rat
Generic manipulation	Transgenic	Transgenic
Promoter	Rat *HTT*	Human *HTT*
PolyQ size	51 CAG	97 CAG/CAA
Protein context	Amino acids 1-727 of mixed human/rat sequence	Full-length human protein
Protein expression level (relative to endogenous Hdh)	Not stated, but lower than endogenous	TG5 line: 450% TG9 line: 250%
Repeat stability	Stable	Stable
Body weight	Generally unchanged, although stunted growth has been reported	Unchanged, although rats are obese
Brain atrophy	Conflicting results, although several indications of neuropathology	Frequently found to have smaller brains, although interplay between progressive atrophy and developmental deficits is unclear
Aggregate formation	Throughout most of CNS	Throughout most of CNS
Neuropathology	Structural alterations of brain regions, neuron shrinkage and loss	Structural alterations, and presence of deteriorating neurons
Premature death	Yes, around 24 months of age	Nothing reported until age of 16 months
Motor phenotypes	Chorea-like movements, impaired performance on rotarod and beam walk tests	Impaired rotarod performance and gait abnormalities
Psychiatric phenotypes	Reduced exploration anxiety	Reduced exploration anxiety
Cognitive phenotypes	Indications of impaired performance in several tasks. Impaired spatial working and reference memory as attentional deficits appear to be robust	Indications of impaired performance in several tasks, although most results are preliminary and not extensively reproduced
Onset and progression of phenotypes	Visible aggregate formation starts at around 6 months of age. Onset of behavioural phenotypes varies, with anxiolytic behaviour appearing as early as 2 months of age, while motor and cognitive phenotypes become apparent at around 6–9 months	Visible aggregate formation starts at around 3 months of age. Onset of behavioural phenotypes varies with impairments on rotarod being detectable after 1 month of age and anxiolytic behaviour appearing at 4 months

2.3.2 Transgenic Full-Length Models

Similar to the rat fragment model, there is currently only 1 transgenic full-length rat model available for HD, the BACHD rat. This model was established more recently than the TgHD rats [111]. The rats carry the same genetic construct used to create the BACHD mice [36, 111], and so far all published work has focused on hemizygote rats with Sprague-Dawley background [112–118, 111]. 2 different lines (TG5 and TG9) were initially established, which overexpress the transgene to different extents. However, with the exception of the initial publication, characterization and treatment studies have hitherto been using male rats of the TG5 line [112, 113, 111]. In these rats, the transgene shows a 4.5 fold overexpression when compared to the endogenous rat Htt. Characterization data obtained from these rats are summarized below. Additionally, dysregulation of gene expression in the striatum of BACHD rats was found as early as 3 months in both transgenic lines TG5 and TG9, reduced TFIID formation might contribute to this in a certain extent [119].

The transgenic construct is expressed throughout most of the CNS [111]. Development of mHtt aggregates largely follow the expression pattern of the protein, initially becoming apparent at around 3 months of age, then increasing in both number and size as the animals grow older [111]. At 13 months of age, most brain regions show aggregate formation, although the cerebral cortex, hippocampus, amygdala and nucleus accumbens are among the most heavily affected areas, while the striatum shows a relatively lower amount of aggregates [113, 111]. Other noted indications of neuropathology are the presence of darkly stained degenerated neurons in several brain regions, a change in the surface area of the striosome compartment of the striatum, a decrease in D2R availability (detectable at 18 months of age) [111] and an impaired auditory gating response at 4 months of age [120].

BACHD rats of the TG5 lines have frequently been found to have an early and progressive impairment on the rotarod with the phenotype becoming apparent already at 2 months of age [121, 113, 111]. In addition, a phenotype of disturbed walking gait appears to be present at 14 months [111] but not earlier [121, 111]. Similar to the TgHD rats, TG5 rats have been found to be less anxious than WT rats when tested on the elevated plus maze. This phenotype appears at around the age of 4 months, and becomes more pronounced with age [111]. When considering more cognition-oriented characterization, the BACHD rats have been found to show discreet impairments in reversal learning [122, 113] and reduced fear conditioning response [121] at 4–6 months of age. Moreover, impaired performance on operant conditioning tests that are sensitive to fronto-striatal lesions was observed already at 4 months of age [114]. Furthermore, BACHD rats have been found to show some impulsivity disorder at 3 months of age [112, 117]. A recent study reported an altered reactivity of central amygdala to $GABA_AR$ antagonist picrotoxin in BACHD rats at 4.5 months of age [116]. Considering the metabolic aspects of the BACHD rats, male rats have been found to be obese without showing an increased

body weight [123]. This interesting phenotype is observed in parallel to the reduced body size and reduced lean mass [123]. The obesity phenotype is maintained despite the fact that BACHD rats consume less food than wild-type rats [123, 111], although it is hard to be conclusive given the reduced lean mass in the transgenic rats. Impairments in cellular metabolism have also been found, indicating glycolysis dysfunction at early ages [124] and mitochondrial dysfunction at older ages [113]. The latter of these phenotypes has been suggested to play a major part in the pathology in BACHD rats and to be linked to increased proteolytic cleavage of mHtt [113]. Reduced life span has not yet been observed in BACHD rats, although mortality beyond the age of 17–18 months of age has not yet been evaluated [112, 111].

2.3.3 Knock-in Models

Several knock-in rat models of HD are currently being developed. There is, however, at this time no published data available.

2.4 Concluding Remarks

The generation of genetic rodent models for HD has given us a valuable tool for deciphering the disease mechanism and discovering therapeutic treatments. The wide range of models available since the discovery of the causative mutation in the *HTT* gene has provided us a variety of choices but at the same time poses difficulty on deciding which is the appropriate model for a particular study. Depending on the aim(s) of investigation, the duration of study, the feasible sample size and the extent of correlation to human HD at the biochemical and behavioural aspects should be considered. Consistency and stability of genetic variation due to breeding and background strain is another concern. These considerations are not limited to current utilization but also provide future directions to the generation of new animal models for HD as well as other neurodegenerative diseases.

References

1. The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983
2. Pouladi MA, Morton AJ, Hayden MR (2013) Choosing an animal model for the study of Huntington's disease. Nat Rev Neurosci 14:708–721
3. Chang R, Liu X, Li S, Li XJ (2015) Transgenic animal models for study of the pathogenesis of Huntington's disease and therapy. Drug Des Devel Ther 9:2179–2188

4. Crook ZR, Housman D (2011) Huntington's disease: can mice lead the way to treatment? Neuron 69:423–435
5. Li JY, Popovic N, Brundin P (2005) The use of the R6 transgenic mouse models of Huntington's disease in attempts to develop novel therapeutic strategies. NeuroRx 2: 447–464
6. Switonski PM, Szlachcic WJ, Gabka A, Krzyzosiak WJ, Figiel M (2012) Mouse models of polyglutamine diseases in therapeutic approaches: review and data table Part II. Mol Neurobiol 46:430–466
7. Heng MY, Duong DK, Albin RL, Tallaksen-Greene SJ, Hunter JM, Lesort MJ, Osmand A, Paulson HL, Detloff PJ (2010) Early autophagic response in a novel knock-in model of Huntington disease. Hum Mol Genet 19:3702–3720
8. Jucker M (2010) The benefits and limitations of animal models for translational research in neurodegenerative diseases. Nat Med 16:1210–1214
9. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87:493–506
10. Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90: 537–548
11. Mangiarini L, Sathasivam K, Mahal A, Mott R, Seller M, Bates GP (1997) Instability of highly expanded CAG repeats in mice transgenic for the Huntington's disease mutation. Nat Genet 15:197–200
12. Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuk JA, Slunt HH, Ratovitski T, Cooper JK, Jenkins NA, Copeland NG, Price DL, Ross CA, Borchelt DR (1999) Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Hum Mol Genet 8:397–407
13. Graham RK, Deng Y, Slow EJ, Haigh B, Bissada N, Lu G, Pearson J, Shehadeh J, Bertram L, Murphy Z, Warby SC, Doty CN, Roy S, Wellington CL, Leavitt BR, Raymond LA, Nicholson DW, Hayden MR (2006) Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. Cell 125:1179–1191
14. Tebbenkamp AT, Green C, Xu G, Denovan-Wright EM, Rising AC, Fromholt SE, Brown HH, Swing D, Mandel RJ, Tessarollo L, Borchelt DR (2011) Transgenic mice expressing caspase-6-derived N-terminal fragments of mutant huntingtin develop neurologic abnormalities with predominant cytoplasmic inclusion pathology composed largely of a smaller proteolytic derivative. Hum Mol Genet 20:2770–2782
15. Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ (1999) Characterization of progressive motor deficits in mice transgenic for the human Huntington's disease mutation. J Neurosci 19:3248–3257
16. Stack EC, Kubilus JK, Smith K, Cormier K, Del Signore SJ, Guelin E, Ryu H, Hersch SM, Ferrante RJ (2005) Chronology of behavioral symptoms and neuropathological sequela in R6/2 Huntington's disease transgenic mice. J Comp Neurol 490:354–370
17. Sathasivam K, Hobbs C, Mangiarini L, Mahal A, Turmaine M, Doherty P, Davies SW, Bates GP (1999) Transgenic models of Huntington's disease. Philos Trans R Soc Lond B Biol Sci 354:963–969
18. Young D, Mayer F, Vidotto N, Schweizer T, Berth R, Abramowski D, Shimshek DR, van der Putten PH, Schmid P (2013) Mutant huntingtin gene-dose impacts on aggregate deposition, DARPP32 expression and neuroinflammation in HdhQ150 mice. PLoS ONE 8: e75108
19. Schilling G, Savonenko AV, Klevytska A, Morton JL, Tucker SM, Poirier M, Gale A, Chan N, Gonzales V, Slunt HH, Coonfield ML, Jenkins NA, Copeland NG, Ross CA, Borchelt DR (2004) Nuclear-targeting of mutant huntingtin fragments produces Huntington's disease-like phenotypes in transgenic mice. Hum Mol Genet 13:1599–1610

20. Waldron-Roby E, Ratovitski T, Wang X, Jiang M, Watkin E, Arbez N, Graham RK, Hayden MR, Hou Z, Mori S, Swing D, Pletnikov M, Duan W, Tessarollo L, Ross CA (2012) Transgenic mouse model expressing the caspase 6 fragment of mutant huntingtin. J Neurosci 32:183–193
21. Hickey MA, Gallant K, Gross GG, Levine MS, Chesselet MF (2005) Early behavioral deficits in R6/2 mice suitable for use in preclinical drug testing. Neurobiol Dis 20:1–11
22. Paulsen JS (2011) Cognitive impairment in Huntington disease: diagnosis and treatment. Curr Neurol Neurosci Rep 11:474–483
23. Snowden JS, Craufurd D, Thompson J, Neary D (2002) Psychomotor, executive, and memory function in preclinical Huntington's disease. J Clin Exp Neuropsychol 24:133–145
24. Lione LA, Carter RJ, Hunt MJ, Bates GP, Morton AJ, Dunnett SB (1999) Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. J Neurosci 19:10428–10437
25. Bolivar VJ, Manley K, Messer A (2003) Exploratory activity and fear conditioning abnormalities develop early in R6/2 Huntington's disease transgenic mice. Behav Neurosci 117:1233–1242
26. Morton AJ, Skillings E, Bussey TJ, Saksida LM (2006) Measuring cognitive deficits in disabled mice using an automated interactive touchscreen system. Nat Methods 3:767
27. Ramaswamy S, McBride JL, Kordower JH (2007) Animal models of Huntington's disease. ILAR J 48:356–373
28. Chiu CT, Liu G, Leeds P, Chuang DM (2011) Combined treatment with the mood stabilizers lithium and valproate produces multiple beneficial effects in transgenic mouse models of Huntington's disease. Neuropsychopharmacology 36:2406–2421
29. Epping EA, Paulsen JS (2011) Depression in the early stages of Huntington disease. Neurodegener Dis Manag 1:407–414
30. Pla P, Orvoen S, Saudou F, David DJ, Humbert S (2014) Mood disorders in Huntington's disease: from behavior to cellular and molecular mechanisms. Front Behav Neurosci 8:135
31. Lunkes A, Lindenberg KS, Ben-Haiem L, Weber C, Devys D, Landwehrmeyer GB, Mandel JL, Trottier Y (2002) Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. Mol Cell 10: 259–269
32. Schilling G, Klevytska A, Tebbenkamp AT, Juenemann K, Cooper J, Gonzales V, Slunt H, Poirer M, Ross CA, Borchelt DR (2007) Characterization of huntingtin pathologic fragments in human Huntington disease, transgenic mice, and cell models. J Neuropathol Exp Neurol 66:313–320
33. Vonsattel JP, DiFiglia M (1998) Huntington disease. J Neuropathol Exp Neurol 57:369–384
34. Schilling G, Jinnah HA, Gonzales V, Coonfield ML, Kim Y, Wood JD, Price DL, Li XJ, Jenkins N, Copeland N, Moran T, Ross CA, Borchelt DR (2001) Distinct behavioral and neuropathological abnormalities in transgenic mouse models of HD and DRPLA. Neurobiol Dis 8:405–418
35. Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, Oh R, Bissada N, Hossain SM, Yang YZ, Li XJ, Simpson EM, Gutekunst CA, Leavitt BR, Hayden MR (2003) Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Hum Mol Genet 12:1555–1567
36. Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, Tao J, Yamazaki I, Li SH, Sun YE, Li XJ, Levine MS, Yang XW (2008) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. J Neurosci 28:6182–6195
37. Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, Smith DJ, Bissada N, McCutcheon K, Nasir J, Jamot L, Li XJ, Stevens ME, Rosemond E, Roder JC, Phillips AG, Rubin EM, Hersch SM, Hayden MR (1999) A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron 23:181–192

38. Wegrzynowicz M, Bichell TJ, Soares BD, Loth MK, McGlothan JS, Mori S, Alikhan FS, Hua K, Coughlin JM, Holt HK, Jetter CS, Pomper MG, Osmand AP, Guilarte TR, Bowman AB (2015) Novel BAC mouse model of Huntington's disease with 225 CAG repeats exhibits an early widespread and stable degenerative phenotype. J Huntingtons Dis 4:17–36
39. Van Raamsdonk JM, Pearson J, Rogers DA, Bissada N, Vogl AW, Hayden MR, Leavitt BR (2005) Loss of wild-type huntingtin influences motor dysfunction and survival in the YAC128 mouse model of Huntington disease. Hum Mol Genet 14:1379–1392
40. Aziz NA, Van Der Burg JM, Landwehrmeyer GB, Brundin P, Stijnen T, Group ES, Roos RAC (2008) Weight loss in Huntington disease increases with higher CAG repeat number. Neurology 71(19):1506–1513
41. Pouladi MA, Stanek LM, Xie Y, Franciosi S, Southwell AL, Deng Y, Butland S, Zhang W, Cheng SH, Shihabuddin LS, Hayden MR (2012) Marked differences in neurochemistry and aggregates despite similar behavioural and neuropathological features of Huntington disease in the full-length BACHD and YAC128 mice. Hum Mol Genet 21:2219–2232
42. Baldo B, Cheong RY, Petersen A (2014) Effects of deletion of mutant huntingtin in steroidogenic factor 1 neurons on the psychiatric and metabolic phenotype in the BACHD mouse model of Huntington disease. PLoS ONE 9:e107691
43. Van Raamsdonk JM, Gibson WT, Pearson J, Murphy Z, Lu G, Leavitt BR, Hayden MR (2006) Body weight is modulated by levels of full-length huntingtin. Hum Mol Genet 15:1513–1523
44. Southwell AL, Franciosi S, Villanueva EB, Xie Y, Winter LA, Veeraraghavan J, Jonason A, Felczak B, Zhang W, Kovalik V, Waltl S, Hall G, Pouladi MA, Smith ES, Bowers WJ, Zauderer M, Hayden MR (2015) Anti-semaphorin 4D immunotherapy ameliorates neuropathology and some cognitive impairment in the YAC128 mouse model of Huntington disease. Neurobiol Dis 76:46–56
45. Van Raamsdonk JM, Murphy Z, Selva DM, Hamidizadeh R, Pearson J, Petersen A, Bjorkqvist M, Muir C, Mackenzie IR, Hammond GL, Vogl AW, Hayden MR, Leavitt BR (2007) Testicular degeneration in Huntington disease. Neurobiol Dis 26:512–520
46. Hult S, Soylu R, Bjorklund T, Belgardt BF, Mauer J, Bruning JC, Kirik D, Petersen A (2011) Mutant huntingtin causes metabolic imbalance by disruption of hypothalamic neurocircuits. Cell Metab 13:428–439
47. Lalic NM, Maric J, Svetel M, Jotic A, Stefanova E, Lalic K, Dragasevic N, Milicic T, Lukic L, Kostic VS (2008) Glucose homeostasis in Huntington disease: abnormalities in insulin sensitivity and early-phase insulin secretion. Arch Neurol 65:476–480
48. Pouladi MA, Xie Y, Skotte NH, Ehrnhoefer DE, Graham RK, Kim JE, Bissada N, Yang XW, Paganetti P, Friedlander RM, Leavitt BR, Hayden MR (2010) Full-length huntingtin levels modulate body weight by influencing insulin-like growth factor 1 expression. Hum Mol Genet 19:1528–1538
49. Aziz NA, Pijl H, Frolich M, van der Graaf AW, Roelfsema F, Roos RA (2009) Increased hypothalamic-pituitary-adrenal axis activity in Huntington's disease. J Clin Endocrinol Metab 94:1223–1228
50. Markianos M, Panas M, Kalfakis N, Vassilopoulos D (2005) Plasma testosterone in male patients with Huntington's disease: relations to severity of illness and dementia. Ann Neurol 57:520–525
51. Menalled L, El-Khodor BF, Patry M, Suarez-Farinas M, Orenstein SJ, Zahasky B, Leahy C, Wheeler V, Yang XW, MacDonald M, Morton AJ, Bates G, Leeds J, Park L, Howland D, Signer E, Tobin A, Brunner D (2009) Systematic behavioral evaluation of Huntington's disease transgenic and knock-in mouse models. Neurobiol Dis 35:319–336
52. Chen X, Wu J, Lvovskaya S, Herndon E, Supnet C, Bezprozvanny I (2011) Dantrolene is neuroprotective in Huntington's disease transgenic mouse model. Mol Neurodegener 6:81
53. Mantovani S, Gordon R, Li R, Christie DC, Kumar V, Woodruff TM (2016) Motor deficits associated with Huntington's disease occur in the absence of striatal degeneration in BACHD transgenic mice. Hum Mol Genet 25:1780–1791

54. Lichter DG, Hershey LA (2010) Before chorea: pre-Huntington mild cognitive impairment. Neurology 75:490–491
55. Giralt A, Saavedra A, Alberch J, Perez-Navarro E (2012) Cognitive dysfunction in Huntington's disease: humans, mouse models and molecular mechanisms. J Huntingtons Dis 1:155–173
56. Doria JG, Silva FR, de Souza JM, Vieira LB, Carvalho TG, Reis HJ, Pereira GS, Dobransky T, Ribeiro FM (2013) Metabotropic glutamate receptor 5 positive allosteric modulators are neuroprotective in a mouse model of Huntington's disease. Br J Pharmacol 169:909–921
57. Southwell AL, Ko J, Patterson PH (2009) Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. J Neurosci 29:13589–13602
58. Van Raamsdonk JM, Pearson J, Slow EJ, Hossain SM, Leavitt BR, Hayden MR (2005) Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci 25:4169–4180
59. Abada YS, Schreiber R, Ellenbroek B (2013) Motor, emotional and cognitive deficits in adult BACHD mice: a model for Huntington's disease. Behav Brain Res 238:243–251
60. Aharony I, Ehrnhoefer DE, Shruster A, Qiu X, Franciosi S, Hayden MR, Offen D (2015) A Huntingtin-based peptide inhibitor of caspase-6 provides protection from mutant Huntingtin-induced motor and behavioral deficits. Hum Mol Genet 24:2604–2614
61. Brooks S, Higgs G, Janghra N, Jones L, Dunnett SB (2012) Longitudinal analysis of the behavioural phenotype in YAC128 (C57BL/6 J) Huntington's disease transgenic mice. Brain Res Bull 88:113–120
62. Brooks SP, Janghra N, Higgs GV, Bayram-Weston Z, Heuer A, Jones L, Dunnett SB (2012) Selective cognitive impairment in the YAC128 Huntington's disease mouse. Brain Res Bull 88:121–129
63. Hult Lundh S, Nilsson N, Soylu R, Kirik D, Petersen A (2013) Hypothalamic expression of mutant huntingtin contributes to the development of depressive-like behavior in the BAC transgenic mouse model of Huntington's disease. Hum Mol Genet 22:3485–3497
64. Pouladi MA, Graham RK, Karasinska JM, Xie Y, Santos RD, Petersen A, Hayden MR (2009) Prevention of depressive behaviour in the YAC128 mouse model of Huntington disease by mutation at residue 586 of huntingtin. Brain 132:919–932
65. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277:1990–1993
66. Carroll JB, Lerch JP, Franciosi S, Spreeuw A, Bissada N, Henkelman RM, Hayden MR (2011) Natural history of disease in the YAC128 mouse reveals a discrete signature of pathology in Huntington disease. Neurobiol Dis 43:257–265
67. Teo RT, Hong X, Yu-Taeger L, Huang Y, Tan LJ, Xie Y, To XV, Guo L, Rajendran R, Novati A, Calaminus C, Riess O, Hayden MR, Nguyen HP, Chuang KH, Pouladi MA (2016) Structural and molecular myelination deficits occur prior to neuronal loss in the YAC128 and BACHD models of Huntington disease. Hum Mol Genet 25:2621–2632
68. Gatto RG, Chu Y, Ye AQ, Price SD, Tavassoli E, Buenaventura A, Brady ST, Magin RL, Kordower JH, Morfini GA (2015) Analysis of YFP(J16)-R6/2 reporter mice and postmortem brains reveals early pathology and increased vulnerability of callosal axons in Huntington's disease. Hum Mol Genet 24:5285–5298
69. Jin J, Peng Q, Hou Z, Jiang M, Wang X, Langseth AJ, Tao M, Barker PB, Mori S, Bergles DE, Ross CA, Detloff PJ, Zhang J, Duan W (2015) Early white matter abnormalities, progressive brain pathology and motor deficits in a novel knock-in mouse model of Huntington's disease. Hum Mol Genet 24:2508–2527
70. Rosas HD, Tuch DS, Hevelone ND, Zaleta AK, Vangel M, Hersch SM, Salat DH (2006) Diffusion tensor imaging in presymptomatic and early Huntington's disease: Selective white matter pathology and its relationship to clinical measures. Mov Disord 21:1317–1325

71. Heng MY, Tallaksen-Greene SJ, Detloff PJ, Albin RL (2007) Longitudinal evaluation of the Hdh(CAG)150 knock-in murine model of Huntington's disease. J Neurosci 27:8989–8998
72. Wheeler VC, Auerbach W, White JK, Srinidhi J, Auerbach A, Ryan A, Duyao MP, Vrbanac V, Weaver M, Gusella JF, Joyner AL, MacDonald ME (1999) Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Hum Mol Genet 8:115–122
73. Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF (2003) Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. J Comp Neurol 465:11–26
74. Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, Ruiz M, Mushlin R, Alosio W, McConnell K, Connor D, Murphy C, Oakeshott S, Kwan M, Beltran J, Ghavami A, Brunner D, Park LC, Ramboz S, Howland D (2012) Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175. PLoS ONE 7:e49838
75. Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, Ren S, Li XJ, Albin RL, Detloff PJ (2001) Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet 10:137–144
76. Holter SM et al (2013) A broad phenotypic screen identifies novel phenotypes driven by a single mutant allele in Huntington's disease CAG knock-in mice. PLoS ONE 8:e80923
77. Phan J, Hickey MA, Zhang P, Chesselet MF, Reue K (2009) Adipose tissue dysfunction tracks disease progression in two Huntington's disease mouse models. Hum Mol Genet 18:1006–1016
78. Wheeler VC, White JK, Gutekunst CA, Vrbanac V, Weaver M, Li XJ, Li SH, Yi H, Vonsattel JP, Gusella JF, Hersch S, Auerbach W, Joyner AL, MacDonald ME (2000) Long glutamine tracts cause nuclear localization of a novel form of huntingtin in medium spiny striatal neurons in HdhQ92 and HdhQ111 knock-in mice. Hum Mol Genet 9:503–513
79. Hickey MA, Kosmalska A, Enayati J, Cohen R, Zeitlin S, Levine MS, Chesselet MF (2008) Extensive early motor and non-motor behavioral deficits are followed by striatal neuronal loss in knock-in Huntington's disease mice. Neuroscience 157:280–295
80. Lawrence AD, Sahakian BJ, Hodges JR, Rosser AE, Lange KW, Robbins TW (1996) Executive and mnemonic functions in early Huntington's disease. Brain 119:1633–1645
81. Curtin PC, Farrar AM, Oakeshott S, Sutphen J, Berger J, Mazzella M, Cox K, He D, Alosio W, Park LC, Howland D, Brunner D (2015) Cognitive training at a young age attenuates deficits in the zQ175 mouse model of HD. Front Behav Neurosci 9:361
82. Heikkinen T, Lehtimaki K, Vartiainen N, Puolivali J, Hendricks SJ, Glaser JR, Bradaia A, Wadel K, Touller C, Kontkanen O, Yrjanheikki JM, Buisson B, Howland D, Beaumont V, Munoz-Sanjuan I, Park LC (2012) Characterization of neurophysiological and behavioral changes, MRI brain volumetry and 1H MRS in zQ175 knock-in mouse model of Huntington's disease. PLoS ONE 7:e50717
83. Smith GA, Rocha EM, McLean JR, Hayes MA, Izen SC, Isacson O, Hallett PJ (2014) Progressive axonal transport and synaptic protein changes correlate with behavioral and neuropathological abnormalities in the heterozygous Q175 KI mouse model of Huntington's disease. Hum Mol Genet 23:4510–4527
84. Brooks S, Higgs G, Jones L, Dunnett SB (2012) Longitudinal analysis of the behavioural phenotype in Hdh(CAG)150 Huntington's disease knock-in mice. Brain Res Bull 88:182–188
85. Orvoen S, Pla P, Gardier AM, Saudou F, David DJ (2012) Huntington's disease knock-in male mice show specific anxiety-like behaviour and altered neuronal maturation. Neurosci Lett 507:127–132
86. Ciamei A, Detloff PJ, Morton AJ (2015) Progression of behavioural despair in R6/2 and Hdh knock-in mouse models recapitulates depression in Huntington's disease. Behav Brain Res 291:140–146
87. Wheeler VC, Gutekunst CA, Vrbanac V, Lebel LA, Schilling G, Hersch S, Friedlander RM, Gusella JF, Vonsattel JP, Borchelt DR, MacDonald ME (2002) Early phenotypes that

presage late-onset neurodegenerative disease allow testing of modifiers in Hdh CAG knock-in mice. Hum Mol Genet 11:633–640
88. Lerner RP, Trejo Martinez Ldel C, Zhu C, Chesselet MF, Hickey MA (2012) Striatal atrophy and dendritic alterations in a knock-in mouse model of Huntington's disease. Brain Res Bull 87:571–578
89. Cummings DM, Cepeda C, Levine MS (2010) Alterations in striatal synaptic transmission are consistent across genetic mouse models of Huntington's disease. ASN Neuro 2:e00036
90. Yu ZX, Li SH, Evans J, Pillarisetti A, Li H, Li XJ (2003) Mutant huntingtin causes context-dependent neurodegeneration in mice with Huntington's disease. J Neurosci 23:2193–2202
91. Baldo B, Paganetti P, Grueninger S, Marcellin D, Kaltenbach LS, Lo DC, Semmelroth M, Zivanovic A, Abramowski D, Smith D, Lotz GP, Bates GP, Weiss A (2012) TR-FRET-based duplex immunoassay reveals an inverse correlation of soluble and aggregated mutant huntingtin in huntington's disease. Chem Biol 19:264–275
92. Marcellin D, Abramowski D, Young D, Richter J, Weiss A, Marcel A, Maassen J, Kauffmann M, Bibel M, Shimshek DR, Faull RL, Bates GP, Kuhn RR, Van der Putten PH, Schmid P, Lotz GP (2012) Fragments of HdhQ150 mutant huntingtin form a soluble oligomer pool that declines with aggregate deposition upon aging. PLoS ONE 7:e44457
93. Squitieri F, Gellera C, Cannella M, Mariotti C, Cislaghi G, Rubinsztein DC, Almqvist EW, Turner D, Bachoud-Levi AC, Simpson SA, Delatycki M, Maglione V, Hayden MR, Donato SD (2003) Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course. Brain 126:946–955
94. Heng MY, Detloff PJ, Paulson HL, Albin RL (2010) Early alterations of autophagy in Huntington disease-like mice. Autophagy 6:1206–1208
95. Dougherty SE, Reeves JL, Lesort M, Detloff PJ, Cowell RM (2013) Purkinje cell dysfunction and loss in a knock-in mouse model of Huntington disease. Exp Neurol 240:96–102
96. Ehrlich ME (2012) Huntington's disease and the striatal medium spiny neuron: cell-autonomous and non-cell-autonomous mechanisms of disease. Neurotherapeutics 9:270–284
97. Zuccato C, Cattaneo E (2007) Role of brain-derived neurotrophic factor in Huntington's disease. Prog Neurobiol 81:294–330
98. Niccolini F, Politis M (2014) Neuroimaging in Huntington's disease. World J Radiol 6:301–312
99. von Horsten S et al (2003) Transgenic rat model of Huntington's disease. Hum Mol Genet 12:617–624
100. Kirch RD, Meyer PT, Geisler S, Braun F, Gehrig S, Langen KJ, von Horsten S, Nikkhah G, Cassel JC, Dobrossy MD (2013) Early deficits in declarative and procedural memory dependent behavioral function in a transgenic rat model of Huntington's disease. Behav Brain Res 239:15–26
101. Nguyen HP, Kobbe P, Rahne H, Worpel T, Jager B, Stephan M, Pabst R, Holzmann C, Riess O, Korr H, Kantor O, Petrasch-Parwez E, Wetzel R, Osmand A, von Horsten S (2006) Behavioral abnormalities precede neuropathological markers in rats transgenic for Huntington's disease. Hum Mol Genet 15:3177–3194
102. Petrasch-Parwez E, Nguyen HP, Lobbecke-Schumacher M, Habbes HW, Wieczorek S, Riess O, Andres KH, Dermietzel R, Von Horsten S (2007) Cellular and subcellular localization of Huntingtin [corrected] aggregates in the brain of a rat transgenic for Huntington disease. J Comp Neurol 501:716–730
103. Kantor O, Temel Y, Holzmann C, Raber K, Nguyen HP, Cao C, Turkoglu HO, Rutten BP, Visser-Vandewalle V, Steinbusch HW, Blokland A, Korr H, Riess O, von Horsten S, Schmitz C (2006) Selective striatal neuron loss and alterations in behavior correlate with impaired striatal function in Huntington's disease transgenic rats. Neurobiol Dis 22:538–547
104. Winkler C, Gil JM, Araujo IM, Riess O, Skripuletz T, von Horsten S, Petersen A (2006) Normal sensitivity to excitotoxicity in a transgenic Huntington's disease rat. Brain Res Bull 69:306–310
105. Fink KD, Rossignol J, Crane AT, Davis KK, Bavar AM, Dekorver NW, Lowrance SA, Reilly MP, Sandstrom MI, von Horsten S, Lescaudron L, Dunbar GL (2012) Early cognitive

dysfunction in the HD 51 CAG transgenic rat model of Huntington's disease. Behav Neurosci 126:479–487
106. Casteels C, Vandeputte C, Rangarajan JR, Dresselaers T, Riess O, Bormans G, Maes F, Himmelreich U, Nguyen H, Van Laere K (2011) Metabolic and type 1 cannabinoid receptor imaging of a transgenic rat model in the early phase of Huntington disease. Exp Neurol 229:440–449
107. Kandasamy M, Couillard-Despres S, Raber KA, Stephan M, Lehner B, Winner B, Kohl Z, Rivera FJ, Nguyen HP, Riess O, Bogdahn U, Winkler J, von Horsten S, Aigner L (2010) Stem cell quiescence in the hippocampal neurogenic niche is associated with elevated transforming growth factor-beta signaling in an animal model of Huntington disease. J Neuropathol Exp Neurol 69:717–728
108. Cao C, Temel Y, Blokland A, Ozen H, Steinbusch HW, Vlamings R, Nguyen HP, von Horsten S, Schmitz C, Visser-Vandewalle V (2006) Progressive deterioration of reaction time performance and choreiform symptoms in a new Huntington's disease transgenic ratmodel. Behav Brain Res 170:257–261
109. Urbach YK, Raber KA, Canneva F, Plank AC, Andreasson T, Ponten H, Kullingsjo J, Nguyen HP, Riess O, von Horsten S (2014) Automated phenotyping and advanced data mining exemplified in rats transgenic for Huntington's disease. J Neurosci Methods 234:38–53
110. Zeef DH, van Goethem NP, Vlamings R, Schaper F, Jahanshahi A, Hescham S, von Horsten S, Prickaerts J, Temel Y (2012) Memory deficits in the transgenic rat model of Huntington's disease. Behav Brain Res 227:194–198
111. Yu-Taeger L, Petrasch-Parwez E, Osmand AP, Redensek A, Metzger S, Clemens LE, Park L, Howland D, Calaminus C, Gu X, Pichler B, Yang XW, Riess O, Nguyen HP (2012) A novel BACHD transgenic rat exhibits characteristic neuropathological features of Huntington disease. J Neurosci 32:15426–15438
112. Adjeroud N, Yague S, Yu-Taeger L, Bozon B, Leblanc-Veyrac P, Riess O, Allain P, Nguyen HP, Doyere V, El Massioui N (2015) Reduced impact of emotion on choice behavior in presymptomatic BACHD rats, a transgenic rodent model for Huntington Disease. Neurobiol Learn Mem 125:249–257
113. Clemens LE, Weber JJ, Wlodkowski TT, Yu-Taeger L, Michaud M, Calaminus C, Eckert SH, Gaca J, Weiss A, Magg JC, Jansson EK, Eckert GP, Pichler BJ, Bordet T, Pruss RM, Riess O, Nguyen HP (2015) Olesoxime suppresses calpain activation and mutant huntingtin fragmentation in the BACHD rat. Brain 138:3632–3653
114. Clemensson EK, Clemensson LE, Riess O, Nguyen HP (2017) The BACHD Rat model of huntington disease shows signs of fronto-striatal dysfunction in two operant conditioning tests of short-term memory. PLoS ONE 12:e0169051
115. El Massioui N, Lamirault C, Yague S, Adjeroud N, Garces D, Maillard A, Tallot L, Yu-Taeger L, Riess O, Allain P, Nguyen HP, von Horsten S, Doyere V (2016) Impaired Decision making and loss of inhibitory-control in a rat model of Huntington disease. Front Behav Neurosci 10:204
116. Lamirault C, Yu-Taeger L, Doyere V, Riess O, Nguyen HP, El Massioui N (2017) Altered reactivity of central amygdala to GABAAR antagonist in the BACHD rat model of Huntington disease. Neuropharmacology 123:136–147
117. Manfre G, Doyere V, Bossi S, Riess O, Nguyen HP, El Massioui N (2016) Impulsivity trait in the early symptomatic BACHD transgenic rat model of Huntington disease. Behav Brain Res 299:6–10
118. Weber JJ, Ortiz Rios MM, Riess O, Clemens LE, Nguyen HP (2016) The calpain-suppressing effects of olesoxime in Huntington's disease. Rare Dis 4:e1153778
119. Yu-Taeger L, Bonin M, Stricker-Shaver J, Riess O, Nguyen HH (2017) Dysregulation of gene expression in the striatum of BACHD rats expressing full-length mutant huntingtin and associated abnormalities on molecular and protein levels. Neuropharmacology 117:260–272

120. Nagy D, Tingley FD 3rd, Stoiljkovic M, Hajos M (2015) Application of neurophysiological biomarkers for Huntington's disease: evaluating a phosphodiesterase 9A inhibitor. Exp Neurol 263:122–131
121. Abada YS, Nguyen HP, Schreiber R, Ellenbroek B (2013) Assessment of motor function, sensory motor gating and recognition memory in a novel BACHD transgenic rat model for huntington disease. PLoS ONE 8:e68584
122. Abada YS, Nguyen HP, Ellenbroek B, Schreiber R (2013) Reversal learning and associative memory impairments in a BACHD rat model for Huntington disease. PLoS ONE 8:e71633
123. Jansson EK, Clemens LE, Riess O, Nguyen HP (2014) Reduced motivation in the BACHD rat model of Huntington disease is dependent on the choice of food deprivation strategy. PLoS ONE 9:e105662
124. Gouarne C, Tardif G, Tracz J, Latyszenok V, Michaud M, Clemens LE, Yu-Taeger L, Nguyen HP, Bordet T, Pruss RM (2013) Early deficits in glycolysis are specific to striatal neurons from a rat model of huntington disease. PLoS ONE 8:e81528

Chapter 3
Mitochondrial Dysfunction in Huntington's Disease

Catarina Carmo, Luana Naia, Carla Lopes and A. Cristina Rego

Abstract Mitochondrial dysfunction has been described as an early pathological mechanism delineating the selective neurodegeneration that occurs in Huntington's disease (HD), a polyglutamine-expansion disorder that largely affects the striatum and the cerebral cortex. Over the years, mitochondria roles in eukaryotic cells (e.g. in neurons) have largely diverged from the classically attributed cell power source; indeed, mitochondria not only contribute for synthesis of several metabolites, but are also dynamic organelles that fragment and fuse to achieve a maximal bioenergetic performance, are transported along microtubules, regulate intracellular calcium homeostasis through the interaction with the endoplasmic reticulum, produce free radicals and participate in cell death processes. Indeed, most of these activities have been demonstrated to be affected in HD, potentially contributing for the neuronal dysfunction in pre-symptomatic stages. This chapter resumes some of the evidences that pose mitochondria as a main regulatory organelle in HD-affected neurons, uncovering some potentially therapeutic mitochondrial-based relevant targets.

Keywords Calcium dyshomeostasis · Oxidative stress · Metabolic deficits Mitochondrial dynamics · Cell death

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Abbreviations

$\Delta\psi_m$	Mitochondrial membrane potential
α-KGDH	α-ketoglutarate dehydrogenase
3-NP	3-nitropropionic acid
AIF	Apoptosis inducing factor
Apaf-1	Apoptotic protease-activating factor 1
ATP	Adenosine triphosphate
Bcl-2	B-cell lymphoma 2
BDNF	Brain derived neurotrophic factor
BH3	Bcl-2 homology 3
Bid	BH3 interacting-domain death agonist
Bim	Bcl-2 interacting mediator of cell death
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3
CBP	CREB-binding protein
CK	Creatine kinase
CoQ	Coenzyme Q
CREB	cAMP response element-binding protein
Drp1	Dynamin-related protein 1
ETC	Electron transport chain
Fis1	Mitochondrial fission 1
FMN	Flavin mononucleotide
GABA	γ-aminobutyric acid
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Gpx	Glutathione peroxidases
GTP	Guanosine triphosphate
H_2O_2	Hydrogen peroxide
HD	Huntington's disease
hESC	Human embryonic stem cells
HTT/*HTT*	Human huntingtin protein/gene
Htt	Rodent huntingtin protein
IAP1	Inhibitor of Apoptosis Protein-1
iPSCs	Induced pluripotent stem cells
K	Lysine
LC3	Light chain 3
MCU	Mitochondrial calcium uniporter
Mff	Mitochondrial fission factor
Mfn	Mitofusin
mHTT	Human mutant HTT
mHtt	Rodent mutant Htt
MIM	Mitochondrial inner membrane
MIS	Mitochondrial intermembrane space
MOM	Mitochondrial outer membrane
mtDNA	Mitochondrial DNA
NAD	β-nicotinamide adenine dinucleotide

ND5	NADH dehydrogenase subunit 5
NRF	Nuclear respiratory factor
Nrf2	Nuclear factor-erythroid 2-related factor-2
OPA1	Optic atrophy 1
OXPHOS	Oxidative phosphorylation
PCr	Phosphocreatine
PDH	Pyruvate dehydrogenase
PGC-1α	PPARγ—coactivator-1α
PINK1	PTEN-induced putative kinase 1
PolyQ	Polyglutamine
PPAR	Peroxisome proliferator-activated receptor
Prx	Peroxiredoxins
PTEN	Phosphatase and tensin homolog
PTP	Permeability transition pore
PUMA	p53 upregulated modulator of apoptosis
ROS	Reactive oxygen species
SDH	Succinate dehydrogenase
Smac/DIABLO	Second mitochondria derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI
SOD	Superoxide dismutase
TAF	TBP-associated factor 4
TBP	TATA-binding protein
TCA	Tricarboxylic acid
Tfam	Mitochondrial transcription factor A
TIM	Translocase of the inner membrane
TRAK	Trafficking kinesin protein
XIAP	X-linked inhibitor of apoptosis
YAC	Yeast artificial chromosome

3.1 Introduction

Mitochondria are double-membrane organelles that represent the major bioenergetic hub coordinating cell and organism homeostasis. Mitochondria control the production of energy (in the form of adenosine triphosphate, ATP) through oxidative phosphorylation (OXPHOS), supporting the biosynthetic and degradative metabolic requirements of the cells, intracellular calcium (Ca^{2+}) homeostasis, apoptotic and cell signaling pathways, among other cellular processes. The sustained integrity of mitochondria is critical for preserving cell viability; therefore, mitochondrial dysfunction is a common process connecting several neurodegenerative and age-related disorders [1], either due to defects in respiratory function, modified organelle dynamics and degradation, cumulating in DNA and protein damage and/or producing excessive amounts of reactive oxygen species (ROS).

Mitochondrial dysfunction plays a major role in the pathogenesis of Huntington's disease (HD), an autosomal dominant neurodegenerative disorder that initially affects the striatum (mainly the caudate) and later the cortex. HD is characterized by psychiatric disturbances, cognitive deficits, involuntary choreiform movements, dementia and weight loss. All HD cases possess, at least in one of their two copies of the *HTT* gene, a polymorphic CAG repeat tract expansion that encodes for a N-terminal polyglutamine (polyQ) segment of more than 39 residues in the huntingtin protein (HTT) named mutant HTT (mHTT) [2]. Striatal neurodegeneration linked to mitochondrial deregulation has been demonstrated in genetic and toxin-induced animal and cellular models and post-mortem HD human brain tissue [3, 4]. Of relevance, magnetic resonance spectroscopy revealed impaired ATP synthesis and oxidative function in pre-symptomatic HD carriers [5], suggesting that mitochondrial deficits might initiate disease onset.

In this chapter we discuss the role of mitochondrial deregulation in HD-related neuronal dysfunction and degeneration, particularly focusing on changes in energy metabolism, dynamics and movement and the regulation of apoptosis in both central and peripheral HD humanized cell and animal models, in HD human peripheral cells and post-mortem brain samples, as well as in HD human induced pluripotent stem cells (iPSCs), a powerful model to study HD cellular pathogenesis in a pre-differentiation neural stage of the disease and after differentiation into a striatal-like neuronal fate.

3.2 Mitochondrial Dysfunction in HD

Early evidence of mitochondrial defects in HD came from a study demonstrating ultrastructural abnormalities in mitochondria isolated from post-mortem HD cortical tissue [6]. Additionally, the observation that systemic administration of 3-nitropropionic acid (3-NP, an irreversible mitochondrial complex II inhibitor) in rodents or non-human primates produced preferential degeneration in the caudate–putamen that resembled many behavioral and anatomical features of HD, supported the previous evidence [4, 7, 8]. Defects in complexes II, III and IV activities from HD patients' striatum were demonstrated simultaneously [9]. A few years later, mHTT was shown to directly interact with the mitochondrial outer membrane (MOM) [10–12], triggering Ca^{2+} release and abnormal mitochondrial morphology and trafficking, as shown in *postmortem* HD patient's brain specimens, in human HD lymphoblasts or mice neurons expressing the expanded exon 1 of *HTT* [12–14]. Furthermore, interaction of N-terminal fragments of mHTT with the translocase of the inner membrane TIM23 was shown recently, culminating in the inhibition of protein import machinery and neuronal death. Mitochondria from brain synaptosomes of presymptomatic HD mice also exhibited a protein import defect, but not liver mitochondria, suggesting an early and tissue-specific event of the disease [15]. Considering these observations, this section will focus on reports describing how mHTT may cause mitochondrial dysfunction by either a direct interaction with the

organelle and modulation of respiration, mitochondrial membrane potential and Ca^{2+} buffering and mitochondrial bioenergetics, which further impact on ROS production and oxidative damage.

3.2.1 *Altered Mitochondrial Membrane Potential and Impaired Mitochondrial Respiratory Chain Complex Activity*

Electron flow along the respiratory complexes I–IV, localized at the mitochondrial inner membrane (MIM), is coupled to proton translocation into the mitochondrial intermembrane space (MIS), creating an electrochemical proton gradient (proton motive force) and thus a mitochondrial transmembrane potential ($\Delta\psi_m$) of −150 to −180 mV that drives ATP synthesis. This energy production requires $\Delta\psi_m$ to be maintained at 80–90% of its maximum value ([16], for review). However, in HD mitochondria this percentage is not preserved. Brain mitochondria isolated from two lines of YAC72 mice expressing "low" and "high" levels of full lenght-mHTT displayed depolarized membrane, with mitochondria from YAC72 high expressor depolarizing faster after Ca^{2+} stimulation [11]. Similar defect in $\Delta\psi_m$ was found in mitochondria from chimeric human-mouse mHTT-expressing cells in response to increasing Ca^{2+} concentrations [17]. Remarkably, a large amount of evidence has shown that HD mitochondria from human lymphoblasts are highly susceptible to decreased $\Delta\psi_m$ [11, 18], which was correlated with increased glutamine repeats [19], suggesting that the adverse effect of mHTT is not limited to neurons. Indeed, results obtained in our laboratory in symptomatic HD cybrids (an ex vivo peripheral model obtained from the fusion of HD human platelets with mtDNA-depleted rho0 cells) *versus* control cybrids and in HD human B-lymphocytes evidenced significant changes in $\Delta\psi_m$ linked to apoptotic events [20, 21]. Interestingly, constitutive HTT phosphorylation at serine 421 completely abrogated the deregulation of $\Delta\psi_m$ in HD human lymphoblasts [18], linking the neuroprotective effects of HTT phosphorylation in this residue (e.g. [22]) to improved mitochondrial function.

A dramatically decrease in the activity of complex II (succinate dehydrogenase, SDH)/III (cytochrome *c* reductase) and mildly complex IV (cytochrome *c* oxidase) in the caudate or putamen were observed in *postmortem* studies of symptomatic HD patients or HD models, namely immortalized striatal cell lines, animal model brains or human peripheral cells [9, 23, 24, 25, 26, 27], and may contribute for decreased $\Delta\psi_m$. Additionally, the decrease in complex II in humans, rodents or primates following administration of 3-NP or malonate (complex II reversible inhibitor) causes HD-like symptoms in animal models and striatal cytotoxicity ([28], for review). Although the selective inhibition of the striatum by a chemical compound such as 3-NP has posed several questions, reduced activity of mitochondrial respiratory chain complexes can largely contribute to accelerate mitochondrial dysfuncton in HD.

3.2.2 Defects in Mitochondrial Ca^{2+} Handling

The MIM possesses a Ca^{2+} uniporter (MCU, mitochondrial calcium uniporter), providing cells with a protective high capacity for Ca^{2+} buffering. Moreover, interaction of mHTT with the MOM may induce the opening of a high conductance pathway, the mitochondrial permeability transition pore (PTP), which is triggered by Ca^{2+}, ROS or decreased adenine nucleotide levels, causing mitochondrial swelling, depolarization and, eventually, cell death [10, 11, 17]. Thus, deficits in mitochondrial Ca^{2+} handling likely contribute to HD neurodegeneration. Studies performed by Panov and colleagues demonstrated that mitochondria isolated from lymphoblasts of HD patients and from brains of transgenic yeast artificial chromosome (YAC) mice expressing full-length mHTT with 72 glutamines (YAC72) exhibited pronounced defects in Ca^{2+} handling. Importantly, these defects persisted even in the presence of PTP inhibitors [11]. Impaired mitochondrial Ca^{2+} homeostasis was also confirmed in intact HD human lymphoblasts following exposure to hydrogen peroxide (H_2O_2) [18]. Moreover, mitochondria obtained from liver of homozygous knock-in $Hdh^{150/150}$ mice or treated with recombinant truncated mHTT protein showed augmented predisposition to Ca^{2+}-stimulated PTP induction [10]. Contrariwise, increased Ca^{2+} uptake was observed in isolated brain non-synaptic mitochondria from R6/2 mice (the most commonly used HD model expressing human *HTT* exon 1 with $\sim$150 CAG repeats) and YAC128 HD mice, when compared to mitochondria from wild-type mice [29]. Indeed, two years earlier, Brustovetsky and colleagues had shown that striatal mitochondria from R6/2 exhibited increased resistance to Ca^{2+}, while in striatal mitochondria from littermate controls, lower doses of Ca^{2+} consistently evoked PTP more easily. Mitochondrial from knock-in HD mice also became more resistant to Ca^{2+} with increasing age and retained these levels of sensitivity throughout life [30], suggesting that mitochondria are capable of compensatory changes towards neuroprotection. Meanwhile, recent data obtained with isolated brain synaptic and non-synaptic mitochondria from YAC128 mice suggest that increased Ca^{2+} uptake capacity can be directly correlated with the amount of mHTT associated with the mitochondrial membrane [31]. In addition, HD-iPSC-derived neuronal-like cells expressing both GABA (γ-aminobutyric acid)-A receptor and ionotropic glutamate receptors (obtained from fibroblasts of symptomatic HD patients retaining 60 or 180 CAGs) revealed a clear CAG expansion-dependent decrease in Ca^{2+} uptake following a chronic glutamate stimulus [32].

3.2.3 Energy Metabolic Deficits

Neurons are highly dependent on mitochondrial ATP production to maintain normal synaptic communication. Therefore, they are very sensitive to disturbed energy metabolism. A number of studies, from HD *postmortem* brains [33] to transgenic

HD mouse brain [34, 35], revealed mHTT-related abnormal ATP/ADP and phosphocreatine/inorganic phosphate (PCr/Pi) ratios and energy charges. This reduction in mitochondrial ATP levels might be linked to increased Ca^{2+} influx through *N*-methyl-D-aspartate receptors, since ATP/ADP ratio could be normalized by blocking Ca^{2+} influx in mHTT-expressing striatal cells [36]. Moreover, a poor creatine kinase (CK)/PCr system in HD brain might also contribute for this reduction [37] (Fig. 3.1). As described before for changes in $\Delta\psi_m$, bioenergetic defects in HD are not only confined to the brain, but are also observed in peripheral tissues, such as muscle [5, 38] or lymphoblasts [36]. These findings raise the possibility that the ubiquitous expression of mHTT may place other cell types at risk, particularly those with high metabolic demand. Remarkably, recent studies conducted in our laboratory showed that glucose deprivation did not exacerbate the defects in ATP/ADP ratio in cortical primary cultures derived from YAC128 mice, contrarily to wild-type neurons, pointing to an abnormal glycolytic pathway linked

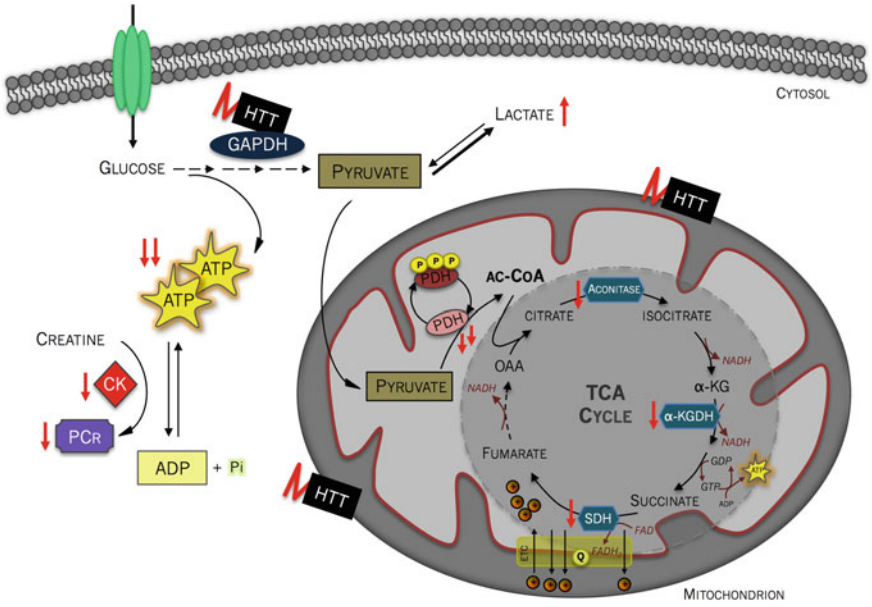

Fig. 3.1 Mutant huntingtin induces energy metabolic failure. Glycolysis is the metabolic pathway that converts glucose into pyruvate, generating ATP and NADH. In the presence of mHTT, ATP production is decreased, along with poor PCr/Pi ratio due to decreased CK activity. Alongside, mHTT interacts with the sixth step glycolysis enzyme GAPDH. Although with compromised glycolysis, pyruvate can still accumulate due to increased phosphorylation/inhibition of PDH, stimulating the conversion to lactate instead of entering in the TCA cycle as acetyl-CoA. Defects in TCA cycle intermediate enzymes such as α-KGDH, aconitase and SDH, as well as the susceptibility of SDH to mHTT interaction severely compromises the generation of reduced equivalents to feed the ETC, which may also explain decreased activities of mitochondrial respiratory chain complexes

to deficient ATP generation [39]. Studies on mitochondrial oxidative metabolism in presymptomatic and symptomatic HD patients previously detected a selective defect in glycolysis in early HD striatum [40–42], suggesting that metabolic deficits in HD may precede neuropathology and clinical symptoms. Indeed, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has an essential function in glycolysis, binding both normal and mHTT, but with a preferential interaction to cleaved polyQ domain, enhancing nuclear translocation of mHTT and cytotoxicity [43]. Interestingly, studies using an allelic series of murine CAG knock-in embryonic stem cell (ESC) lines have shown dominant CAG-length dependent reduction in energy metabolism [44]. In addition, human derived HD specific neural stem cells showed significantly decreased intracellular ATP [32], supporting the hypothesis that bioenergetic dysfunction is an early event in HD.

Elevated levels of lactate and increased lactate/pyruvate ratio have been also described in striatum, cortex and cerebrospinal fluid from HD patients [45–47], as well as in brains from YAC128 and R6/2 transgenic mouse models [34, 48]. Concordantly, we observed that HD cybrid lines exhibited increased lactate/pyruvate levels, which were correlated with a large decrease in the activity and protein levels of pyruvate dehydrogenase (PDH) [49], a protein complex located in the mitochondrial matrix, responsible for catalyzing the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA (Fig. 3.1). Decreased mitochondrial metabolism in caudate (in particular) and putamen of HD patients was previously assessed through a progressive decrement in PDH complex [50, 51], whereas dichloroacetate, a PDH kinase inhibitor previously shown to increase PDH activity, prevented the increase of cerebral lactate concentrations and attenuated the development of striatal neuron atrophy in R6/2 and N171-82Q transgenic HD models [52]. Besides the PDH complex, tricarboxylic acid (TCA) cycle enzymes that provide the main pathway for generating reducing equivalents, such as aconitase, α-ketoglutarate dehydrogenase (α-KGDH) or SDH are also compromised in central HD tissues [53, 54] (Fig. 3.1). Cultured striatal neurons expressing N-terminal HTT fragment showed decreased SDH activity, the only enzyme that participates in both TCA cycle and electron transport chain (ETC) [23]. Contrariwise, in peripheral HD cybrids α-KGDH enzymatic activity was increased [49, 55, 56], suggesting a compensatory mechanism to re-establish NADH levels.

3.3 Oxidative Dysregulation

Under physiological conditions about 1% of O_2 consumed generates superoxide anion ($O_2^{\bullet -}$) at the mitochondrial respiratory chain due to the leakage of electrons at the level of complexes I and III. Indeed, mitochondria are both sources (at the level of complexes I and III) and targets of ROS, contributing to mitochondrial damage in several pathologies. In complex I (reduced β-nicotinamide adenine dinucleotide (NADH)-ubiquinone:oxidoreductase), flavin mononucleotide (FMN) accepts electrons from NADH passing them through a chain of iron–sulfur centers

to the ubiquinone (CoQ, coenzyme Q) reduction site. Complex I-dependent ROS production is influenced by the matrix redox potential ($NADH/NAD^+$ ratio) and is enhanced by its selective inhibitor, rotenone, which prevents the transfer of electrons to the CoQ-binding site [57]. Complex III catalyzes the reduction of cytochrome *c* by oxidation of CoQ, which has two reaction centers, the ubiquinol-oxidation center (Qo site) and the ubiquinone-reduction center (Qi site). Inhibition of Qi site by antimycin A prompts the production of large amounts of $O_2^{\bullet-}$ on both sides of the MIM [58]. $O_2^{\bullet-}$ is highly membrane impermeable, however it can be readily dismutated to H_2O_2 by superoxide dismutases (SOD); in contrast, H_2O_2 diffuses across membranes [59]. Mitochondria have their own antioxidant defense system, thus minimizing the deleterious effects exerted by ROS. The first line of defense involves the dismutation of $O_2^{\bullet-}$ to H_2O_2 by metal-containing enzymes, comprising Mn-SOD (SOD2), located in the mitochondrial matrix, and Cu,Zn-SOD (SOD1) at the MIS and cytosol. H_2O_2 can also be effectively detoxified by mitochondrial antioxidant enzymes like glutathione peroxidases (Gpx), which are also present in the cytosol and by peroxiredoxins (Prx) and catalase, the latter located in the peroxisomes, which convert H_2O_2 into H_2O and O_2 [60, 61].

The major cause for energetic deficits in HD involves mitochondrial abnormalities and ROS production triggered by mHTT-mitochondria interactions (in a polyQ length-dependent manner), which impair oxidative phosphorylation and ATP production [62]. Aconitase is an iron-sulphur enzyme of the TCA cycle that is particularly susceptible to ROS. Several studies found that it is strongly affected in *postmortem* human HD caudate, putamen and cortex [55, 63, 64] and in striatum from R6/2 mice [65].

A relationship between inhibition of complexes I and III and enhanced levels of mitochondrial-generated $O_2^{\bullet-}$ was previously confirmed by us in HD striatal cells [66]. Enhanced ROS formation occurring mainly via mitochondria, observed in knock-in striatal cells expressing mHtt ($ST\mathit{Hdh}^{Q111/Q111}$), was related with altered activities and levels of antioxidant defense systems, and decreased antioxidant response to exogenous stressors associated to impaired nuclear factor-erythroid 2-related factor-2 (Nrf2) transcriptional activity [66, 67]. An exhaustive proteomic analysis of human HD samples from striatum and cortex showed increased oxidative stress response, defined by the induction of several antioxidant enzymes, namely Prx 1, 2, and 6, and Gpx 1 and 6. Concomitantly, catalase activity was enhanced and SOD2 showed a significant increase of both protein and activity levels in cortex and striatum [63]. Moreover, protein carbonyl formation (a marker of protein oxidation) of glial fibrillary acidic protein, γ-enolase, and CK B was increased in human HD samples from striatum and cortex [63].

Preceding studies in iPSC lines generated from HD patients fibroblasts, ranging from 33 to 180 CAGs, and control fibroblasts, also described increased cell death in HD-iPSC lines in response to toxic stressors, suggesting that HD-iPSCs are increasingly susceptible to ROS [32]. In HD-iPSCs reprogrammed from a juvenile HD patient carrying 72 CAG repeats, Chae and colleagues (2012) shed some light

on protein expression profiles that are key regulators of oxidative stress, DNA damage and expression of cytoskeleton associated proteins [68]. These authors found lower levels of SOD1, GST and Gpx1 in HD-iPSC lines, when compared to human embryonic stem cells (hESCs), in contrast with the upregulation of Prx family members, including Prx1, 2 and 6, which have been implicated as important indicators for cellular ROS signals [68].

In summary, mitochondrial dysfunction leads to increased production of ROS, which has a major role in both necrotic and apoptotic mechanisms of cell death (detailed in Sect. 3.6).

3.4 Mitochondrial Biogenesis

Mitochondrial network is constantly being renewed through an equilibrium between biogenesis and degradation of damaged mitochondria (mitophagy) [69]. Mitochondria biogenesis comprises a multistep process, where mtDNA transcription and translation, along with translation of nuclear-encoded mitochondrial-related transcripts, mitochondrial protein import and overall assembly into a mitochondrial network must proceed correctly [70].

cAMP response element-binding protein (CREB)/TATA-binding protein (TBP)-associated factor (TAF)4 signaling pathway regulates various mitochondrial genes, such as NADH dehydrogenase subunit 5 (ND5) that codes for a subunit of complex I, and is severely disrupted in HD. In fact, mHTT can interact with several transcription factors involved in this pathway, such as CREB-binding protein (CBP) or TAF4/TAFII130 [71, 72]. Ultimately, CREB/TAF4 signaling pathway regulates the expression of peroxisome proliferator-activated receptor γ—PPARγ—coactivator-1α (PGC-1α), one of the major transcriptional regulators of organelle biogenesis [73]. PGC-1α is a major regulator of mitochondrial function, mediating mitochondrial biogenesis and respiration. Being a transcriptional co-activator, it regulates the expression of nuclear-encoded subunits of each of the electron transport-chain complexes, along with genes involved in antioxidant response such as ATP synthase or SOD2 [74]. PGC-1α also regulates nuclear respiratory factor (NRF)1 and 2 and PPAR α, δ and γ by forming heteromeric complexes, sharing a role in the expression of genes such as cytochrome *c*, complexes I-V and the mitochondrial transcription factor A (Tfam), the major transcriptional regulator of mtDNA [75]. In HD in vitro and in vivo models PGC-1α was found to be repressed, partially due to the direct interaction of mHTT with the signaling pathway mentioned above that regulates its expression, but also by a direct binding to its promoter [73]. HD patients also showed reduced levels of Tfam and PGC-1α as disease severity increases, along with evidences of mitochondrial loss [3, 73, 76, 77] (Fig. 3.2).

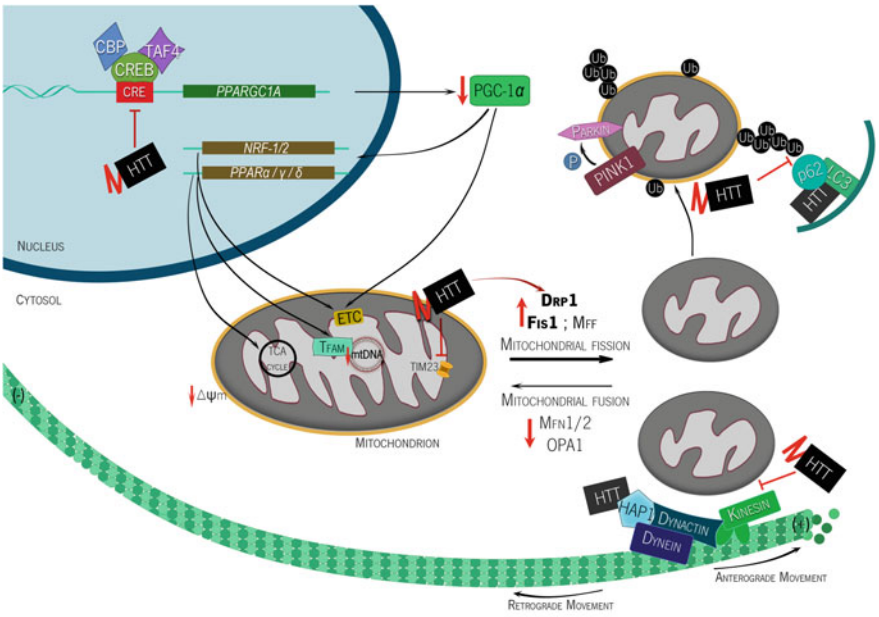

Fig. 3.2 Mutant huntingtin severely impacts mitochondrial turnover. mHTT disrupts CREB/TAF4 signaling culminating in decreased PGC-1α levels and activity. Ultimately, signaling pathways regulated by downstream targets of this transcriptional coactivator may be affected, namely NRF-1, NRF-2, PPARα, PPARδ and PPARγ. Expression of genes involved in TCA cycle, ETC and Tfam are significantly impaired, with compromised mitochondrial biogenesis. By directly interacting with mitochondria, mHTT inhibits mitochondrial protein import, a major factor not only for mitochondrial biogenesis, but also for overall mitochondrial function. Moreover, mHTT disrupts the balance between fission and fusion, with increased mitochondrial fragmentation due to decreased levels of Mfn1, Mfn2 and OPA1 and increased levels of Drp1 and Fis1. Additionally, mHTT interacts with and increases Drp1's GTPase activity. Mitochondrial trafficking is impaired in a retrograde (dynactin/dynein-dependent) and anterograde (kinesin-dependent) manner. mHTT may also interfere with the selective degradation of dysfunctional mitochondria, namely by hampering HTT's function in aiding p62 to associate with LC3

3.5 Mitochondrial Dynamics and Mitophagy

Mitochondria are dynamic organelles with frequent changes in size, shape, number and even cellular distribution that directly relate with their function in response to cellular needs or to diverse stimuli [78]. Increasing evidence suggests that unbalanced mitochondrial dynamics take an important role in neurodegeneration in HD ([79] for review). The presence of mHTT appears to reduce the number of mitochondria and leads to their fragmentation, with defects in anterograde and retrograde transport and velocity, ultimately causing neuronal death [80].

3.5.1 Mitochondrial Fission/Fusion Balance

Mitochondria hold the ability to divide (fission) and unite (fusion) in response to diverse stimuli. Both processes allow the exchange of membranes and intramitochondrial content or mobility of the organelle to specific subcellular locations; moreover, fission facilitates apoptosis by regulating the release of MIS proteins into the cytosol, such as cytochrome *c* [78].

Fission/fusion balance has been reported to be altered in HD, with altered expression of genes involved in these processes, culminating in abnormal mitochondrial morphology and consequently in neuronal dysfunction [81]. Dynamin-related protein 1 (Drp1) takes control of mitochondrial fission. This is a cytosolic protein that can transit towards the MOM upon a fission stimulus, having an effector guanosine triphosphate (GTP)ase domain [82]. Mitochondrial fission 1 (Fis1) and mitochondrial fission factor (Mff), two integral proteins of the MOM, serve as adaptors for Drp1, allowing the recruitment of Drp1 to the effector sites [83]. Meanwhile, fusion counts with machinery of both the MIM and MOM. Mitofusins (Mfn) 1 and 2 are also GTPases, located at the MOM, which are responsible for the fusion of MOMs of the juxtaposing mitochondria. Optic atrophy 1 (OPA1) is the regulator for the MIM fusion process; OPA1 is found at the MIS and shows association with the MIM. Maintenance of $\Delta\psi_m$ is required for mitochondrial fusion. As such, after dissipation of $\Delta\psi_m$, fusion is inhibited, but fission can still occur and mitochondrial fragmentation becomes a dominant morphology [84].

A visible decrease in the number of mitochondria in striatal spiny neurons derived from neostriatal tissue of HD patients appears to directly correlate with disease severity [3]. Increased levels of Drp1 and Fis1, along with decreased levels of Mfn1, Mfn2 and OPA1 were found in striatum and cortex of several HD animal models and patients, resulting in excessive mitochondrial fragmentation [80, 85]. In addition to increased expression, interaction of Drp1 with mHTT results in increased GTPase activity [12] (Fig. 3.2), leading to fragmented and less efficient mitochondria that culminates in loss of energy required for neuronal function.

3.5.2 Mitochondrial Trafficking

Impairment in mitochondrial transport along neuronal processes, with slower translocation of the organelle has been associated to HD. Mitochondria trafficking along the cells allows the organelle to be present in subcellular compartments that are in need of a high energy demand. This process is critically important when considering neurons that need energy outside the regular bioenergetic requirements, such as for synaptic transmission. In fact, mHTT-induced fragmented mitochondria are mainly localized in the cell body, not being able to be transported to dendrites, axons or synapses (anterograde movement), which consequently results in low ATP levels at these sites and in overall synaptic degeneration [76].

The processes of mitochondrial fusion and fission can be directly related to their motility. Fission allows for smaller mitochondria to be separated from the rest of the network and to be transported along the microtubules with the aid of dynein, dynactin (retrograde transport) and kinesins motors (anterograde transport) [86]. Mitochondria enlist motor adaptors such as trafficking kinesin protein (TRAK)1 and TRAK2 that bind Miro (MOM protein) to kynesin motors and ensures targeted and precise trafficking in response to neuronal activity [87]. Both N-terminal fragments and full-length mHTT can directly affect mitochondria motility in either anterograde or retrograde movement, resulting in accumulation of the organelle in the soma [76, 88, 89] (Fig. 3.2). Sequestration of mitochondrial transport machinery and blockage by mHTT aggregates may take place, being impossible for mitochondria to move through narrow neuronal projections, as seen in cortical neurons overexpressing mHTT and in HD striatal neurons [89, 90]. Moreover, Orr and colleagues reported altered mitochondrial trafficking in the absence of mHTT aggregates, suggesting an early impairment due to reduced association of mitochondria with motor proteins [76, 88, 89].

3.5.3 Mitophagy

Accumulation of damaged mitochondria occurs in HD cells and can be due to the loss in $\Delta\psi_m$, oxidative stress, impaired OXPHOS, excessive fragmentation and/or decreased biogenesis. Selective mitochondrial degradation by macroautophagy (hereafter termed mitophagy) ensures mitochondrial quality control and recycling, but must be balanced with biogenesis [91]. Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)/Parkin-dependent mitophagy pathway is the most well characterized mitophagy pathway, although PINK1/Parkin-independent mitophagy can also occur [92, 93]. One example comes from the redistribution of cardiolipin, naturally in the MIM, to the MOM, signaling damaged mitochondria from neuronal cells to undergo degradation. This allows cardiolipin to interact with autophagosome-associated light chain 3 (LC3) [94].

PINK1 is a serine/threonine kinase that localizes in the cytosol and is normally imported into MIM where it is degraded by mitochondrial proteases [95–97]. In the presence of damaged mitochondria exhibiting decreased $\Delta\psi_m$, PINK1 is stabilized at the MOM, inducing Parkin translocation to mitochondria [98]. Since Parkin is an E3 ubiquitin ligase, it ligates ubiquitin chains on MOM proteins that are recognized by autophagy adaptors such as p62 [99]. Khalil and colleagues reported a protective role for PINK1 overexpression in HD flies and ST*Hdh*$^{Q111/Q111}$ cells [100]. Nevertheless, the significance of alterations in PINK1/Parkin-dependent mitophagy has remained elusive in HD.

HTT was proposed to have a role in the control of autophagosome dynamics, along with huntingtin-associated protein 1 (HAP1), through the regulation of dynein and kinesin. Moreover, axonal transport of autophagosomes was found to be impaired in the presence of mHTT. It ultimately ended in inefficient degradation of

internalized mitochondria, probably due to inhibition of autophagosome/lysosome fusion [101]. Additionally, wild-type HTT may serve as a scaffold protein in selective autophagy (not just mitophagy), aiding autophagic adaptor p62 to associate with LC3 and lysine 63 (K63)-linked ubiquitinated substrates [102]. PolyQ-expanded HTT may thus impede this scaffold function (Fig. 3.2). Indeed, expression of a mHTT transgene in a mice expressing mitochondria-targeted Keima, a protein exhibiting pH-dependent excitation, reduced the levels of mitophagy [103].

3.6 Mitochondrial-Dependent Apoptosis

Intrinsic apoptosis is triggered following the loss of integrity of the MOM, which allows the release of pro-apoptotic factors to the cytosol. The release of proteins such as cytochrome *c* and Smac/DIABLO (second mitochondria derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI) induces the formation of the apoptosome. After release from the MIS, cytochrome *c* forms a complex with deoxy-ATP, Apaf-1 (apoptotic protease-activating factor 1) and the initiator caspase 9, which when activated further activates the executioner caspases (3, 6 and 7) and induces the well-recognized nuclear apoptotic features, such as DNA fragmentation and chromatin condensation [104–106]. In neurons, p53 has also been implicated in upregulating the pro-apoptotic proteins Bax and the BH3-only proteins PUMA (p53 upregulated modulator of apoptosis) and Noxa [107].

Different evidences reinforce the deleterious influence of mHTT in the apoptotic pathway, particularly by activating several BH3-only proteins. Changes in Bcl-2 (B-cell lymphoma 2), an anti-apoptotic protein, in HD are controversial. In several cell lines with endogenous expression of mHTT or induced to overexpress mHTT, Bcl-2 family protein dysregulation was common; several studies showed decreased Bcl-2 protein and mRNA levels, Bax activation and translocation from the cytosol to mitochondria, followed by Bim (Bcl-2 interacting mediator of cell death) activation [108–112] (Fig. 3.3). However, in animal models of HD, the results are largely controversial, particularly when considering the anti-apoptotic proteins. Initial studies showed that Bcl-2 protein levels were unchanged in total brain or in mitochondrial fractions from R6/1 and R6/2 mice [113–115]. Conversely, other studies reported down-regulation of Bcl-2 levels in R6/2 mouse brain [109, 116] and in striatal tissue from N171-82Q mice [108]. Importantly, overexpression of Bcl-2 was shown to slow the disease progression [117]. For other pro-survival proteins, such as Bcl-XL, no major differences were found in total brain and striatum lysates or mitochondrial fractions of R6/1 (control and with low levels of the neurotrophin brain derived neurotrophic factor, BDNF), HD94 (tetracycline inducible expression of mHtt) and R6/2 mouse models [113, 117]. Moreover, higher Smac/DIABLO levels were found in the cytosol of cells overexpressing mHTT and this release was responsible for the degradation of anti-apoptotic IAP1

(Inhibitor of apoptosis protein-1) and XIAP (X-linked inhibitor of apoptosis) proteins in the cytosol of HD striatal cells [118]. The mitochondrial levels of AIF (apoptosis inducing factor), which induces apoptotic cell death through a caspase-independent pathway, were also found to be reduced in HD mouse striatal cells [119].

More consistent data has been observed in pro-apoptotic proteins examined in HD animal models and patient samples. In mitochondrial brain fractions of R6/1 and R6/2 mice the pro-apoptotic protein Bax was increased; moreover, increased Bax mRNA was described in the cortex and cerebellum of R6/1 mice [113, 115, 117]. A decrease in phosphorylated Bad and activation of caspases-1 and -3 was also reported in R6/2 mice models [117]. In caudate samples from HD patients neostriatal neurodegeneration was associated with enhanced Bax levels, weak caspase-3 immunostaining and cells exhibiting apoptotic morphology, in comparison with controls without neurological disease [120]. These changes seem not to be specific for HD human brain, since increased Bax levels were also described in peripheral B and T lymphocytes and monocytes derived from symptomatic HD patients [20] (Fig. 3.3). Moreover, untreated HD human cybrid lines showed increased mitochondrial Bim and Bak levels, and a slight release in cytochrome c, evidencing their increased susceptibility to intrinsic apoptosis [21]. Another mitochondrial localized BH3-only protein implicated in HD is BNIP3; when activated, BNIP3 causes loss in $\Delta\psi_m$ and induces mitochondrial fragmentation prior to mitophagy [112]. Although the molecular mechanisms for BNIP3 activation are not well understood, BNIP3 levels were described to be increased in total lysates of HD myoblasts [121].

Numerous studies in vitro and in vivo support the interaction between caspases and HTT in vulnerable cell types in the striatum and cortex leading to increase cell death [122–125]. Increased expression of caspase-2 was correlated with decreased BDNF levels in the cortex and striatum of YAC72 mice. Htt can be recruited and cleaved by initiator caspase-2, which induces neuritic degeneration. The generated N-terminal toxic fragment was shown to associate with synaptic vesicles and inhibit glutamate uptake. Caspase-7, which is specifically expressed in medium-sized neurons and enriched in the striatum, may also associate with Htt, triggering the activity of other caspases, namely caspase-6, and accelerating the production of Htt fragments and the induction of apoptosis [122, 124]. Two active caspase-3 sites and one caspase-6 site have been largely studied in HTT. Caspase 6 cleavage at the 586 cysteine residue of Htt, but not caspase 3 cleavage, was shown to be required for the development of the characteristic behavioral and neuropathological features of HD [123, 126, 127]. However, recent studies showed that the cleavage pattern was unaltered when mHtt expressing mice were crossed onto caspase knockout background [128, 129], raising the question of whether other caspases might be involved in the generation of cytotoxic mHTT fragments.

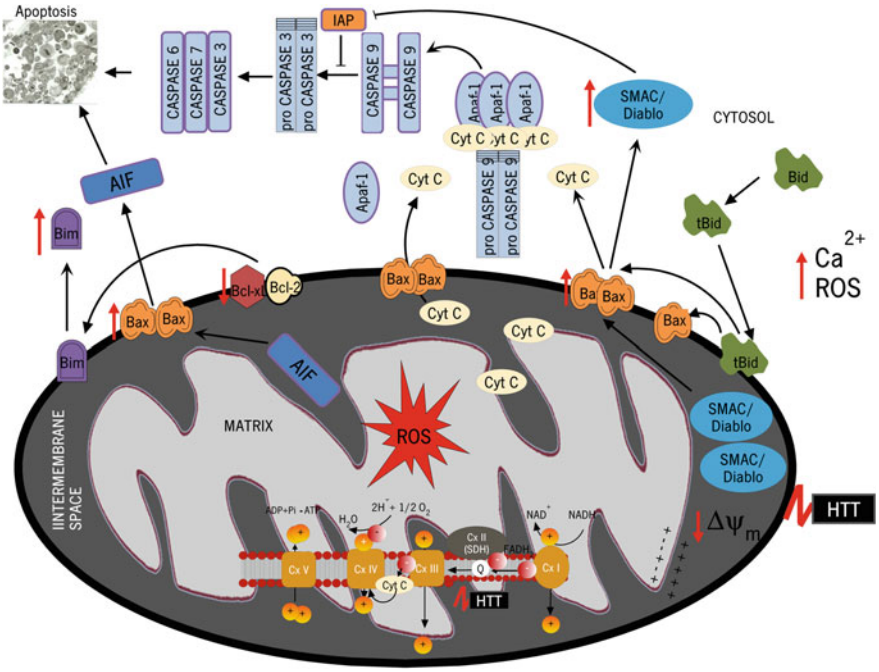

Fig. 3.3 Intrinsic apoptotic pathway is triggered by intracellular stimuli such as Ca^{2+} overload and excessive ROS levels. Following the stress signals, the apoptotic process is controlled by Bcl-2-protein family, classified into anti-apoptotic and pro-apoptotic members. The pro-apoptotic effector proteins, Bak and Bax, can be activated by pro-apoptotic BH3-only proteins —Bim and active Bid (truncated Bid, tBid)—or by a noxious stimulus, upon which these proteins form oligomers that insert into the MOM, being responsible for the loss of membrane integrity. Destabilization of the mitochondrial membrane causes the release of apoptotic factors such as cytochrome *c* and Smac/Diablo, the latter promoting apoptosis by neutralizing the inhibitory effect of IAPs on caspases activity. After being released, cytochrome *c* assembles into a multiprotein caspase activating complex formed by procaspase-9, Apaf-1 and deoxy-ATP, the apoptosome. Subsequently, activated caspase 9 cleaves and activates the downstream executioner caspases 3, 7 and 6 to induce cell death. Other apoptotic factors are released from the MIS into the cytosol, namely AIF, which acts independently of caspases. Stress factors such as increased intracellular Ca^{2+} levels and ROS production can trigger the opening of the PTP (*not shown*)

3.7 Concluding Remarks

In conclusion, numerous studies have shed some light on the role of mitochondria in the regulatory mechanism of biochemical/molecular functions involved in HD pathophysiology. Increasing evidence support the contribution of mitochondria and oxidative phosphorylation defects in HD patients and cellular and animal HD models as a trigger event for the irreversible cascade of events involved in disease phenotype, as demonstrated by some of the most relevant publications that were generated in this field since 1978 until 2015 (Fig. 3.4). However, many questions

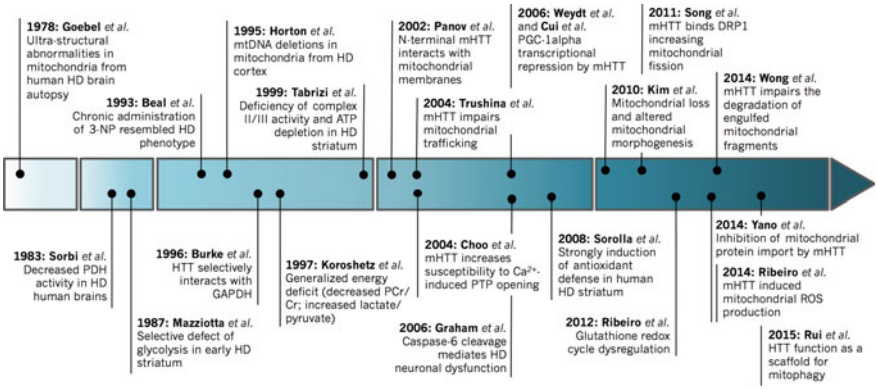

Fig. 3.4 **Key discoveries involving mitochondria in HD pathogenesis**. This timeline resumes some of the major discoveries involving mitochondrial dysfunction in HD and related oxidative stress changes, from dysfunctional glycolysis described in the late 80 s, to deficiency of respiratory chain complexes in the late 90 s, which was later explained by the interaction of mHTT with mitochondria and by transcriptional deregulation. More recently, research has focused on the role of both wild-type and mHTT on mitochondrial dynamics and turnover. All studies are indicated in the reference list

remain to be answered regarding the role of mitochondria dysfunction in HD and its influence on other pathological processes and thus on disease progression. The full understanding of how mHTT targets mitochondria leading to a dysfunctional organelle can be foreseen an important advance to identify new molecular pathways and eventually therapeutic targets. So far, few clinical trials aimed to directly improve mitochondrial function in HD, although the results have not been very promising. A study concerning coenzyme Q10 intake was canceled at phase III due to the lack of results. Other bioenergetic agents tested include creatine and cysteamine bitartrate, the latter the neuroactive metabolite of cystamine. The present chapter clearly points out several molecular changes that can be the targets of more efficient mitochondrial-based therapies and may impact on the organelle physiology. Considering the diversity of changes operated just in a single organelle such as mitochondria, development of combined therapies aiming to improve these alterations may help to delay HD onset and/or disease progression.

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References

1. Chaturvedi RK, Flint Beal M (2013) Mitochondrial diseases of the brain. Free Radic Biol Med 63:1–29
2. Collaborative THsD (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. In Cell 971–983
3. Kim J, Moody JP, Edgerly CK, Bordiuk OL, Cormier K, Smith K, Beal MF, Ferrante RJ (2010) Mitochondrial loss, dysfunction and altered dynamics in Huntington's disease. Hum Mol Genet 19:3919–3935
4. Kodsi MH, Swerdlow NR (1997) Mitochondrial toxin 3-nitropropionic acid produces startle reflex abnormalities and striatal damage in rats that model some features of Huntington's disease. Neurosci Lett 231:103–107
5. Saft C, Zange J, Andrich J, Muller K, Lindenberg K, Landwehrmeyer B, Vorgerd M, Kraus PH, Przuntek H, Schols L (2005) Mitochondrial impairment in patients and asymptomatic mutation carriers of Huntington's disease. Mov Disord Off J Mov Disord Soc 20:674–679
6. Goebel HH, Heipertz R, Scholz W, Iqbal K, Tellez-Nagel I (1978) Juvenile Huntington chorea: clinical, ultrastructural, and biochemical studies. Neurology 28:23–31
7. Beal MF, Brouillet E, Jenkins BG, Ferrante RJ, Kowall NW, Miller JM, Storey E, Srivastava R, Rosen BR, Hyman BT (1993) Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J Neurosci Off J Soc Neurosci 13:4181–4192
8. Brouillet E, Hantraye P, Ferrante RJ, Dolan R, Leroy-Willig A, Kowall NW, Beal MF (1995) Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. Proc Natl Acad Sci USA 92:7105–7109
9. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH (1996) Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 39:385–389
10. Choo YS, Johnson GV, MacDonald M, Detloff PJ, Lesort M (2004) Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. Hum Mol Genet 13:1407–1420
11. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT (2002) Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci 5:731–736
12. Song W, Chen J, Petrilli A, Liot G, Klinglmayr E, Zhou Y, Poquiz P, Tjong J, Pouladi MA, Hayden MR et al (2011) Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. Nat Med 17:377–382
13. Napoli E, Wong S, Hung C, Ross-Inta C, Bomdica P, Giulivi C (2013) Defective mitochondrial disulfide relay system, altered mitochondrial morphology and function in Huntington's disease. Hum Mol Genet 22:989–1004
14. Squitieri F, Cannella M, Sgarbi G, Maglione V, Falleni A, Lenzi P, Baracca A, Cislaghi G, Saft C, Ragona G et al (2006) Severe ultrastructural mitochondrial changes in lymphoblasts homozygous for Huntington disease mutation. Mech Ageing Dev 127:217–220
15. Yano H, Baranov SV, Baranova OV, Kim J, Pan Y, Yablonska S, Carlisle DL, Ferrante RJ, Kim AH, Friedlander RM (2014) Inhibition of mitochondrial protein import by mutant huntingtin. Nat Neurosci 17:822–831
16. Nicholls DG, Ward MW (2000) Mitochondrial membrane potential and neuronal glutamate excitotoxicity: mortality and millivolts. Trends Neurosci 23(4):166–174
17. Milakovic T, Quintanilla RA, Johnson GV (2006) Mutant huntingtin expression induces mitochondrial calcium handling defects in clonal striatal cells: functional consequences. J Biol Chem 281:34785–34795

18. Naia L, Ferreira IL, Cunha-Oliveira T, Duarte AI, Ribeiro M, Rosenstock TR, Laco MN, Ribeiro MJ, Oliveira CR, Saudou F et al (2015) Activation of IGF-1 and insulin signaling pathways ameliorate mitochondrial function and energy metabolism in Huntington's Disease human lymphoblasts. Mol Neurobiol 51:331–348
19. Sawa A, Wiegand GW, Cooper J, Margolis RL, Sharp AH, Lawler JF Jr, Greenamyre JT, Snyder SH, Ross CA (1999) Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. Nat Med 5:1194–1198
20. Almeida S, Sarmento-Ribeiro AB, Januario C, Rego AC, Oliveira CR (2008) Evidence of apoptosis and mitochondrial abnormalities in peripheral blood cells of Huntington's disease patients. Biochem Biophys Res Commun 374:599–603
21. Ferreira IL, Nascimento MV, Ribeiro M, Almeida S, Cardoso SM, Grazina M, Pratas J, Santos MJ, Januario C, Oliveira CR et al (2010) Mitochondrial-dependent apoptosis in Huntington's disease human cybrids. Exp Neurol 222:243–255
22. Humbert S, Bryson EA, Cordelieres FP, Connors NC, Datta SR, Finkbeiner S, Greenberg ME, Saudou F (2002) The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. Dev Cell 2:831–837
23. Benchoua A, Trioulier Y, Zala D, Gaillard MC, Lefort N, Dufour N, Saudou F, Elalouf JM, Hirsch E, Hantraye P et al (2006) Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin. Mol Biol Cell 17:1652–1663
24. Browne SE (2008) Mitochondria and Huntington's disease pathogenesis: insight from genetic and chemical models. Ann N Y Acad Sci 1147:358–382
25. Maksimovic ID, Jovanovic MD, Colic M, Mihajlovic R, Micic D, Selakovic V, Ninkovic M, Malicevic Z, Rusic-Stojiljkovic M, Jovicic A (2001) Oxidative damage and metabolic dysfunction in experimental Huntington's disease: selective vulnerability of the striatum and hippocampus. Vojnosanit Pregl 58:237–242
26. Pandey M, Varghese M, Sindhu KM, Sreetama S, Navneet AK, Mohanakumar KP, Usha R (2008) Mitochondrial NAD + -linked State 3 respiration and complex-I activity are compromised in the cerebral cortex of 3-nitropropionic acid-induced rat model of Huntington's disease. J Neurochem 104:420–434
27. Silva AC, Almeida S, Laco M, Duarte AI, Domingues J, Oliveira CR, Januario C, Rego AC (2013) Mitochondrial respiratory chain complex activity and bioenergetic alterations in human platelets derived from pre-symptomatic and symptomatic Huntington's disease carriers. Mitochondrion 13:801–809
28. Chakraborty J, Rajamma U, Mohanakumar KP (2014) A mitochondrial basis for Huntington's disease: therapeutic prospects. Mol Cell Biochem 389:277–291
29. Oliveira JM, Jekabsons MB, Chen S, Lin A, Rego AC, Goncalves J, Ellerby LM, Nicholls DG (2007) Mitochondrial dysfunction in Huntington's disease: the bioenergetics of isolated and in situ mitochondria from transgenic mice. J Neurochem 101:241–249
30. Brustovetsky N, LaFrance R, Purl KJ, Brustovetsky T, Keene CD, Low WC, Dubinsky JM (2005) Age-dependent changes in the calcium sensitivity of striatal mitochondria in mouse models of Huntington's Disease. J Neurochem 93:1361–1370
31. Pellman JJ, Hamilton J, Brustovetsky T, Brustovetsky N (2015) Ca(2+) handling in isolated brain mitochondria and cultured neurons derived from the YAC128 mouse model of Huntington's disease. J Neurochem 134:652–667
32. HD iPSC Consortium (2012) Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. Cell stem cell 11:264–278
33. Mochel F, N'Guyen TM, Deelchand D, Rinaldi D, Valabregue R, Wary C, Carlier PG, Durr A, Henry PG (2012) Abnormal response to cortical activation in early stages of Huntington disease. Movement disorders: official journal of the Movement Disorder Society 27:907–910
34. Lopes C, Ribeiro M, Duarte AI, Humbert S, Saudou F, Pereira de Almeida L, Hayden M, Rego AC (2014) IGF-1 intranasal administration rescues Huntington's disease phenotypes in YAC128 mice. Mol Neurobiol 49:1126–1142

35. Mochel F, Durant B, Meng X, O'Callaghan J, Yu H, Brouillet E, Wheeler VC, Humbert S, Schiffmann R, Durr A (2012) Early alterations of brain cellular energy homeostasis in Huntington disease models. J Biol Chem 287:1361–1370
36. Seong IS, Ivanova E, Lee JM, Choo YS, Fossale E, Anderson M, Gusella JF, Laramie JM, Myers RH, Lesort M et al (2005) HD CAG repeat implicates a dominant property of huntingtin in mitochondrial energy metabolism. Hum Mol Genet 14:2871–2880
37. Zhang SF, Hennessey T, Yang L, Starkova NN, Beal MF, Starkov AA (2011) Impaired brain creatine kinase activity in Huntington's disease. Neurodegener Dis 8:194–201
38. Lodi R, Schapira AH, Manners D, Styles P, Wood NW, Taylor DJ, Warner TT (2000) Abnormal in vivo skeletal muscle energy metabolism in Huntington's disease and dentatorubropallidoluysian atrophy. Ann Neurol 48:72–76
39. Naia L, Ribeiro M, Rodrigues J, Duarte AI, Lopes C, Rosenstock TR, Hayden MR, Rego AC (2016) Insulin and IGF-1 regularize energy metabolites in neural cells expressing full-length mutant huntingtin. Neuropeptides
40. Antonini A, Leenders KL, Spiegel R, Meier D, Vontobel P, Weigell-Weber M, Sanchez-Pernaute R, de Yebenez JG, Boesiger P, Weindl A et al (1996) Striatal glucose metabolism and dopamine D2 receptor binding in asymptomatic gene carriers and patients with Huntington's disease. Brain A J Neurol 119(Pt 6):2085–2095
41. Mazziotta JC, Phelps ME, Pahl JJ, Huang SC, Baxter LR, Riege WH, Hoffman JM, Kuhl DE, Lanto AB, Wapenski JA et al (1987) Reduced cerebral glucose metabolism in asymptomatic subjects at risk for Huntington's disease. The New England journal of medicine 316:357–362
42. Powers WJ, Haas RH, Le T, Videen TO, Hershey T, McGee-Minnich L, Perlmutter JS (2007) Normal platelet mitochondrial complex I activity in Huntington's disease. Neurobiology of disease 27:99–101
43. Burke JR, Enghild JJ, Martin ME, Jou YS, Myers RM, Roses AD, Vance JM, Strittmatter WJ (1996) Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. Nat Med 2:347–350
44. Jacobsen JC, Gregory GC, Woda JM, Thompson MN, Coser KR, Murthy V, Kohane IS, Gusella JF, Seong IS, MacDonald ME et al (2011) HD CAG-correlated gene expression changes support a simple dominant gain of function. Hum Mol Genet 20:2846–2860
45. Jenkins BG, Koroshetz WJ, Beal MF, Rosen BR (1993) Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized 1H NMR spectroscopy. Neurology 43:2689–2695
46. Koroshetz WJ, Jenkins BG, Rosen BR, Beal MF (1997) Energy metabolism defects in Huntington's disease and effects of coenzyme Q10. Ann Neurol 41:160–165
47. Martin WR, Wieler M, Hanstock CC (2007) Is brain lactate increased in Huntington's disease? J Neurol Sci 263:70–74
48. Tsang TM, Woodman B, McLoughlin GA, Griffin JL, Tabrizi SJ, Bates GP, Holmes E (2006) Metabolic characterization of the R6/2 transgenic mouse model of Huntington's disease by high-resolution MAS 1H NMR spectroscopy. J Proteome Res 5:483–492
49. Ferreira IL, Cunha-Oliveira T, Nascimento MV, Ribeiro M, Proenca MT, Januario C, Oliveira CR, Rego AC (2011) Bioenergetic dysfunction in Huntington's disease human cybrids. Exp Neurol 231:127–134
50. Butterworth J, Yates CM, Reynolds GP (1985) Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase and gamma-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. J Neurol Sci 67:161–171
51. Sorbi S, Bird ED, Blass JP (1983) Decreased pyruvate dehydrogenase complex activity in Huntington and Alzheimer brain. Ann Neurol 13:72–78
52. Andreassen OA, Ferrante RJ, Huang HM, Dedeoglu A, Park L, Ferrante KL, Kwon J, Borchelt DR, Ross CA, Gibson GE et al (2001) Dichloroacetate exerts therapeutic effects in transgenic mouse models of Huntington's disease. Ann Neurol 50:112–117

53. Klivenyi P, Starkov AA, Calingasan NY, Gardian G, Browne SE, Yang L, Bubber P, Gibson GE, Patel MS, Beal MF (2004) Mice deficient in dihydrolipoamide dehydrogenase show increased vulnerability to MPTP, malonate and 3-nitropropionic acid neurotoxicity. J Neurochem 88:1352–1360
54. Tabrizi SJ, Cleeter MW, Xuereb J, Taanman JW, Cooper JM, Schapira AH (1999) Biochemical abnormalities and excitotoxicity in Huntington's disease brain. Ann Neurol 45:25–32
55. Naseri NN, Xu H, Bonica J, Vonsattel JP, Cortes EP, Park LC, Arjomand J, Gibson GE (2015) Abnormalities in the tricarboxylic Acid cycle in Huntington disease and in a Huntington disease mouse model. J Neuropathol Exp Neurol 74:527–537
56. Sorolla MA, Rodriguez-Colman MJ, Tamarit J, Ortega Z, Lucas JJ, Ferrer I, Ros J, Cabiscol E (2010) Protein oxidation in Huntington disease affects energy production and vitamin B6 metabolism. Free Radic Biol Med 49:612–621
57. Vinogradov AD, Grivennikova VG (2015) Oxidation of NADH and ROS production by respiratory complex I. Biochim Biophys Acta
58. Bleier L, Drose S (2013) Superoxide generation by complex III: from mechanistic rationales to functional consequences. Biochim Biophys Acta 1827:1320–1331
59. Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417:1–13
60. Flynn JM, Melov S (2013) SOD2 in mitochondrial dysfunction and neurodegeneration. Free Radic Biol Med 62:4–12
61. Rabilloud T, Heller M, Rigobello MP, Bindoli A, Aebersold R, Lunardi J (2001) The mitochondrial antioxidant defence system and its response to oxidative stress. Proteomics 1:1105–1110
62. Hands S, Sajjad MU, Newton MJ, Wyttenbach A (2011) In vitro and in vivo aggregation of a fragment of huntingtin protein directly causes free radical production. J Biol Chem 286:44512–44520
63. Sorolla MA, Reverter-Branchat G, Tamarit J, Ferrer I, Ros J, Cabiscol E (2008) Proteomic and oxidative stress analysis in human brain samples of Huntington disease. Free Radic Biol Med 45:667–678
64. Tabrizi SJ, Schapira AH (1999) Secondary abnormalities of mitochondrial DNA associated with neurodegeneration. Biochem Soc Symp 66:99–110
65. Tabrizi SJ, Workman J, Hart PE, Mangiarini L, Mahal A, Bates G, Cooper JM, Schapira AH (2000) Mitochondrial dysfunction and free radical damage in the Huntington R6/2 transgenic mouse. Ann Neurol 47:80–86
66. Ribeiro M, Rosenstock TR, Oliveira AM, Oliveira CR, Rego AC (2014) Insulin and IGF-1 improve mitochondrial function in a PI-3 K/Akt-dependent manner and reduce mitochondrial generation of reactive oxygen species in Huntington's disease knock-in striatal cells. Free Radic Biol Med 74:129–144
67. Ribeiro M, Rosenstock TR, Cunha-Oliveira T, Ferreira IL, Oliveira CR, Rego AC (2012) Glutathione redox cycle dysregulation in Huntington's disease knock-in striatal cells. Free Radic Biol Med 53:1857–1867
68. Chae JI, Kim DW, Lee N, Jeon YJ, Jeon I, Kwon J, Kim J, Soh Y, Lee DS, Seo KS et al (2012) Quantitative proteomic analysis of induced pluripotent stem cells derived from a human Huntington's disease patient. Biochem J 446:359–371
69. Stotland A, Gottlieb RA (2015) Mitochondrial quality control: easy come, easy go. Biochim Biophys Acta 1853:2802–2811
70. Zhu J, Wang KZ, Chu CT (2013) After the banquet: mitochondrial biogenesis, mitophagy, and cell survival. Autophagy 9:1663–1676
71. Jin YN, Johnson GV (2010) The interrelationship between mitochondrial dysfunction and transcriptional dysregulation in Huntington disease. J Bioenerg Biomembr 42:199–205
72. Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci USA 97:6763–6768

73. Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D (2006) Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell 127:59–69
74. Johri A, Chandra A, Beal MF (2013) PGC-1alpha, mitochondrial dysfunction, and Huntington's disease. Free Radic Biol Med 62:37–46
75. Palikaras K, Tavernarakis N (2014) Mitochondrial homeostasis: the interplay between mitophagy and mitochondrial biogenesis. Exp Gerontol 56:182–188
76. Shirendeb UP, Calkins MJ, Manczak M, Anekonda V, Dufour B, McBride JL, Mao P, Reddy PH (2012) Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. Hum Mol Genet 21:406–420
77. Weydt P, Pineda VV, Torrence AE, Libby RT, Satterfield TF, Lazarowski ER, Gilbert ML, Morton GJ, Bammler TK, Strand AD et al (2006) Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. Cell Metab 4:349–362
78. Detmer SA, Chan DC (2007) Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol 8:870–879
79. Rosenstock TR, Brett AC, Rego AC (2012) Modified mitochondrial dynamics, turnover and function in neurodegeneration: a focus on Huntington's Disease. Cell Bioenerg Health Dis New Perspect Mitochondrial Biol, 149–194
80. Shirendeb U, Reddy AP, Manczak M, Calkins MJ, Mao P, Tagle DA, Reddy PH (2011) Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. Hum Mol Genet 20:1438–1455
81. Reddy PH, Shirendeb UP (2012) Mutant huntingtin, abnormal mitochondrial dynamics, defective axonal transport of mitochondria, and selective synaptic degeneration in Huntington's disease. Biochim Biophys Acta 1822:101–110
82. Knott AB, Perkins G, Schwarzenbacher R, Bossy-Wetzel E (2008) Mitochondrial fragmentation in neurodegeneration. Nat Rev Neurosci 9:505–518
83. Loson OC, Song Z, Chen H, Chan DC (2013) Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. Mol Biol Cell 24(5):659–667
84. Griffin EE, Detmer SA, Chan DC (2006) Molecular mechanism of mitochondrial membrane fusion. Biochim Biophys Acta 1763:482–489
85. Costa V, Giacomello M, Hudec R, Lopreiato R, Ermak G, Lim D, Malorni W, Davies KJ, Carafoli E, Scorrano L (2010) Mitochondrial fission and cristae disruption increase the response of cell models of Huntington's disease to apoptotic stimuli. EMBO Mol Med 2:490–503
86. Zinsmaier KE, Babic M, Russo GJ (2009) Mitochondrial transport dynamics in axons and dendrites. Results Probl Cell Differ 48:107–139
87. Lin MY, Sheng ZH (2015) Regulation of mitochondrial transport in neurons. Exp Cell Res 334:35–44
88. Orr AL, Li S, Wang CE, Li H, Wang J, Rong J, Xu X, Mastroberardino PG, Greenamyre JT, Li XJ (2008) N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. J Neurosci Off J Soc Neurosci 28:2783–2792
89. Trushina E, Dyer RB, Badger JD 2nd, Ure D, Eide L, Tran DD, Vrieze BT, Legendre-Guillemin V, McPherson PS, Mandavilli BS et al (2004) Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. Mol Cell Biol 24:8195–8209
90. Chang TWD, Rintoul GL, Pandipati S, Reynolds IJ (2006) Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. Neurobiol Dis 22(2): 388–400
91. Twig G, Shirihai OS (2011) The interplay between mitochondrial dynamics and mitophagy. Antioxid Redox Signal 14:1939–1951

92. Allen GF, Toth R, James J, Ganley IG (2013) Loss of iron triggers PINK1/Parkin-independent mitophagy. EMBO Rep 14:1127–1135
93. Strappazzon F, Nazio F, Corrado M, Cianfanelli V, Romagnoli A, Fimia GM, Campello S, Nardacci R, Piacentini M, Campanella M et al (2015) AMBRA1 is able to induce mitophagy via LC3 binding, regardless of PARKIN and p62/SQSTM1. Cell Death Differ 22:517
94. Chu CT, Ji J, Dagda RK, Jiang JF, Tyurina YY, Kapralov AA, Tyurin VA, Yanamala N, Shrivastava IH, Mohammadyani D et al (2013) Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat Cell Biol 15:1197–1205
95. Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, Loh SH, Renton AE, Harvey RJ, Whitworth AJ, Martins LM et al (2011) PINK1 cleavage at position A103 by the mitochondrial protease PARL. Hum Mol Genet 20:867–879
96. Greene AW, Grenier K, Aguileta MA, Muise S, Farazifard R, Haque ME, McBride HM, Park DS, Fon EA (2012) Mitochondrial processing peptidase regulates PINK1 processing, import and Parkin recruitment. EMBO Rep 13:378–385
97. Okatsu K, Kimura M, Oka T, Tanaka K, Matsuda N (2015) Unconventional PINK1 localization to the outer membrane of depolarized mitochondria drives Parkin recruitment. J Cell Sci 128:964–978
98. Kazlauskaite A, Martinez-Torres RJ, Wilkie S, Kumar A, Peltier J, Gonzalez A, Johnson C, Zhang J, Hope AG, Peggie M et al (2015) Binding to serine 65-phosphorylated ubiquitin primes Parkin for optimal PINK1-dependent phosphorylation and activation. EMBO Rep 16:939–954
99. Narendra D, Tanaka A, Suen DF, Youle RJ (2008) Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol 183:795–803
100. Khalil B, El Fissi N, Aouane A, Cabirol-Pol MJ, Rival T, Lievens JC (2015) PINK1-induced mitophagy promotes neuroprotection in Huntington's disease. Cell Death Dis 6:e1617
101. Wong YC, Holzbaur EL (2014) The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. J Neurosci Off J Soc Neurosci 34:1293–1305
102. Rui YN, Xu Z, Patel B, Chen Z, Chen D, Tito A, David G, Sun Y, Stimming EF, Bellen HJ et al (2015) Huntingtin functions as a scaffold for selective macroautophagy. Nat Cell Biol 17:262–275
103. Sun N, Yun J, Liu J, Malide D, Liu C, Rovira II, Holmstrom KM, Fergusson MM, Yoo YH, Combs CA et al (2015) Measuring In Vivo Mitophagy. Mol Cell 60:685–696
104. Kuwana T, Newmeyer DD (2003) Bcl-2-family proteins and the role of mitochondria in apoptosis. Curr Opin Cell Biol 15:691–699
105. Parsons MJ, Green DR (2010) Mitochondria in cell death. Essays Biochem 47:99–114
106. Tait SW, Green DR (2012) Mitochondria and cell signalling. J Cell Sci 125:807–815
107. Culmsee C, Mattson MP (2005) p53 in neuronal apoptosis. Biochem Biophys Res Commun 331:761–777
108. Duan W, Peng Q, Masuda N, Ford E, Tryggestad E, Ladenheim B, Zhao M, Cadet JL, Wong J, Ross CA (2008) Sertraline slows disease progression and increases neurogenesis in N171-82Q mouse model of Huntington's disease. Neurobiol Dis 30:312–322
109. Ju TC, Chen HM, Lin JT, Chang CP, Chang WC, Kang JJ, Sun CP, Tao MH, Tu PH, Chang C et al (2011) Nuclear translocation of AMPK-alpha1 potentiates striatal neurodegeneration in Huntington's disease. J Cell Biol 194:209–227
110. Kong PJ, Kil MO, Lee H, Kim SS, Johnson GV, Chun W (2009) Increased expression of Bim contributes to the potentiation of serum deprivation-induced apoptotic cell death in Huntington's disease knock-in striatal cell line. Neurol Res 31:77–83
111. Leon R, Bhagavatula N, Ulukpo O, McCollum M, Wei J (2010) BimEL as a possible molecular link between proteasome dysfunction and cell death induced by mutant huntingtin. Eur J Neurosci 31:1915–1925

112. Sassone J, Colciago C, Marchi P, Ascardi C, Alberti L, Di Pardo A, Zippel R, Sipione S, Silani V, Ciammola A (2010) Mutant Huntingtin induces activation of the Bcl-2/adenovirus E1B 19-kDa interacting protein (BNip3). Cell Death Dis 1:e7
113. Garcia-Martinez JM, Perez-Navarro E, Xifro X, Canals JM, Diaz-Hernandez M, Trioulier Y, Brouillet E, Lucas JJ, Alberch J (2007) BH3-only proteins Bid and Bim(EL) are differentially involved in neuronal dysfunction in mouse models of Huntington's disease. J Neurosci Res 85:2756–2769
114. Hansson O, Castilho RF, Korhonen L, Lindholm D, Bates GP, Brundin P (2001) Partial resistance to malonate-induced striatal cell death in transgenic mouse models of Huntington's disease is dependent on age and CAG repeat length. J Neurochem 78:694–703
115. Teles AV, Rosenstock TR, Okuno CS, Lopes GS, Bertoncini CR, Smaili SS (2008) Increase in bax expression and apoptosis are associated in Huntington's disease progression. Neurosci Lett 438:59–63
116. Chiang MC, Chen CM, Lee MR, Chen HW, Chen HM, Wu YS, Hung CH, Kang JJ, Chang CP, Chang C et al (2010) Modulation of energy deficiency in Huntington's disease via activation of the peroxisome proliferator-activated receptor gamma. Hum Mol Genet 19:4043–4058
117. Zhang Y, Ona VO, Li M, Drozda M, Dubois-Dauphin M, Przedborski S, Ferrante RJ, Friedlander RM (2003) Sequential activation of individual caspases, and of alterations in Bcl-2 proapoptotic signals in a mouse model of Huntington's disease. J Neurochem 87:1184–1192
118. Goffredo D, Rigamonti D, Zuccato C, Tartari M, Valenza M, Cattaneo E (2005) Prevention of cytosolic IAPs degradation: a potential pharmacological target in Huntington's Disease. Pharmacol Res 52:140–150
119. Rosenstock TR, de Brito OM, Lombardi V, Louros S, Ribeiro M, Almeida S, Ferreira IL, Oliveira CR, Rego AC (2011) FK506 ameliorates cell death features in Huntington's disease striatal cell models. Neurochem Int 59:600–609
120. Vis JC, Schipper E, de Boer-van Huizen RT, Verbeek MM, de Waal RM, Wesseling P, ten Donkelaar HJ, Kremer B (2005) Expression pattern of apoptosis-related markers in Huntington's disease. Acta Neuropathol 109:321–328
121. Ciammola A, Sassone J, Alberti L, Meola G, Mancinelli E, Russo MA, Squitieri F, Silani V (2006) Increased apoptosis, Huntingtin inclusions and altered differentiation in muscle cell cultures from Huntington's disease subjects. Cell Death Differ 13:2068–2078
122. Graham RK, Deng Y, Carroll J, Vaid K, Cowan C, Pouladi MA, Metzler M, Bissada N, Wang L, Faull RL et al (2010) Cleavage at the 586 amino acid caspase-6 site in mutant huntingtin influences caspase-6 activation in vivo. J Neurosci 30:15019–15029
123. Graham RK, Deng Y, Slow EJ, Haigh B, Bissada N, Lu G, Pearson J, Shehadeh J, Bertram L, Murphy Z et al (2006) Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. Cell 125:1179–1191
124. Hermel E, Gafni J, Propp SS, Leavitt BR, Wellington CL, Young JE, Hackam AS, Logvinova AV, Peel AL, Chen SF et al (2004) Specific caspase interactions and amplification are involved in selective neuronal vulnerability in Huntington's disease. Cell Death Differ 11:424–438
125. Warby SC, Doty CN, Graham RK, Carroll JB, Yang YZ, Singaraja RR, Overall CM, Hayden MR (2008) Activated caspase-6 and caspase-6-cleaved fragments of huntingtin specifically colocalize in the nucleus. Hum Mol Genet 17:2390–2404
126. Tebbenkamp AT, Green C, Xu G, Denovan-Wright EM, Rising AC, Fromholt SE, Brown HH, Swing D, Mandel RJ, Tessarollo L et al (2011) Transgenic mice expressing caspase-6-derived N-terminal fragments of mutant huntingtin develop neurologic abnormalities with predominant cytoplasmic inclusion pathology composed largely of a smaller proteolytic derivative. Hum Mol Genet 20:2770–2782

127. Waldron-Roby E, Ratovitski T, Wang X, Jiang M, Watkin E, Arbez N, Graham RK, Hayden MR, Hou Z, Mori S et al (2012) Transgenic mouse model expressing the caspase 6 fragment of mutant huntingtin. J Neurosci 32:183–193
128. Gafni J, Papanikolaou T, Degiacomo F, Holcomb J, Chen S, Menalled L, Kudwa A, Fitzpatrick J, Miller S, Ramboz S et al (2012) Caspase-6 activity in a BACHD mouse modulates steady-state levels of mutant huntingtin protein but is not necessary for production of a 586 amino acid proteolytic fragment. J Neurosci 32:7454–7465
129. Landles C, Weiss A, Franklin S, Howland D, Bates G (2012) Caspase-6 does not contribute to the proteolysis of mutant huntingtin in the HdhQ150 knock-in mouse model of Huntington's disease. PLoS Curr 4, e4fd085bfc9973

Chapter 4
RNA Related Pathology in Huntington's Disease

Andreas Neueder and Gillian P. Bates

Abstract This chapter summarises research investigating the expression of huntingtin sense and anti-sense transcripts, the effect of the mutation on huntingtin processing as well as the more global effect of the mutation on the coding and non-coding transcriptomes. The huntingtin gene is ubiquitously expressed, although expression levels vary between tissues and cell types. A SNP that affects NF-κB binding in the huntingtin promoter modulates the expression level of huntingtin transcripts and is associated with the age of disease onset. Incomplete splicing between exon 1 and exon 2 has been shown to result in the expression of a small polyadenylated mRNA that encodes the highly pathogenic exon 1 huntingtin protein. This occurs in a CAG-repeat length dependent manner in all full-length mouse models of HD as well as HD patient post-mortem brains and fibroblasts. An anti-sense transcript to huntingtin is generated that contains a CUG repeat that is expanded in HD patients. In myotonic dystrophy, expanded CUG repeats form RNA foci in cell nuclei that bind specific proteins (e.g. MBL1). Short, pure CAG RNAs of approximately 21 nucleotides that have been processed by DICER can inhibit the translation of other CAG repeat containing mRNAs. The HD mutation affects the transcriptome at the level of mRNA expression, splicing and the expression of non-coding RNAs. Finally, expanded repetitive stretched of nucleotides can lead to RAN translation, in which the ribosome translates from the expanded repeat in all possible reading frames, producing proteins with various poly-amino acid tracts. The extent to which these events contribute to HD pathogenesis is largely unknown.

Keywords Huntingtin transcripts · Antisense RNA · Non-coding RNA
Huntingtin splicing · RAN translation

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Huntington's disease (HD) was first described in 1872 by George Huntington as a 'dancing' disorder due to involuntary movements, the chorea being the most obvious motoric symptom [1]. However, HD had been known since the Middle Ages under the name 'St. Vitas dance'. This name also included dancing and St. Vitas, the saint to whom people prayed for help. Their grimaces, the chorea and mental impairments were the reasons people believed HD patients were possessed by the devil. Some of the victims burned as witches in the Middle Ages were probably suffering from HD.

The discoveries of DNA and genes as units of hereditary information were milestones that ultimately led to the identification of a marker on chromosome 4 linked to HD in 1983 [2]. This discovery allowed the eventual identification of the disease causing gene in a large-scale collaborative effort a decade later in 1993 [3]. The publication describes an unstable CAG trinucleotide expansion in the huntingtin gene (*HTT*) and shows that this expansion is the sole genetic cause of HD. Genetic testing in the following years established that patients with a repeat length of 40 or more CAGs inevitably develop HD, with a mean age of onset in the 40 s. There is also a more severe, fast progressing form of HD with an age of onset in childhood or adolescence caused by longer CAG repeats in the juvenile onset range.

The CAG repeat encodes for a stretch of glutamines (polyQ) in the HTT protein. This polyQ tract in turn leads to the appearance of proteinaceous, very large aggregates, the so called inclusion bodies, a pathological hallmark in a variety of diseases. A wide variety of proteins co-localise with these aggregates and the proteins that are sequestered are most probably not able to fulfil their normal role in cell homeostasis. The observation of aggregates forming and the absorption of other proteins into those led to the hypothesis of a 'toxic gain of function' of mutant huntingtin. In contrast to this, the poly-glutamine stretch could disrupt the normal function of HTT leading to a 'loss of function' [4].

There were very early indications that N-terminal fragments of HTT, that include the polyQ tract, were an integral unit of the aggregates. In *post mortem* HD brains nuclear aggregates could only be detected with antibodies against the N-terminus of HTT [5, 6]. Furthermore, when aggregated proteins were released by formic acid treatment, N-terminal fragments could also be identified [7]. In cell models HTT can be cleaved to release the small N-terminal fragments cleavage products (cp)-A and (cp-B) [7] or cp-1 and cp-2 [8, 9]. A comprehensive study in a knock-in mouse model revealed 14 N-terminal fragments of HTT [10], the smallest of which was encoded by exon 1 of the *Htt* gene. Exon 1 HTT is extremely aggregation prone and is the most toxic species of naturally occurring HTT fragments [11].

4.1 Regulation of *HTT* Transcription

Although *HTT* is ubiquitously expressed [12], neuronal tissue is especially vulnerable in HD. A major determining factor of protein expression is the promoter driving expression of the respective gene. A difference in the promoter activity and

by this expression levels of *HTT*, which in turn would lead to higher concentration of the mutated protein and an increase in disease burden, could be one explanation for this selective vulnerability. We can deduce from the functional expression of the exon 1 *HTT* transgene in the R6/mouse model that an upstream sequence of approximately 1 kilobase of the *HTT* gene is sufficient to induce transcription of the transgene in an animal model [13]. The human *HTT* promoter has a CpG island [14], lacks the canonical TATA and CAAT boxes, but retains the SP1, AP-2 and AP-4 core transcription factor binding sites [15]. An in vitro analysis of different *HTT* promoter constructs driving a luciferase assay revealed a selectively higher reporter signal in a neuronal versus a non-neuronal cell line [16]. A recent study identified an additional NF-κB binding site in the *HTT* promoter and could show that a single nucleotide polymorphism (SNP) in this binding site correlated with the age of disease onset [17]. Reduced binding of NF-κB to the promoter due to the transition of a G to A led to reduced expression of HTT and a delayed onset of HD. Additional detailed studies of the interplay of transcription factors inducing expression of *HTT*, identification and analysis of possible enhancer regions of the *HTT* gene, as well as identification of other cis and trans acting factors influencing expression of *HTT* could lead to the development of new therapeutic avenues in the future. As proof-of-principle, decreasing the levels of NF-κB resulted in lower levels of *HTT* expression [17]. One could also imagine allele specific *HTT* lowering therapies by targeting SNPs [18, 17].

CAG trinucleotide expansions have the propensity to form highly stable self-complementary structures. Newly synthesised mRNA could interact with the DNA template and thus might require additional DNA-dependent RNA polymerase II (PolI) subunits for efficient transcription. These DNA/RNA hybrid structures are called R-loops, which play an important role e.g. in transcription regulation, DNA replication and genome stability including repeat instability. They have already been shown to exist in several repeat expansion diseases where it was proposed that they induce gene silencing [19, 20]. In HD, R-loops have not yet been identified. However, the yeast transcription factor Spt4 and its mammalian homologue Supt4 h were found to be required for efficient transcription through the CAG repeat [21]. Inhibition of this transcription factor led to reduced expression of *Htt*, while the transcription of other genes seemed to be largely unaffected.

4.2 Alternative Splice Forms of the *HTT* mRNA

Perhaps the most striking evidence for the extreme pathogenicity of the exon 1 HTT protein as compared to other HTT fragments, is the rapidly progressing phenotype displayed by the R6/mouse lines [13], in particular line R6/2. These were created by transgene integration into the mouse genome; the transgene consisting of about 1 kb of the human *HTT* promoter, human *HTT* exon 1 with an elongated CAG repeat and part of human *HTT* intron 1. R6/2 mice have been shown to develop very comparable molecular and phenotypic signatures at later stages of disease

progression when compared to full-length knock-in mouse models such as the *Hdh*Q150 mice [22], in which an expanded CAG repeat is integrated into the full-length endogenous mouse gene [23]. However, the length of time to reach the end stage of the disease is more than 20 months in homozygous *Hdh*Q150 mice as compared to approximately 14 weeks in the R6/2 model. The comparably higher levels of exon 1 HTT in the R6/2 mice, as compared to that of HTT fragments in the knock-in lines, was therefore sufficient to cause this vastly accelerated disease progression.

When exon 1 HTT was first detected in brain lysates from knock-in mice, it was assumed that it was generated through proteolytic cleavage of full-length HTT, similar to the generation of other HTT fragments [10]. However, more recent evidence showed that exon 1 HTT is generated through the incomplete splicing of the *HTT* mRNA [24]. In all full-length knock-in mouse models of HD with pathogenic repeat sizes, a transcript could be detected that consists of *Htt* exon 1 and a few hundred base pairs of intron 1 (HTT exon 1 mRNA). The production of the HTT exon 1 transcript was shown to be CAG repeat length dependent, with longer repeats producing higher levels of HTT exon 1 mRNA. The authors further showed that a general, serine, arginine rich splicing factor (SRSF6) bound tightly to the elongated CAG repeat. Serine, arginine rich splicing factors interact with U1 RNA containing small nuclear spliceosomal ribonucleoprotein complexes (U1 snRNP), also parts of the general splicing machinery [25]. U1 snRNP, in addition to initiating the formation of the spliceosome at the 5′ splice site, protects cryptic polyadenylation sites (polyA sites) from being recognised and thus inhibits the formation of aberrantly spliced, shorter transcripts [26]. A shortage in the levels of U1 snRNP therefore leads to the generation of shorter mRNAs, a phenomenon that can be observed during organismal development [27]. However, in a fully developed organism, generation of these aberrant transcripts is in the best case unproductive leading to mRNA decay, or in the worst case produces the message for toxic proteins, as is the case in HD. The increased binding of SRSF6 to the mutated CAG repeats very likely depletes the local pool of U1 snRNP by sequestering the spliceosomal component. This in turn could inhibit the formation of the spliceosome at the 5′ splice site and not mutually exclusive, also expose cryptic polyA sites in *HTT* intron 1. The reduced efficiency in splicing and the partly exposed cryptic polyA sites open a kinetic window in which cleavage and polyadenylation factors can recognise the normally protected sites in *HTT* intron 1, cleave and synthesise a polyA tail to generate a functional 3′ mRNA end. Moreover, the HTTexon 1 transcript was shown to be associated with poly-ribosomes indicating nuclear export and functional integrity. Finally, the authors showed that in all mouse models that produced the small transcript, the presence of an exon 1 HTT protein could be detected.

The incomplete splicing of the *Htt* message to create the HTT exon 1 mRNA is not limited to the mouse *Htt* gene. There are several mouse models of HD in which the mouse sequence of exon 1 and short sequences of intron 1 have been exchanged with their human counterparts. These chimeric models are based on two genetic constructs, which slightly differ in the amount of human *HTT* intron 1 insertion/

deletion of mouse *Htt* intron 1 [28–30]. Independently of these small differences, an incompletely spliced HTT exon 1 mRNA and exon 1 HTT protein was detected in all chimeric models with pathogenic repeat sizes [24]. Taking it one step further to the full length human gene, the authors also analysed BAC (bacterial artificial chromosome) and YAC (yeast artificial chromosome) mouse models of HD. Both models express a full length copy of the human *HTT* gene from artificial chromosomes that also includes large up- and downstream sequences. Strikingly, the same transcript of *HTT* exon 1 and partial intron 1 could also be found in these models. To exclude that the generation of the HTT exon 1 mRNA for some strange reason was restricted to mouse models of HD, Sathasivam et al. analysed samples from human HD patients. They identified usage of the cryptic polyA site in HTT intron 1 for patient derived fibroblasts and *post mortem* brain samples. Intronic sequences, consistent with the incomplete splicing of exon 1 to exon 2 have recently been detected in *post mortem* brain samples and fibroblast lines from juvenile HD patients (Neueder et al. unpublished).

In addition to the generation of HTT exon 1 mRNA through incomplete splicing, novel isoforms of mouse and human *HTT* have been identified [31–33]. These murine isoforms seem to be ubiquitously expressed and either lack exon 28 or 29, or retain part of intron 28. Interestingly, the *Htt* isoform lacking exon 29 was under-represented in the cerebellum of the *Hdh*Q150 HD mouse model [31]. Various isoforms have been identified in human brains [32] and cell lines [33]. The differences from the canonical full length HTT mRNA were: inclusion and exclusion of exons, retention of intronic sequences and inclusion of a novel hominid specific exon. Furthermore, the authors used protein homology modelling of the novel isoforms and suggested that the splice changes would lead to loss of protein-protein interactions and potential alterations in post-translational modifications of HTT. In any case, the impact of the respective isoforms on HD pathogenesis needs to be determined.

4.3 Implications of Aberrant Splicing of the *HTT* mRNA

These findings have major implications for our understanding of the molecular mechanisms that initiate the occurrence of HD symptoms. The HTT exon 1 mRNA that is present in all mouse models, as well as in human patients, is almost identical to the transgene expressed in the R6/mouse lines. Given the very rapid progression of the phenotype in these mouse models, the production of exon 1 HTT through the generation of an incompletely spliced *HTT* message might be expected to make a significant contribution to disease onset and progression in humans. One of the main questions which need to be answered is therefore: how much does generation of exon 1 HTT contribute to HD pathogenesis [34]? Novel mouse models in which the production of exon 1 HTT is inhibited or increased will certainly help to answer this question. It will also be very interesting to see if there is a correlation of HTT exon 1 mRNA levels in human patients with age of disease onset, rate of disease

progression, or severity of symptoms. Additional factors acting in trans, which could influence the rate of HTT exon 1 mRNA production, might be uncovered by genome wide association studies (GWAS). The first high powered GWAS analysis in HD found a locus on chromosome 15, which could either hasten or delay, and a locus on chromosome 8, which hastened the age of onset of HD (Genetic Modifiers of Huntington's Disease [35]. Additionally, in their analysis of over-represented pathways, the authors found that DNA maintenance and repair mechanisms were enriched. It has been shown that these pathways influence somatic instability of the CAG repeat, with striatum and cortex, the two most affected tissues in HD, showing the largest instability [36]. Since the production of HTT exon 1 mRNA is clearly CAG repeat length dependent [24], somatic instability would enhance the generation of exon 1 HTT.

One of the most promising strategies to counter HD is to lower HTT levels, either by targeting both the normal and mutated alleles, or preferably only the levels of the mutated allele [37, 38]. There are mounting data to suggest that by lowering HTT levels many phenotypical symptoms in HD model systems can be improved [39–42]. Lowering HTT levels in a non-allele specific way seemed to be well tolerated for at least 6 months in a non-human primate [43]. The first clinical trial to lower mutant huntingtin levels with an antisense oligonucleotide was initiated during 2015 (Ionis Pharmaceuticals in collaboration with Roche). However, these strategies do not target the production of the HTT exon 1 mRNA. While a reduction of mutated HTT in general has therapeutic potential, inhibiting the generation of exon 1 HTT and by this the probable source for nucleation of aggregation might have an even greater therapeutic value.

4.4 Antisense Transcription from the *HTT* Locus

Naturally occurring antisense transcription is a very common phenomenon acting as an important transcriptional regulator [44]. An antisense RNA (*HTTAS*), which is produced from the *HTT* locus and was identified in human brain tissue, influences sense *HTT* transcript levels [45]. Chung et al. showed that *HTTAS* is comprised of 3 exons, with the transcription start site at +300 base pairs relative to the transcription start site of the *HTT* gene. In addition *HTTAS* contains a 5′-cap and is polyadenylated, giving it all the essential features of a mature mRNA. The antisense RNA is differentially spliced into two isoforms: a longer one including the first exon, which is mostly complementary to *HTT* exon 1 including the CAG repeat, and a shorter one without this exon. Interestingly, only the longer isoform was able to repress *HTT* transcription in a reporter cell line in a CAG repeat length dependent manner. Its levels were also reduced in the frontal cortex of HD patients. The long isoform of HTTAS contains a CUG repeat, the same repeat as in patients of myotonic dystrophy type 1 and spinocerebellar ataxia type 8. These CUG repeats sequester proteins like CUG-BP (hNab50) [46] and prominently MBNL1 [47, 48] resulting in the inhibition of its function and induction of wide spread pathogenic

splicing changes [49]. RNA binding proteins like MBNL1 that bind to double stranded RNA hairpins recognize an RNA structure rather than a defined RNA sequence [50]. CAG repeats, as CUG repeats, seem to adopt a double stranded structure and were shown to also co-localize with MBNL1 [51, 52]. A comparison of splicing changes between *myotonic* dystrophy type 1 and induction of changes through expression of long CAG repeat containing reporter RNAs revealed a high similarity in the cellular response to both [53]. The existing evidence of RNA binding proteins being sequestered to the mutated CAG repeat in *HTT* suggests that there is a high probability of additional factors being involved. These experiments will certainly shed further light on the possibility of HD belonging to the class of 'RNAopathies' [54].

4.5 Small RNAs Are Produced from the *HTT* mRNA

Cellular compartmentalization and transport between these compartments constitutes an important regulatory layer in cellular homeostasis. Full length HTT mRNA, as well as HTT exon 1 mRNA are exported from the nucleus and are translated in the cytoplasm. However, elongated CAG repeat containing reporter RNAs [51, 53], *as well as full length HTT RNA* can form nuclear foci [51, 55]. These nuclear foci co-localize with MBNL1 and by increasing the levels of MBNL1, the amount of retention of HTT in the nucleus is increased as well. Higher levels of U2AF2, part of the U2AF splicing RNP, counteract this phenomenon and lead to more efficient export of *HTT* mRNA [55]. Whether these nuclear RNA foci are a quality control step in the maturation of the *HTT* mRNA, or whether they are a pathological feature induced by the elongated CAG repeat still needs to be clarified. Once the *HTT* mRNA is exported it can be engaged by Dicer, which normally processes double stranded (ds) pre-RNA into short (21-24 nucleotides) ds-RNAs that regulate gene expression [56]. Dicer recognizes the secondary structure of the elongated CAG repeat, which resembles the hairpin structure of its normal substrates [57]. It then processes the elongated CAG repeat into short, pure CAG RNAs of about 21 nucleotide length (sCAG RNAs) in a CAG repeat length dependent manner [58]. The authors could furthermore show that the levels of these sCAG RNAs were increased in post mortem brain tissue of HD patients. Since these sCAG RNAs potentially act as transcriptional repressors of other CAG containing mRNAs in the cell they can induce cytotoxicity. It is also conceivable that they are part of a feedback loop reducing the expression of *HTT* itself.

4.6 Mutant *HTT* Affects General Splicing

Transcriptome wide dysregulation is one of the hallmark features of HD and has been extensively studied in HD models and *post mortem* HD brains [59]. Historically, the striatum has been considered to be the most affected tissue.

However, transcriptional dysregulation is qualitatively very similar between different tissues (caudate nucleus, frontal cortex and cerebellum) in *post mortem* brains of HD patients, although its effect size is different [54]. An interesting possibility how mutant HTT could induce transcriptional dysregulation has been raised by the group of Jang-Ho Cha [60]. They found that HTT was bound to promoter DNA in a CAG repeat length dependent manner. Although wild type HTT also occupied some promoters, mutant HTT bound more strongly and to promoters of different genes. The elongated polyQ region is sufficient to confer DNA binding competence on exon 1 of HTT. The DNA binding competence seems to distort DNA structure, as well as interfere with the binding of other transcription factors.

As mentioned before, abnormal binding of the general splicing factor SRSF6 to the elongated CAG repeat is an important step in the induction of incomplete splicing of *HTT* mRNA and production of exon 1 HTT. Furthermore, MBNL1 and possibly U2AF2 bind to the elongated CAG repeat and regulate the localization of *HTT* mRNA. It is therefore easy to imagine that the mutant transcript sequesters additional RNA binding factors leading to attenuation of their function and eventually splicing changes. SRSF6 is, besides its role in *HTT* splicing, is implicated in the alternative splicing of tau where it regulates the inclusion of exon 10 coding for the fourth microtubule binding domain of the microtubule associated protein tau (MAPT, henceforth tau) [61]. Tau is a protein that is essential to stabilize microtubules, especially in the brain and its function is closely tied into its phosphorylation state [62]. There is a large, diverse group of diseases linked to the function and dysfunction of tau, the tauopathies, in which Alzheimer's disease is the most prominent one. In most tauopathies, toxicity is induced by tau forming self-propagating fibrils. However, dysregulation of its microtubule stabilizing function also leads to disease. The four repeat tau isoforms (tau 4R) exhibit a higher propensity to stabilize microtubules than the three repeat iso forms (tau 3R). A switch in the ratio of the 3R to 4R isoforms is sufficient to cause neurodegeneration [63]. This switch could contribute to disease pathogenesis in HD, as a greater amount of the four repeat isoforms and filamentous tau structures were observed in the striatum and cortex of HD patients [64]. Furthermore, it has recently been shown that an aberrant interaction of tau with mutated HTT in vitro and in HD mouse models led to hyperphosphorylation and mis-localization of tau [65]. Intriguingly, the same phenomenon has been observed in HD patients [66]. The authors also proposed that mutations in the *MAPT* gene are a clinical risk factor for increased disease progression as measured by cognitive decline; however, not for the age of onset of HD.

An indirect way of analysing the dysregulation of splicing changes and identifying the causative factors is motif analysis of transcriptome wide alternative splicing events [67]. Lin and colleagues used transcriptome wide sequencing data from the BA4 region of HD patients and controls to map alternative splicing events in HD. Bioinformatics analysis revealed a list of 15 potential regulatory factors,

including SRSF6, many of which are themselves dysregulated in HD. Another factor, polypyrimidine tract binding protein 1 (PTBP1), involved in repression of neuronal specific genes, was transcriptionally dysregulated in grade 2 [68] HD brains.

4.7 Mutant HTT Affects the Balance of Non-coding RNAs

In addition to the gain of novel interactions through the mutated polyQ domain, mutant huntingtin also loses the capability to bind to some of its normal interacting proteins. One example is the loss of interaction with REST (R element-1 silencing transcription factor) [69, 70]. REST is usually kept in an inactive state in the cytoplasm through its sequestration into a complex including wild type HTT [71, 72]. The loss of binding to the inhibitory complex leads to translocation of REST into the nucleus and repression of a variety of neuronal genes, amongst those e.g. neurotrophic factors like brain derived neurotrophic factor (BDNF) [73, 70]. Ever since the identification of BDNF dysregulation in HD [74], it has been a prominent target of investigations, partly because it could explain the higher vulnerability of neurons observed in HD. Increasing the levels of BDNF in the forebrain [75, 76] and inhibiting REST [77, 78] has conferred some therapeutic value in models of HD.

Abnormal regulation of REST is also linked to changes in microRNA (miRNA) levels in HD [79, 80]. MicroRNAs are non-coding RNAs that can bind to other transcripts and induce degradation of the miRNA/RNA hybrid, thus representing an important post-transcriptional regulatory mechanism [81]. They are important regulators of a variety of genes that are dysregulated in HD, e.g. genes encoding for synaptic proteins [82]. Dysregulation of various miRNA species has been observed in HD mouse models [83], monkeys [84] and more recently in human *post mortem* brains [85–87] and in peripheral tissue [88, 89]. However, maybe due to technical difficulties, or representing a real variance in the expression levels of the population, the overlap between the individual studies is only small and in need of further evaluation.

MicroRNAs and other small non-coding RNAs need to be in a protein complex with argonaute proteins to be catalytically active [90]. A pull-down experiment of wild type or mutated HTT expressed in a HELA cell line showed co-purification of two argonaute proteins (AGO1, AGO2) independent of the elongated CAG repeat [91]. Moreover, HTT co-localized with AGO2 in cytoplasmic foci, so called processing-bodies or p-bodies. P-bodies are a rendezvous point for ribonucleoprotein complexes and accessory factors playing a role in mRNA surveillance, degradation or silencing [92]. Despite its co-localization with p-bodies, the possible roles of HTT for p-body assembly, function or mRNA stability remain unknown.

4.8 Mutant *HTT* Affects Translation

Interestingly, the expanded CAG repeat in the *HTT* mRNA can act as a binding platform for a protein complex that enhances the translation of the mutant mRNA [93]. This regulatory complex consists of midline 1 (MID1), protein phosphatase 2A (PPP2A) and ribosomal protein S6 kinase (S6 K). Krauss and colleagues could show that binding of this complex was CAG repeat length dependent and its stimulatory effect on translation increased with CAG repeat length. Furthermore, a knockdown of MID1 resulted in decreased protein levels of mutant HTT, suggesting that targeting this mechanism could prove to be a valuable therapeutic approach. Expanded, repetitive stretches of nucleotides confuse the translation machinery in a fascinating way. They lead to so called repeat associated non-ATG (RAN) translation, where the ribosome starts translating from the expanded repeat in all possible reading frames creating proteins with different amino-acid expansions [94]. This phenomenon was discovered in spinocerebellar ataxia type 8 [95], but has now been described for a multitude of repeat expansion diseases [94], including for HD very recently [96]. The group could show that all four possible RAN proteins—polyAla (sense and antisense), polySer (sense), polyCys (antisense) and polyLeu (antisense)—were present in post mortem brain tissue of HD patients and a HD mouse model (N171 [97]; expresses the first 171 amino acids of human HTT with about 82 glutamines. The production was CAG repeat length dependent and the expression levels correlated with the severity of HD symptoms in the different tissues. Since several of these RAN proteins are implicated in disease [94] and some of them like polyAla show higher toxicity than polyGln in cell models [96], it is likely that they also contribute to HD pathogenesis. The extent of their contribution however, needs to be addressed.

4.9 Summary

Classically, polyglutamine expansion diseases like HD are categorized as ‘proteinopathies’, disorders in which abnormally folded proteins cause the disease by loss-of-function and/or gain-of-toxic-function mechanisms. It is without doubt that pathological symptoms of HD are caused by the mutant HTT protein, however, as described in this book chapter, HD shares some features with disorders in which the pathology is caused by mutations in non-coding regions, in particular through RNA gain-of-toxic-function mechanisms. Members of these ‘RNAopathies’ include myotonic dystrophy type 1 (DM1) and type 2 (DM2) [98] and the repeat expansion in *C9orf72*, which is the most common cause of familial and sporadic ALS and frontotemporal lobar degeneration [99, 100]. An unusual feature in HD is that *HTT* pre-mRNA splicing itself is altered in a way that multiple shorter versions of the HTT are produced; amongst these, exon 1 HTT is the most toxic N-terminal fragment. Understanding the underlying molecular mechanisms of RNA related

pathology in HD will certainly help to gain novel insights into the processes that cause and drive the disease. In the future these findings could then help to design new drugs and avenues of clinical intervention to treat Huntington's disease.

References

1. Huntington G (2003) On chorea. George Huntington, M.D. J Neuropsychiatry Clinical Neurosciences 15(1):109–112
2. Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY et al (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306(5940):234–238
3. The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Huntington's Dis Collaborative Res Group. Cell 72(6):971–983
4. Cattaneo E, Zuccato C, Tartari M (2005) Normal huntingtin function: an alternative approach to Huntington's disease. Nat Rev Neurosci 6(12):919–930
5. DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277(5334):1990–1993
6. Schilling G, Klevytska A, Tebbenkamp AT, Juenemann K, Cooper J, Gonzales V, Slunt H, Poirer M, Ross CA, Borchelt DR (2007) Characterization of huntingtin pathologic fragments in human Huntington disease, transgenic mice, and cell models. J Neuropathol Exp Neurol 66(4):313–320
7. Lunkes A, Lindenberg KS, Ben-Haiem L, Weber C, Devys D, Landwehrmeyer GB, Mandel JL, Trottier Y (2002) Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. Mol Cell 10(2):259–269
8. Ratovitski T, Gucek M, Jiang H, Chighladze E, Waldron E, D'Ambola J, Hou Z, Liang Y, Poirier MA, Hirschhorn RR, Graham R, Hayden MR, Cole RN, Ross CA (2009) Mutant huntingtin N-terminal fragments of specific size mediate aggregation and toxicity in neuronal cells. J Biol Chem 284(16):10855–10867
9. Ratovitski T, Nakamura M, D'Ambola J, Chighladze E, Liang Y, Wang W, Graham R, Hayden MR, Borchelt DR, Hirschhorn RR, Ross CA (2007) N-terminal proteolysis of full-length mutant huntingtin in an inducible PC12 cell model of Huntington's disease. Cell Cycle 6(23):2970–2981
10. Landles C, Sathasivam K, Weiss A, Woodman B, Moffitt H, Finkbeiner S, Sun B, Gafni J, Ellerby LM, Trottier Y, Richards WG, Osmand A, Paganetti P, Bates GP (2010) Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington disease. J Biol Chem 285(12):8808–8823
11. Barbaro BA, Lukacsovich T, Agrawal N, Burke J, Bornemann DJ, Purcell JM, Worthge SA, Caricasole A, Weiss A, Song W, Morozova OA, Colby DW, Marsh JL (2015) Comparative study of naturally occurring huntingtin fragments in Drosophila points to exon 1 as the most pathogenic species in Huntington's disease. Hum Mol Genet 24(4):913–925
12. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson A, Kampf C, Sjostedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Ponten F (2015) Proteomics. Tissue-based map of the human proteome. Science 347(6220):1260419

13. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 87(3):493–506
14. Ng CW, Yildirim F, Yap YS, Dalin S, Matthews BJ, Velez PJ, Labadorf A, Housman DE, Fraenkel E (2013) Extensive changes in DNA methylation are associated with expression of mutant huntingtin. Proc Natl Acad Sci U S A 110(6):2354–2359
15. Holzmann C, Schmidt T, Thiel G, Epplen JT, Riess O (2001) Functional characterization of the human Huntington's disease gene promoter. Brain Res Mol Brain Res 92(1–2):85–97
16. Coles R, Caswell R, Rubinsztein DC (1998) Functional analysis of the Huntington's disease (HD) gene promoter. Hum Mol Genet 7(5):791–800
17. Becanovic K, Norremolle A, Neal SJ, Kay C, Collins JA, Arenillas D, Lilja T, Gaudenzi G, Manoharan S, Doty CN, Beck J, Lahiri N, Portales-Casamar E, Warby SC, Connolly C, De Souza RA, Network RIotEHsD, Tabrizi SJ, Hermanson O, Langbehn DR, Hayden MR, Wasserman WW, Leavitt BR (2015) A SNP in the HTT promoter alters NF-kappaB binding and is a bidirectional genetic modifier of Huntington disease. Nat Neurosci 18(6):807–816
18. Bates GP, Dorsey R, Gusella JF, Hayden MR, Kay C, Leavitt BR, Nance M, Ross CA, Scahill RI, Wetzel R, Wild EJ, Tabrizi SJ (2015) Huntington disease. Nat Rev Dis Primers 1:15005
19. Duan R, Sharma S, Xia Q, Garber K, Jin P (2014) Towards understanding RNA-mediated neurological disorders. J Genet Genomics = Yi chuan xue bao 41(9):473–484
20. Groh M, Gromak N (2014) Out of balance: R-loops in human disease. PLoS Genet 10(9): e1004630
21. Liu CR, Chang CR, Chern Y, Wang TH, Hsieh WC, Shen WC, Chang CY, Chu IC, Deng N, Cohen SN, Cheng TH (2012) Spt4 is selectively required for transcription of extended trinucleotide repeats. Cell 148(4):690–701
22. Woodman B, Butler R, Landles C, Lupton MK, Tse J, Hockly E, Moffitt H, Sathasivam K, Bates GP (2007) The Hdh(Q150/Q150) knock-in mouse model of HD and the R6/2 exon 1 model develop comparable and widespread molecular phenotypes. Brain Res Bull 72(2–3):83–97
23. Lin CH, Tallaksen-Greene S, Chien WM, Cearley JA, Jackson WS, Crouse AB, Ren S, Li XJ, Albin RL, Detloff PJ (2001) Neurological abnormalities in a knock-in mouse model of Huntington's disease. Hum Mol Genet 10(2):137–144
24. Sathasivam K, Neueder A, Gipson TA, Landles C, Benjamin AC, Bondulich MK, Smith DL, Faull RL, Roos RA, Howland D, Detloff PJ, Housman DE, Bates GP (2013) Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. Proc Natl Acad Sci U S A 110(6):2366–2370
25. Cho S, Hoang A, Sinha R, Zhong XY, Fu XD, Krainer AR, Ghosh G (2011) Interaction between the RNA binding domains of Ser-Arg splicing factor 1 and U1-70 K snRNP protein determines early spliceosome assembly. Proc Natl Acad Sci U S A 108(20):8233–8238
26. Kaida D, Berg MG, Younis I, Kasim M, Singh LN, Wan L, Dreyfuss G (2010) U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. Nature 468(7324):664–668
27. Berg MG, Singh LN, Younis I, Liu Q, Pinto AM, Kaida D, Zhang Z, Cho S, Sherrill-Mix S, Wan L, Dreyfuss G (2012) U1 snRNP determines mRNA length and regulates isoform expression. Cell 150(1):53–64
28. Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, Ruiz M, Mushlin R, Alosio W, McConnell K, Connor D, Murphy C, Oakeshott S, Kwan M, Beltran J, Ghavami A, Brunner D, Park LC, Ramboz S, Howland D (2012) Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175. PLoS ONE 7(12):e49838
29. Wheeler VC, Auerbach W, White JK, Srinidhi J, Auerbach A, Ryan A, Duyao MP, Vrbanac V, Weaver M, Gusella JF, Joyner AL, MacDonald ME (1999) Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Hum Mol Genet 8(1):115–122

30. White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, Joyner AL, MacDonald ME (1997) Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet 17(4):404–410
31. Hughes AC, Mort M, Elliston L, Thomas RM, Brooks SP, Dunnett SB, Jones L (2014) Identification of novel alternative splicing events in the huntingtin gene and assessment of the functional consequences using structural protein homology modelling. J Mol Biol 426 (7):1428–1438
32. Mort M, Carlisle FA, Waite AJ, Elliston L, Allen ND, Jones L, Hughes AC (2015) Huntingtin exists as multiple splice forms in human brain. J Huntington's Dis 4(2):161–171
33. Ruzo A, Ismailoglu I, Popowski M, Haremaki T, Croft GF, Deglincerti A, Brivanlou AH (2015) Discovery of novel isoforms of huntingtin reveals a new hominid-specific exon. PLoS ONE 10(5):e0127687
34. Gipson TA, Neueder A, Wexler NS, Bates GP, Housman D (2013) Aberrantly spliced HTT, a new player in Huntington's disease pathogenesis. RNA Biol 10(11):1647–1652
35. Genetic Modifiers of Huntington's Disease C (2015) Identification of genetic factors that modify clinical onset of Huntington's disease. Cell 162(3):516–526
36. Larson E, Fyfe I, Morton AJ, Monckton DG (2015) Age-, tissue-and length-dependent bidirectional somatic CAG*CTG repeat instability in an allelic series of R6/2 Huntington disease mice. Neurobiology of disease 76:98–111
37. Aronin N, DiFiglia M (2014) Huntingtin-lowering strategies in Huntington's disease: antisense oligonucleotides, small RNAs, and gene editing. Movement disorders: official journal of the Movement Disorder Society 29(11):1455–1461
38. Wild EJ, Tabrizi SJ (2014) Targets for future clinical trials in Huntington's disease: what's in the pipeline? Movement Disorders. Official J Mov Disord Soc 29(11):1434–1445
39. Carroll JB, Warby SC, Southwell AL, Doty CN, Greenlee S, Skotte N, Hung G, Bennett CF, Freier SM, Hayden MR (2011) Potent and selective antisense oligonucleotides targeting single-nucleotide polymorphisms in the Huntington disease gene/allele-specific silencing of mutant huntingtin. Mol Ther J Am Soc Gene Ther 19(12):2178–2185
40. Harper SQ, Staber PD, He X, Eliason SL, Martins IH, Mao Q, Yang L, Kotin RM, Paulson HL, Davidson BL (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proc Natl Acad Sci U S A 102(16): 5820–5825
41. Stanek LM, Sardi SP, Mastis B, Richards AR, Treleaven CM, Taksir T, Misra K, Cheng SH, Shihabuddin LS (2014) Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. Hum Gene Ther 25(5):461–474
42. Trager U, Andre R, Lahiri N, Magnusson-Lind A, Weiss A, Grueninger S, McKinnon C, Sirinathsinghji E, Kahlon S, Pfister EL, Moser R, Hummerich H, Antoniou M, Bates GP, Luthi-Carter R, Lowdell MW, Bjorkqvist M, Ostroff GR, Aronin N, Tabrizi SJ (2014) HTT-lowering reverses Huntington's disease immune dysfunction caused by NFkappaB pathway dysregulation. Brain J Neurol 137(Pt 3):819–833
43. Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, Artates JW, Weiss A, Cheng SH, Shihabuddin LS, Hung G, Bennett CF, Cleveland DW (2012) Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron 74(6):1031–1044
44. Pelechano V, Steinmetz LM (2013) Gene regulation by antisense transcription. Nat Rev Genet 14(12):880–893
45. Chung DW, Rudnicki DD, Yu L, Margolis RL (2011) A natural antisense transcript at the Huntington's disease repeat locus regulates HTT expression. Hum Mol Genet 20(17):3467–3477
46. Philips AV, Timchenko LT, Cooper TA (1998) Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. Science 280(5364):737–741
47. Kanadia RN, Johnstone KA, Mankodi A, Lungu C, Thornton CA, Esson D, Timmers AM, Hauswirth WW, Swanson MS (2003) A muscleblind knockout model for myotonic dystrophy. Science 302(5652):1978–1980

48. Miller JW, Urbinati CR, Teng-Umnuay P, Stenberg MG, Byrne BJ, Thornton CA, Swanson MS (2000) Recruitment of human muscle blind proteins to (CUG)(n) expansions associated with myotonic dystrophy. The EMBO journal 19(17):4439–4448
49. Jog SP, Paul S, Dansithong W, Tring S, Comai L, Reddy S (2012) RNA splicing is responsive to MBNL1 dose. PLoS ONE 7(11):e48825
50. Li X, Kazan H, Lipshitz HD, Morris QD (2014) Finding the target sites of RNA-binding proteins. Wiley Interdisciplinary Rev RNA 5(1):111–130
51. de Mezer M, Wojciechowska M, Napierala M, Sobczak K, Krzyzosiak WJ (2011) Mutant CAG repeats of Huntingtin transcript fold into hairpins, form nuclear foci and are targets for RNA interference. Nucleic Acids Res 39(9):3852–3863
52. Yuan Y, Compton SA, Sobczak K, Stenberg MG, Thornton CA, Griffith JD, Swanson MS (2007) Muscleblind-like 1 interacts with RNA hairpins in splicing target and pathogenic RNAs. Nucleic Acids Res 35(16):5474–5486
53. Mykowska A, Sobczak K, Wojciechowska M, Kozlowski P, Krzyzosiak WJ (2011) CAG repeats mimic CUG repeats in the misregulation of alternative splicing. Nucleic Acids Res 39(20):8938–8951
54. Neueder A, Bates GP (2014) A common gene expression signature in Huntington's disease patient brain regions. BMC Med Genomics 7:60
55. Sun X, Li PP, Zhu S, Cohen R, Marque LO, Ross CA, Pulst SM, Chan HY, Margolis RL, Rudnicki DD (2015) Nuclear retention of full-length HTT RNA is mediated by splicing factors MBNL1 and U2AF65. Scientific reports 5:12521
56. Wilson RC, Doudna JA (2013) Molecular mechanisms of RNA interference. Annual Rev Biophysics 42:217–239
57. Krol J, Fiszer A, Mykowska A, Sobczak K, de Mezer M, Krzyzosiak WJ (2007) Ribonuclease dicer cleaves triplet repeat hairpins into shorter repeats that silence specific targets. Mol Cell 25(4):575–586
58. Banez-Coronel M, Porta S, Kagerbauer B, Mateu-Huertas E, Pantano L, Ferrer I, Guzman M, Estivill X, Marti E (2012) A pathogenic mechanism in Huntington's disease involves small CAG-repeated RNAs with neurotoxic activity. PLoS Genet 8(2):e1002481
59. Valor LM (2015) Transcription, epigenetics and ameliorative strategies in Huntington's disease: a genome-wide perspective. Mol Neurobiol 51(1):406–423
60. Benn CL, Sun T, Sadri-Vakili G, McFarland KN, DiRocco DP, Yohrling GJ, Clark TW, Bouzou B, Cha JH (2008) Huntingtin modulates transcription, occupies gene promoters in vivo, and binds directly to DNA in a polyglutamine-dependent manner. J Neurosci 28(42):10720–10733
61. Yin X, Jin N, Gu J, Shi J, Zhou J, Gong CX, Iqbal K, Grundke-Iqbal I, Liu F (2012) Dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1A) modulates serine/arginine-rich protein 55 (SRp55)-promoted Tau exon 10 inclusion. J Biol Chem 287(36):30497–30506
62. Medina M, Hernandez F, Avila J (2016) New Features about Tau Function and Dysfunction. Biomolecules 6(2)
63. Qian W, Liu F (2014) Regulation of alternative splicing of tau exon 10. Neuroscience Bulletin 30(2):367–377
64. Fernandez-Nogales M, Cabrera JR, Santos-Galindo M, Hoozemans JJ, Ferrer I, Rozemuller AJ, Hernandez F, Avila J, Lucas JJ (2014) Huntington's disease is a four-repeat tauopathy with tau nuclear rods. Nat Med 20(8):881–885
65. Blum D, Herrera F, Francelle L, Mendes T, Basquin M, Obriot H, Demeyer D, Sergeant N, Gerhardt E, Brouillet E, Buee L, Outeiro TF (2015) Mutant huntingtin alters Tau phosphorylation and subcellular distribution. Hum Mol Genet 24(1):76–85
66. Vuono R, Winder-Rhodes S, de Silva R, Cisbani G, Drouin-Ouellet J, Network RIotEHsD, Spillantini MG, Cicchetti F, Barker RA (2015) The role of tau in the pathological process and clinical expression of Huntington's disease. Brain J Neurol 138(Pt 7):1907–1918

67. Lin L, Park JW, Ramachandran S, Zhang Y, Tseng YT, Shen S, Waldvogel HJ, Curtis MA, Faull RL, Troncoso JC, Ross CA, Davidson BL, Xing Y (2016) Transcriptome sequencing reveals aberrant alternative splicing in Huntington's disease. Hum Mol Genet
68. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr (1985) Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 44(6): 559–577
69. Zuccato C, Belyaev N, Conforti P, Ooi L, Tartari M, Papadimou E, MacDonald M, Fossale E, Zeitlin S, Buckley N, Cattaneo E (2007) Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. J Neurosci 27(26):6972–6983
70. Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, Cataudella T, Leavitt BR, Hayden MR, Timmusk T, Rigamonti D, Cattaneo E (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 35(1): 76–83
71. Schiffer D, Caldera V, Mellai M, Conforti P, Cattaneo E, Zuccato C (2014) Repressor element-1 silencing transcription factor (REST) is present in human control and Huntington's disease neurones. Neuropathol Appl Neurobiol 40(7):899–910
72. Shimojo M (2008) Huntingtin regulates RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) nuclear trafficking indirectly through a complex with REST/NRSF-interacting LIM domain protein (RILP) and dynactin p150 Glued. J Biol Chem 283 (50):34880–34886
73. Soldati C, Bithell A, Johnston C, Wong KY, Stanton LW, Buckley NJ (2013) Dysregulation of REST-regulated coding and non-coding RNAs in a cellular model of Huntington's disease. J Neurochem 124(3):418–430
74. Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, MacDonald ME, Friedlander RM, Silani V, Hayden MR, Timmusk T, Sipione S, Cattaneo E (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 293 (5529):493–498
75. Gharami K, Xie Y, An JJ, Tonegawa S, Xu B (2008) Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington's disease phenotypes in mice. J Neurochem 105(2):369–379
76. Xie Y, Hayden MR, Xu B (2010) BDNF overexpression in the forebrain rescues Huntington's disease phenotypes in YAC128 mice. J Neurosci 30(44):14708–14718
77. Conforti P, Mas Monteys A, Zuccato C, Buckley NJ, Davidson B, Cattaneo E (2013) In vivo delivery of DN:REST improves transcriptional changes of REST-regulated genes in HD mice. Gene Ther 20(6):678–685
78. Soldati C, Bithell A, Conforti P, Cattaneo E, Buckley NJ (2011) Rescue of gene expression by modified REST decoy oligonucleotides in a cellular model of Huntington's disease. J Neurochem 116(3):415–425
79. Johnson R, Zuccato C, Belyaev ND, Guest DJ, Cattaneo E, Buckley NJ (2008) A microRNA-based gene dysregulation pathway in Huntington's disease. Neurobiology Dis 29 (3):438–445
80. Packer AN, Xing Y, Harper SQ, Jones L, Davidson BL (2008) The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. J Neurosci 28(53):14341–14346
81. Qiu L, Tan EK, Zeng L (2015) microRNAs and Neurodegenerative Diseases. Adv Exp Med Biol 888:85–105
82. Cohen JE, Lee PR, Chen S, Li W, Fields RD (2011) MicroRNA regulation of homeostatic synaptic plasticity. Proc Natl Acad Sci U S A 108(28):11650–11655
83. Lee ST, Chu K, Im WS, Yoon HJ, Im JY, Park JE, Park KH, Jung KH, Lee SK, Kim M, Roh JK (2011) Altered microRNA regulation in Huntington's disease models. Exp Neurol 227(1):172–179
84. Kocerha J, Xu Y, Prucha MS, Zhao D, Chan AW (2014) microRNA-128a dysregulation in transgenic Huntington's disease monkeys. Molecular brain 7:46

85. Hoss AG, Kartha VK, Dong X, Latourelle JC, Dumitriu A, Hadzi TC, Macdonald ME, Gusella JF, Akbarian S, Chen JF, Weng Z, Myers RH (2014) MicroRNAs located in the Hox gene clusters are implicated in huntington's disease pathogenesis. PLoS Genet 10(2): e1004188
86. Hoss AG, Labadorf A, Latourelle JC, Kartha VK, Hadzi TC, Gusella JF, MacDonald ME, Chen JF, Akbarian S, Weng Z, Vonsattel JP, Myers RH (2015) miR-10b-5p expression in Huntington's disease brain relates to age of onset and the extent of striatal involvement. BMC Med Genomics 8:10
87. Marti E, Pantano L, Banez-Coronel M, Llorens F, Minones-Moyano E, Porta S, Sumoy L, Ferrer I, Estivill X (2010) A myriad of miRNA variants in control and Huntington's disease brain regions detected by massively parallel sequencing. Nucleic Acids Res 38(20):7219–7235
88. Gaughwin PM, Ciesla M, Lahiri N, Tabrizi SJ, Brundin P, Bjorkqvist M (2011) Hsa-miR-34b is a plasma-stable microRNA that is elevated in pre-manifest Huntington's disease. Hum Mol Genet 20(11):2225–2237
89. Mastrokolias A, Ariyurek Y, Goeman JJ, van Duijn E, Roos RA, van der Mast RC, van Ommen GB, den Dunnen JT, t Hoen PA, van Roon-Mom WM (2015) Huntington's disease biomarker progression profile identified by transcriptome sequencing in peripheral blood. European J Human Genet EJHG 23(10):1349–1356
90. Azlan A, Dzaki N, Azzam G (2016) Argonaute: the executor of small RNA function. J Genet Genomics = Yi chuan xue bao
91. Savas JN, Makusky A, Ottosen S, Baillat D, Then F, Krainc D, Shiekhattar R, Markey SP, Tanese N (2008) Huntington's disease protein contributes to RNA-mediated gene silencing through association with Argonaute and P bodies. Proc Natl Acad Sci U S A 105 (31):10820–10825
92. Jain S, Parker R (2013) The discovery and analysis of P Bodies. Adv Exp Med Biol 768:23–43
93. Krauss S, Griesche N, Jastrzebska E, Chen C, Rutschow D, Achmuller C, Dorn S, Boesch SM, Lalowski M, Wanker E, Schneider R, Schweiger S (2013) Translation of HTT mRNA with expanded CAG repeats is regulated by the MID1-PP2A protein complex. Nature communications 4:1511
94. Cleary JD, Ranum LP (2014) Repeat associated non-ATG (RAN) translation: new starts in microsatellite expansion disorders. Curr Opin Genet Dev 26:6–15
95. Zu T, Gibbens B, Doty NS, Gomes-Pereira M, Huguet A, Stone MD, Margolis J, Peterson M, Markowski TW, Ingram MA, Nan Z, Forster C, Low WC, Schoser B, Somia NV, Clark HB, Schmechel S, Bitterman PB, Gourdon G, Swanson MS, Moseley M, Ranum LP (2011) Non-ATG-initiated translation directed by microsatellite expansions. Proc Natl Acad Sci U S A 108(1):260–265
96. Banez-Coronel M, Ayhan F, Tarabochia AD, Zu T, Perez BA, Tusi SK, Pletnikova O, Borchelt DR, Ross CA, Margolis RL, Yachnis AT, Troncoso JC, Ranum LP (2015) RAN translation in Huntington disease. Neuron 88(4):667–677
97. Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuk JA, Slunt HH, Ratovitski T, Cooper JK, Jenkins NA, Copeland NG, Price DL, Ross CA, Borchelt DR (1999) Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Hum Mol Genet 8(3):397–407
98. Caillet-Boudin ML, Fernandez-Gomez FJ, Tran H, Dhaenens CM, Buee L, Sergeant N (2014) Brain pathology in myotonic dystrophy: when tauopathy meets spliceopathy and RNAopathy. Frontiers in molecular neuroscience 6:57
99. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72 (2):245–256

100. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviita VM, Kaivorinne AL, Holtta-Vuori M, Ikonen E, Sulkava R, Benatar M, Wuu J, Chio A, Restagno G, Borghero G, Sabatelli M, Consortium I, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72(2):257–268

Chapter 5
X-Linked Spinal and Bulbar Muscular Atrophy: From Clinical Genetic Features and Molecular Pathology to Mechanisms Underlying Disease Toxicity

Constanza J. Cortes and Albert R. La Spada

Abstract Spinal and Bulbar Muscular Atrophy (SBMA) is an inherited neuromuscular disorder caused by a CAG—polyglutamine (polyQ) repeat expansion in the androgen receptor (AR) gene. Unlike other polyQ diseases, where the function of the native causative protein is unknown, the biology of AR is well understood, and this knowledge has informed our understanding of how native AR function interfaces with polyQ-AR dysfunction. Furthermore, ligand-dependent activation of AR has been linked to SBMA disease pathogenesis, and has led to a thorough study of androgen-mediated effects on polyQ-AR stability, degradation, and post-translational modifications, as well as their roles in the disease process. Transcriptional dysregulation, proteostasis dysfunction, and mitochondrial abnormalities are central to polyQ-AR neurotoxicity, most likely via a 'change-of-function' mechanism. Intriguingly, recent work has demonstrated a principal role for skeletal muscle in SBMA disease pathogenesis, indicating that polyQ-AR toxicity initiates in skeletal muscle and results in secondary motor neuron demise. The existence of robust animal models for SBMA has permitted a variety of preclinical trials, driven by recent discoveries of altered cellular processes, and some of this preclinical work has led to human clinical trials. In this chapter, we review SBMA clinical features and disease biology, discuss our current understanding of the cellular and molecular basis of SBMA pathogenesis, and highlight ongoing efforts toward therapy development.

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Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an X-linked inherited neuromuscular disorder characterized by lower motor neuron degeneration leading to weakness and atrophy of bulbar, facial, and limb muscles [1]. SBMA patients also display signs of androgen insensitivity, and full disease penetrance is restricted to adult males. The causal mutation in SBMA is a CAG trinucleotide repeat expansion in the first exon of the androgen receptor (AR) gene [1]. Unaffected individuals carry anywhere from 5 to 34 CAG repeats in the AR gene, whereas SBMA patients have 37 repeats or more CAG repeats. As with other polyglutamine (polyQ) disorders, there is an inverse correlation between the length of the polyQ tract and disease onset, and symptoms usually manifest between 30–60 years of age. The prevalence of SBMA is estimated to be 1 per 100,000, and a significant fraction of patients are initially misdiagnosed with other neuromuscular disorders, including amyotrophic lateral sclerosis (ALS) or autosomal recessive spinal muscular atrophy (SMA).

SBMA is unique among other polyQ disorders in that the native function of the causal protein is well-characterized. AR is ubiquitously expressed, with particularly high levels in the central nervous system (CNS), skeletal muscle, and primary and secondary sexual organs, where it regulates the transcription of androgen-responsive genes. These tissue-specific high levels of expression may account for the selective vulnerability of certain cell-types to mutant AR toxicity and the characteristic neuromuscular and endocrine symptoms in SBMA.

Today, the field regards SBMA pathogenesis as most likely involving two distinct pathways: a primary gain-of-function toxicity due to production of misfolded polyQ-AR protein, and a secondary loss or alteration of AR normal function. This is supported by two independent observations: first, a variety of animal models of SBMA have been generated in the past three decades via expression of polyglutamine-expanded AR (polyQ-AR), and they have all demonstrated the androgen dependence of polyQ-AR neuromuscular dysfunction (Table 5.1) [2–7]. Second, while endocrine-related phenotypes occur in mice and humans carrying an inactivated AR-gene, there is no documentation of motor neuron toxicity [8, 9], suggesting that loss-of-function of AR alone is not sufficient to drive neuromuscular disease. As we discuss below, understanding the role of native AR and how these functions are altered in the context of the polyQ expansion have led to substantive advances in our understanding of SBMA disease pathogenesis, and are paving the way for provocative therapies.

Table 5.1 Mouse models of SBMA

Name of mouse model	CAG repeat / PolyQ tract size	Promoter	Muscle pathology?	Motor neuron phenotype?	First author & year of publication
AR97Q	97 glutamines	β-actin	Yes	Yes	Katsuno [5]
YAC AR100	100 CAGs	Human AR	Yes	Yes	Sopher [7]
PrP-AR112Q	112 glutamines	Prion protein	No	Yes	Chevalier-Larsen [26]
AR113Q knock-in	113 glutamines	Endogenous mouse AR	Yes	No	Yu [80]
BAC fxAR121	121 CAGs	Human AR	Yes	Yes	Cortes [3]
AR F23A 108Q (FxxFL mutant)	108 glutamines	Prion protein		Yes	Zboray [32]
AR113Q-KRKR knock-in (SUMOylation resistant)	113 glutamines	Endogenous mouse AR			Chua [40]
CMV-stop-AR113Q (*tissue specific expression)	113 glutamines	CMV	Neuro AR -No Myo AR - Yes	Neuro AR -Yes Myo AR - No	Ramzan [6]

5.1 AR Structure and Function

The AR gene is located on the X chromosome short arm (band q11-12) and contains 8 coding exons consisting of three functional domains that are shared among the entire super-family of steroid-binding transcription factors [10]. These include the N-terminal transactivation domain, a DNA-binding domain, and a C-terminal ligand-binding domain (Fig. 5.1). The AR actually contains three glutamine tracts, of which the first one, located in Exon 1, is by far the longest with an average repeat length of ~22 CAGs. This AR CAG repeat is highly polymorphic in human populations, with >99% of females carrying two different repeat length alleles. Expansion beyond 37 repeats causes SBMA, with the longest expansion reported to date to be 70 CAG repeats (Fig. 5.1). Interestingly, while expansion of this CAG repeat tract causes SBMA, shorter CAG tract lengths are associated with an increased risk of prostate cancer [11].

In the absence of ligand, AR exists in a multi-heteromeric inactive cytosolic complex with heat-shock proteins (HSPs). Binding to androgen (testosterone or dihydrotestosterone) leads to dissociation of AR from this chaperone complex, and facilitates an intramolecular interaction of its amino and carboxy terminals (known as N–C interactions), which promotes dimerization and nuclear translocation

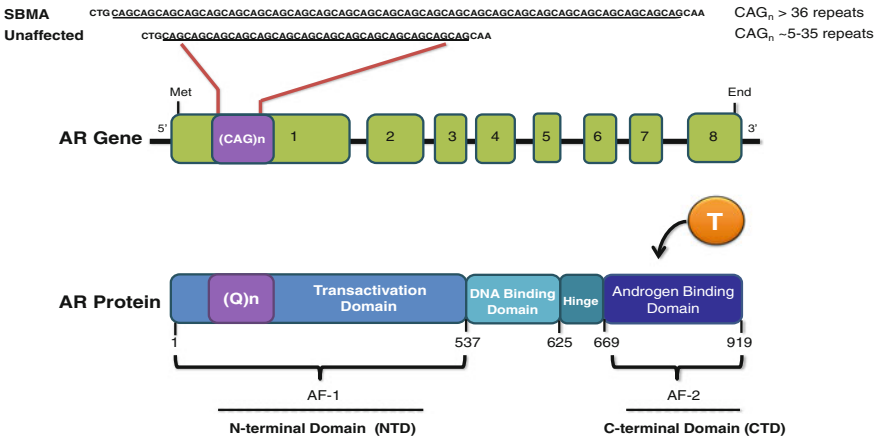

Fig. 5.1 Exon-intron structure of the androgen receptor gene and functional domains of the androgen receptor protein. The androgen receptor (AR) gene consists of eight exons, with the start site of translation and the CAG repeat located within exon 1. Expansion of the CAG repeat is responsible for causing the disease SBMA, with expanded disease-causing alleles greater in length than 36 CAGs. The AR protein is normally 919 amino acids in length, but SBMA patients produce a larger protein due to expansion of the glutamine repeat [(Q)n] tract. The AR protein is a member of the steroid thyroid-hormone nuclear receptor superfamily, with functional domains as shown. The principal ligand for the C-terminal androgen binding domain is testosterone (T) or metabolites thereof

(Fig. 5.1). AR then directly binds to Androgen Responsive Elements (AREs) in target promoter regions, selectively recruiting co-activators and transcriptional machinery for transcription initiation of androgen-responsive genes [10]. Importantly, ligand-induced nuclear translocation, N–C interactions, DNA binding, and co-regulator interactions have all been implicated in SBMA disease pathogenesis. Indeed, the polyQ-expansion may modify the intrinsic properties of the N-terminal transactivation domain, leading to altered co-factor interaction and transcriptional dysregulation (see below).

5.2 SBMA Disease Features and Pathogenesis

SBMA is characterized by degeneration of motor neurons in the anterior horn of the spinal cord and in the brain stem, and to a lesser extent, of sensory neurons in the dorsal root ganglia. Clinically, patients present an adult-onset, slowly progressive muscle weakness, atrophy, and fasciculation of bulbar and limb skeletal muscle. Muscle biopsies show both myogenic and myopathic features, including fiber type grouping, target fiber formation, angulated atrophic fibers, and fiber size variability

with fiber splitting [12]. Nuclear inclusions (NIs), a pathological hallmark of polyQ disorders, are also a cardinal feature of SBMA [13]. These inclusions are highly enriched in N-terminal polyQ-AR fragments, chaperone proteins, and ubiquitin [13, 14], and are found in surviving motor neurons in the spinal cord and brainstem. In SBMA, NIs are also prominent in non-neural tissues, including skeletal muscle, prostate, kidney, and testis [13], suggesting they may intrinsically not represent the primary toxic species in polyQ-AR toxicity.

What is the origin of AR protein inclusions? Atomic Force Microscopy studies on in vitro models indicate that wild-type AR can self-assemble into annular oligomers between 120–170 nm in length, a species that appears distinct from the oligomeric fibrils formed by polyQ-AR, which are 300–600 nm in length [15]. It is thus likely that the polyQ expansion tract in AR interferes with normal protein folding, generating misfolded conformers that enhance the natural propensity of wild-type AR to self-aggregate [15]. As with other aggregation-prone proteins, polyQ-AR inclusion formation most likely occurs in a succession of steps. PolyQ-AR oligomerizes into soluble deposits, cycling through intermediate unstable conformers ('seeding' phase), until maturing into insoluble fibrils ('elongation' phase) and finally NIs. Histopathology studies in SBMA patients and transgenic mice support this seeding model of polyQ-AR aggregation. Analysis of autopsied SBMA patients reveals that diffuse nuclear accumulation of polyQ-AR is more frequently observed than NIs in surviving spinal cord motor neurons [16]. Similarly, polyQ-AR oligomers precede NI formation and symptom onset in SBMA PrP-AR112Q mice, suggesting these intermediate species represent early toxic polyQ-AR entities in vivo [17]. Interestingly, these oligomers are both soluble and androgen-dependent, as they dissipate after testosterone ablation via surgical castration [17]. This strongly suggests that polyQ-AR oligomers are dynamic in nature, representing a state both structurally and temporally distinct from NIs, which are believed to be quite stable [17, 18].

The role of NIs in polyQ toxicity has generated considerable debate in the field. Although initially thought to be pathogenic entities, proteinaceous inclusions are now more often regarded as a protective cellular response, sequestering toxic diffuse species into non-reactive inclusions [19]. Consistent with this idea, genetic overexpression of p62, a marker of cytosolic inclusions and a known AR interactor, can rescue both neuromuscular abnormalities and decreased lifespan in AR97Q transgenic mice by driving inclusion formation, shifting soluble oligomeric AR towards insoluble AR species [20]. Nonetheless, one must also consider the long-term effects of inclusions in a broader cellular context. If unable to be cleared —either due to their inaccessibility for protein degradation pathways, or failure of these pathways with aging—inclusions may ultimately impact physiological pathways and cause some degree of toxicity. Thus, the precise role of aggregates in SBMA (and other polyQ disorders) still remains unclear, and the search for the most toxic polyQ-AR species is still ongoing.

5.3 Ligand-Dependent SBMA Pathogenesis

SBMA is unique among other polyQ disorders in that the disease protein is activated by a specific ligand, and this activation step is necessary for polyQ-AR toxicity [21–24]. This feature explains the gender-selectivity of SBMA, as affected men have higher circulating levels of androgens than carrier females, who are almost always unaffected. This gender-restriction is recapitulated in all transgenic mouse models of SBMA to date (Table 5.1), with only males presenting with the full manifestation of disease [3–5, 7, 25]. It is not surprising then that hormone interventions are important modulators of disease phenotypes. Unaffected transgenic female mice quickly become symptomatic after testosterone administration [5], and chemical or surgical castration to reduce circulating levels of androgens leads to marked improvements in affected males [5, 21, 26]. Similarly, testosterone-dependent toxicity has been demonstrated in *Drosophila* models of SBMA [27], and there is evidence to suggest that testosterone administration may exacerbate neuromuscular disease symptoms in SBMA patients.

How then does ligand activation promote polyQ-AR toxicity? AR can bind to testosterone (T) or its derivative dihydrotestosterone (DHT), and androgen binding has three major effects on AR biology: (a) dissociation from the heteromeric Hsp70/Hsp90 complex, (b) promotion of post-translational modifications, and (c) translocation into the nucleus. Recent studies into the mechanisms of ligand-activation of AR have demonstrated the potential impact of each of these steps for SBMA pathology, and we discuss each of them here.

5.3.1 Dissociation from HSP Proteins

In the absence of ligand, AR coexists in complex with cytosolic heat-shock chaperones Hsp70 and Hsp90. Upon binding to androgen, AR dissociates from its chaperone interactors, dimerizes, and undergoes structural modifications that ultimately lead to full transactivation competence [10]. PolyQ-AR is also member of this cytosolic complex in its inactive state, which most likely masks the polyQ-tract and prevents its self-association and aggregation. In the presence of ligand, polyQ-AR is released from the complex, and subsequent structural modifications expose the polyQ-tract permitting oligomerization to begin. Furthermore, once activated by androgen, polyQ-AR appears to be cleaved by caspase-3, releasing a pro-apoptotic, polyQ-tract containing N-terminal fragment [14, 28, 29]. Susceptibility to this cleavage is dependent on polyQ-tract length, and may be necessary for polyQ-AR inclusion formation and neurotoxicity [14, 28]. Additionally, these self-aggregating polyQ-AR N-terminal fragments may contribute to the ‘seeding’ phase of aggregation. Ligand-binding thus has profound effects on the structural conformation of AR, allowing for the exposure of not just the polyQ-tract, but also motifs that are recognized by proteases and interactors alike.

5.3.2 AR Post-translational Modifications

AR is substantially modified in response to ligand-binding by phosphorylation, acetylation, and SUMOylation (Fig. 5.2) [30]. These post-translational modifications have important downstream consequences, altering AR activity and cellular localization, and are recognized as important SBMA disease modifiers. For example, Akt phosphorylation of AR at serine 215 and 792 reduces polyQ-AR toxicity by blocking ligand binding, an effect that is stimulated by insulin-growth Factor-1 (IGF-1) [31]. Activation of IGF-1/Akt signaling in vivo by overexpression of a muscle-specific isoform of IGF-1 rescues behavioral and neuropathology abnormalities, extends lifespan, and reduces both muscle and spinal cord pathology in SBMA mice [31]. This was accompanied by increased polyQ-AR phosphorylation and decreased AR aggregation [31], suggesting Akt may have important modulatory effects on SBMA pathogenesis. More recently, phosphorylation of serine 16 has been identified as another potential neuroprotective post-translational modification of AR [32], although its relevance in vivo remains unclear (Fig. 5.2).

Phosphorylation may also favor the cellular toxicity of polyQ-AR. Serine 514 phosphorylation by JNK MAP kinase activates caspase-3 mediated cleavage of the N-terminus of polyQ-AR [33], a crucial step in its cytotoxicity [28]. Similarly, testosterone-dependent phosphorylation of AR at serines 81 and 308 by nemo-like

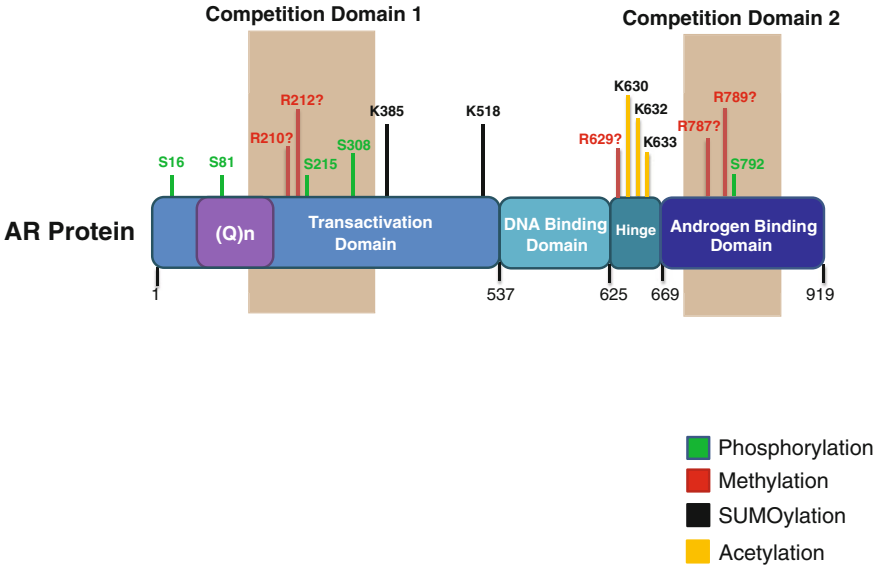

Fig. 5.2 Androgen receptor post-translational modifications implicated in SBMA disease pathogenesis. The AR protein is normally 919 amino acids in length, with a variably sized glutamine repeat [(Q)n] tract, that upon expansion causes the disease SBMA. Here the amino acid regions containing the functional domains of the AR protein are shown, highlighting the various amino acid residues that are subject to different types of post-translational modification. K = lysine; R = arginine; S = serine

kinase (NLK) promotes SBMA pathogenesis in BAC fxAR121 mice, enhancing polyQ-AR aggregation and increasing AR-mediated transcription [34]. NLK is an evolutionarily conserved, mitogen-activated protein kinase-like serine/threonine kinase, which interacts either directly or indirectly with a number of neurodegenerative disease-related proteins, including polyQ-expanded ataxin-1, the causative mutant protein in spinocerebellar ataxia type 1 (SCA1) [35]. Interestingly, loss of one copy of NLK (resulting in a 50% reduction in protein expression) is beneficial in mouse models of both SCA1 and SBMA [34, 35], suggesting that down-regulation of NLK activity or expression can have beneficial effects against polyQ toxicity. Phosphorylation of polyQ-AR can thus have pro-apoptotic or cytoprotective effects in a residue specific manner. Therefore, any attempts at developing phosphorylation-targeting therapies for SBMA will require further studies to understand the delicate interplay between ligand-dependent phosphorylation of AR, AR transactivation function, and protein kinase activation. However, these studies provide a definitive proof-of-principle that manipulation of AR phosphorylation can affect polyQ-AR toxicity, and may thus hold promise for SBMA therapy development.

Small Ubiquitin-like Modifier (or SUMO) proteins are a family of small proteins that modify lysine (K) residues in target proteins, in a process that enzymatically resembles ubiquitination. There are three SUMO family members: SUMO-1, SUMO-2 and SUMO-3, and they are all ubiquitously expressed in mammals. This reversible pathway provides a rapid and efficient way to modulate the subcellular localization, activity, and stability of a wide variety of substrates. SUMOylation has recently attracted considerable attention as a disease modifier for neurodegenerative diseases, including polyQ disorders. Indeed, several lines of evidence suggest that SUMOylation may play a role in SBMA pathogenesis. AR is subject to SUMOylation at two lysine residues (K385 and K518) in an androgen-dependent manner [30, 36] (Fig. 5.2). Mutations in the motifs containing the SUMOylation sites in AR are associated with androgen insensitivity, testicular cancer, and prostate cancer in humans [37], indicating that SUMOylation is indeed an important regulatory mechanism for AR function. Overall enhancement of the SUMOylation pathway reduces hormone-dependent aggregation of polyQ-AR in vitro, and this effect is dependent on intact lysines in AR [38]. Pan-disruption of the SUMOylation pathway worsens SBMA eye toxicity in *Drosophila* [39]. The first direct examination of the role of SUMOylation in SBMA pathogenesis was recently performed using a novel line of SUMOylation resistant SBMA knock-in mice (AR113Q-KRKR) (Table 5.1). These lysine-to-arginine point mutations partially restored transcriptional activity of polyQ-AR, and AR113Q-KRKR mice exhibited substantially improved exercise capacity and extended survival compared to AR113Q mice [40]. These results appear to contradict previous evidence indicating SUMOylation of polyQ-AR reduced toxicity and aggregation. However, due to the low stoichiometry of SUMOylation of AR, initial data generated in vitro required high levels of exogenous SUMO protein to achieve stable AR modification, which may have produced overexpression artifacts. Intriguingly, the KRKR mutation only partially rescued polyQ-AR transcriptional activity. Mitochondrial and energy

metabolism genes returned to normal levels in SUMOylation resistant SBMA mice, whereas denervation-associated gene-expression changes and associated endocrine alterations remained unchanged [40]. SUMOylated-AR transcriptional inhibition appears promoter-selective [36], indicating that: (a) SUMOylation-dependent transcriptional dysfunction may only affect a subset of the AR transcriptome, and (b) only certain AR targets may be rescued by changes to AR SUMOylation status. However, these mice provide evidence that manipulation of AR SUMOylation status may ameliorate SBMA by restoring transcriptional competence.

Arginine methylation is a newly discovered post-translational modification with physiological roles in signal transduction, mRNA splicing, transcription regulation, DNA repair, and protein translocation. Arginine methylation is catalyzed by a family of enzymes known as protein arginine methyltransferases (PRMTs), which differ in their activity, substrate specificity and subcellular localization. PRMTs are known co-factors of AR, although their role in SBMA remains largely unexplored. Recently, PRMT6 was shown to bind to AR through a canonical steroid receptor interaction motif, and this interaction is enhanced with polyQ-AR [41]. AR is potentially methylated by PRMT6 at arginines 210, 212, 629, 787, and 789 (Fig. 5.2). Genetic modulation of PRMT6 levels negatively correlates with polyQ-AR toxicity both in vivo and in vitro, suggesting PRMT6 is a novel regulator of AR function [41]. It is interesting to note that PRMT6 methylates AR at arginine residues spanning the canonical Akt phosphorylation sites (Fig. 5.2). Arginine methylation and serine phosphorylation at these sites is mutually exclusive, suggesting a potential competition for post-translational modifications at the consensus RXRXXS motifs.

Acetylation of lysine residues also has important effects on protein dynamics, modulating function, trafficking, and turnover of target proteins. Furthermore, acetylation has been reported to have varying effects on polyQ proteins, promoting the degradation of huntingtin and inhibiting clearance of ataxin-7 via autophagy [19]. The acetylation state of AR is also testosterone-responsive, and is regulated by cellular acetyltransferases [including p300, p300/CBP-associated factor (P/CAF), and TIF60 (Tat-interactive protein)] and deacetylases (such as HDAC1 and Sirtuin-1). Androgen-induced acetylation of AR can regulate DNA-binding and enhances AR transcriptional activity in a promoter-dependent context [30]. It might appear puzzling then that polyQ-AR is acetylated even in the absence of ligand [42], and ligand-bound polyQ-AR is hyperacetylated [42, 43], as initial reports showed that polyQ tract expansion blunts AR transcriptional activity [42, 44]. Recent evidence suggests that AR transcriptional inhibition or activation is both gene and tissue dependent [3, 4, 45], suggesting a complex interplay of interactors and post-translational modifications regulate polyQ-AR transcriptional competence. Hyperacetylation of polyQ-AR may thus alter transcription of a specific subset of AR target genes, similar to the effect of the SUMOylation modifications described above. Importantly, pharmacological reduction of acetylation or genetic mutation of lysines 630/632/633 to prevent acetylation decreases polyQ-AR aggregation and toxicity in vitro, suggesting modulation of acetylation could be a therapeutic target for SBMA [43]. In agreement with this, Sirtuin-1 can deacetylate polyQ-AR and is

neuroprotective against DHT-mediated polyQ-AR toxicity. Interestingly, similar to arginine methylation, acetylation-resistant mutant polyQ-AR also showed reduced levels of phosphorylation, perhaps indicating a connection between these two distinct post-translational modifications. Finally, acetylation of AR may also alter its N-C interactions, a feature essential for polyQ-AR aggregation and toxicity [46], but a complete analysis of the effect of acetylation on AR intramolecular interactions remains to be performed.

5.3.3 Nuclear Translocation

AR nuclear translocation is promoted by androgen binding, which triggers a conformation change exposing its bipartite nuclear localization signal (NLS). Current evidence strongly suggests a central role for the nucleus in the pathogenesis of polyQ disorders, and nuclear inclusions are a hallmark of SBMA, HD, SCA1, SCA3 and SCA7 [19]. Importantly, retention of the respective mutant proteins in the cytosol, either by mutation of the NLS motifs or by forcing nuclear export by adding a nuclear export signal (NES), significantly rescues disease phenotypes in models of polyQ disease. In SBMA, polyQ-AR inclusions are also present primarily in nuclei [18], and nuclear trafficking is a key step for polyQ-AR toxicity in *Drosophila* and rodent models of SBMA [24, 47]. Similar to findings for SCA1 and SCA3, cytosolic retention of AR via mutation of its NLS sequence ameliorates disease phenotypes in a mouse model of SBMA (PrPAr112Q-dNLS) [47]. Interestingly, while nuclear translocation is necessary for SBMA pathogenesis, it is not sufficient to drive polyQ-AR toxicity. Mutations that inhibit polyQ-AR binding to DNA but do not impede nuclear translocation, prevent degeneration in *Drosophila* models of SBMA, indicating that the AR-DNA interaction is necessary for polyQ-AR toxicity [23]. Furthermore, androgens mediate the intra-molecular N-C interaction and subsequent AR dimerization, which is also required for polyQ-AR toxicity in vivo [32, 46]. Prevention of AR N-C interaction by mutating the intra-molecular interacting motif FxxLF reduces androgen-dependent mutant AR toxicity in primary cultures, and SBMA mice carrying this mutation present substantially delayed motor deficits and reduced pathological changes in spinal motor neurons and muscle [32] (Table 5.1).

5.4 Mechanisms of Mutant AR Toxicity

PolyQ disorders are likely due in part to a toxic gain of function of the polyQ-expanded proteins. Considerable progress has been made towards understanding the native function of polyQ proteins and altered function during disease pathogenesis. SBMA is unique among polyQ disorders, as the native function of AR as an androgen-dependent transcription factor is well understood. It is this

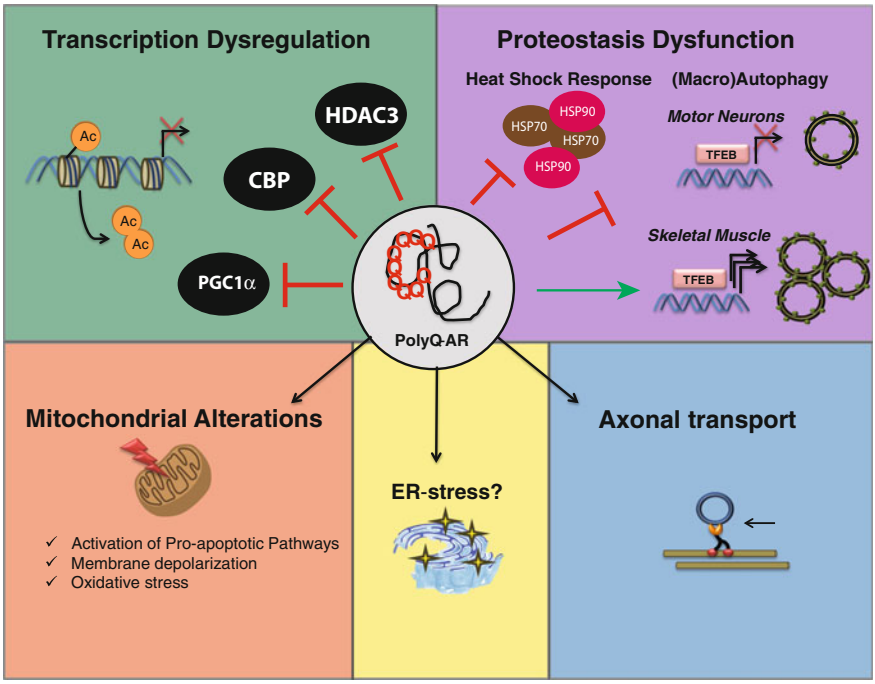

Fig. 5.3 Mechanisms of mutant AR protein toxicity. Mutant AR protein has been found to disrupt a variety of cellular processes. Here we depict pathways for which considerable evidence has accumulated over the last two decades. As impaired functions of these different pathways likely contribute to SBMA disease pathogenesis, therapy development is focusing on modulation of these processes to compensate for observed dysfunctions

knowledge that has driven much of the work into understanding the mechanistic basis of SBMA pathogenesis [19]. In this section, we will discuss our current understanding of disease pathways in SBMA, with particular emphasis on tissue-specific phenotypes (Fig. 5.3).

5.4.1 Transcriptional Dysregulation

Gene expression analysis indicates that transcriptional alterations are an early change in the pathogenic cascade in polyQ disease mouse models. It has thus been suggested that global transcriptome alterations may underlie most polyQ diseases [19]. For no other polyQ disorder is the connection between transcription and disease as evident as for SBMA. AR is a transcription factor, and thus dysfunction of AR transcriptional activity has long been suspected to be a key contributor to SBMA disease pathogenesis [44, 48]. In agreement with this hypothesis, SBMA transgenic mice placed on an AR null background display accelerated motor neuron

degeneration and severe androgen insensitivity in comparison to SBMA transgenic mice on a wild-type background [48], suggesting that impaired AR transactivation contributes to motor neuron demise in SBMA. However, altered AR transcription function is in reality a 'change-of-function', as expression of AR-regulated androgen-responsive genes can be enhanced, inhibited, or unchanged [3, 4, 7]. The net effect is due to a combination of factors, including post-translational modifications, altered AR interactome relationships, and aberrant co-factor sequestration.

One such sequestered factor is CREB-binding protein (CBP), an AR co-activator with histone acetyltransferase activity. As mentioned above, CBP is incorporated into nuclear inclusions in cultured cells, transgenic mice, and SBMA patient tissue [49]. CBP activity is decreased in various models of polyQ disease, and acetylation of nuclear Histone 3 is significantly reduced in SBMA mice [50], strongly suggesting that polyQ-mediated interference with CBP-mediated transcription may underlie global transcriptional dysfunction in SBMA [7, 51] (Fig. 5.3). In agreement with this, expression of vascular endothelial growth factor (VEGF) and transforming growth factor-beta receptor (TGFβ-R), both powerful determinants of motor neuron survival and both known targets of CBP transcription, is markedly reduced in SBMA transgenic mice [7], Katsuno et al. [52]. CBP and polyQ-AR may thus be part of a complex regulatory loop, as polyQ-AR aggregates sequester CBP away from its gene targets, reducing CBP-mediated transcription, while at the same time potentially enhancing CBP-mediated acetylation and aggregation of polyQ-AR itself. Additionally, PRMTs mediated arginine methylation of histones. Although PRMT6 can directly methylate polyQ-AR to modulate its toxicity [41], this novel interaction between polyQ-AR and PRMT6 may also alter histone epigenetic marks, either by altering AR transcriptional function via methylation, or through direct sequestration of PRMT6 into polyQ-AR aggregates, potentially contributing to the transcription dysregulation that characterizes SBMA.

Another target of polyQ-AR transcription interference is peroxisome proliferator-activated receptor gamma co-activator 1-α (PGC-1α), a master regulator of mitochondrial biogenesis and function. Expression of PGC-1α subunits is reduced in models of SBMA [53], indicating that mitochondrial impairment might also be a feature of SBMA disease pathogenesis, although this hypothesis remains to be fully explored (Fig. 5.3). Finally, reductions in expression of dynactin 1, a microtubule motor protein that regulates axonal trafficking, have also been reported in SBMA transgenic mice [54]. Thus, dysregulation of the AR transcriptome in the context of polyQ-AR results in a variety of energetic and metabolic alterations that may underlie different aspects of disease.

5.4.2 Proteostasis Dysfunction

PolyQ-expanded AR misfolds to form inclusions or aggregates, posing a direct challenge to the two main cellular proteostatic systems of the cell: the ubiquitin proteasome system (UPS) and the autophagy-lysosomal pathway. Both neurons and skeletal muscle rely heavily upon maintaining protein quality control through

highly efficient degradation mechanisms, perhaps explaining the selective vulnerability of these tissues to polyQ-AR toxicity. Here we examine our current understanding of UPS and autophagy dysfunction in SBMA, and highlight the novel role of AR as a regulator of lysosomal-mediated clearance.

5.4.2.1 Chaperone and Heat Shock Protein Alterations in SBMA

In SBMA, polyQ-AR forms nuclear inclusions in motor neurons, skeletal muscle, and other tissues. These inclusions are heavily ubiquitinated, suggesting a failure of UPS-mediated degradation [18]. Indeed, many components of the UPS, including subunits of the proteasome and chaperones, are known to co-localize with polyQ-AR NIs. This suggests that NIs represent both the intrinsic aggregation properties of polyQ-AR and an impairment of degradative pathways to process accumulating misfolded species.

Heat-shock proteins (Hsps) play crucial roles in maintaining proper protein folding, assembly, and transport, rapidly responding to cellular proteotoxic stress. In particular, Hsp70 and Hsp90 are essential components of the AR-chaperone cytosolic complex, and they regulate function, nuclear translocation, and degradation of AR [10]. Protein chaperones are often found in polyQ-AR inclusions, suggesting two non-mutually exclusive possibilities: a sequestration of heat-shock proteins, or an attempt by cellular protein quality control systems to clear out these aggregates (Fig. 5.3). PolyQ-AR delays the induction of Hsp70 after heat shock [55], and Hsp expression levels are decreased in models of SBMA [56, 57], suggesting that dysfunction of the heat shock response machinery may underlie polyQ-AR toxicity. A variety of chaperones have been tested for their ability to enhance polyQ-AR clearance, including Hsp40, Hsp70, Hsp90, Hsp105, and DnaJ-like-1 proteins [58–61]. These chaperones work through different mechanisms to ultimately prevent polyQ-AR toxicity, including chaperone-mediated refolding, preventing aggregate formation, and facilitating ubiquitination and UPS-mediated degradation. More recently, in vivo studies have shown beneficial effects in SBMA AR97Q transgenic mice by activating the heat-shock response through lentiviral delivery of Heat-shock factor-1 (HSF-1), a transcriptional regulator of Hsps [57] or by overexpression of Hsp70 [58]. These effects correlated with suppression of polyQ-AR accumulation, suggesting enhanced degradation of the mutant protein, although the cellular degradation pathway responsible for this effect was not clearly delineated in either study. ATP-independent Hsps such as heat shock protein B8 (HspB8), can also reduce polyQ-AR aggregation, in this case by targeting polyQ-AR towards autophagy-mediated degradation [62]. Both HspB8 and its co-chaperone BAG3 are robustly upregulated together with other specific HspB8 interactors in SBMA AR113Q skeletal muscle [63].

The overall power of chaperone proteins to prevent polyQ-AR misfolding and aggregation is evident, and represents an appealing strategy for disease therapy. However, it is important to remember that the chaperone system handles the folding and dynamics of a variety of client proteins, many of which are essential for neuron and cellular function. Thus, rather than targeting the heat-shock protein system as a whole, the goal should be to identify compounds that specifically alter the polyQ-AR - chaperone interaction.

5.4.2.2 Autophagy Dysfunction in SBMA

Macroautophagy (hereafter autophagy) is an evolutionarily conserved lysosomal degradation pathway that functions to eliminate toxic macromolecules and dysfunctional organelles. Cytosolic cargo is enveloped by a double-layered membrane, generating a novel organelle known as an autophagosome. Autophagosomes then undergo a series of intermediate maturation steps, interacting with the endocytic and secretory pathway, until their final fusion with lysosomes to generate degradative autolysosomes. Cargo is degraded in the lysosomal lumen by hydrolytic enzymes, and essential building blocks are translocated back into the cytosol for re-use. Autophagy cargo can include protein aggregates and damaged mitochondria, substrates too large to be threaded through the pore of the proteasome. Furthermore, autophagy is a particularly important degradation pathway in polyQ diseases, as eukaryotic proteasomes may not efficiently degrade long polyQ tracts. Instead, autophagy can degrade both aggregated and soluble forms of polyQ proteins, indicating that autophagy is likely a preferential degradation pathway in polyQ disease. Recent discoveries further implicate polyQ proteins in the normal regulation of autophagy, suggesting polyQ proteins play a dual role as both autophagy substrates and autophagy regulators [19, 64] (Fig. 5.3).

Evidence has revealed a potential role for autophagy dysregulation in the pathogenesis of SBMA. Over-expression of polyQ-AR leads to the accumulation of LC3 puncta, a classical autophagy marker associated with increased autophagosome numbers [65]. This correlated with accumulations of electron dense AVs in close proximity to lysosomal structures in cultured cells overexpressing polyQ-AR [66]. In a *Drosophila* model of SBMA, polyQ-AR eye toxicity is accompanied by AV and multivesicular-body accumulation [27]. Additionally, motor neurons of the YAC AR100 transgenic mouse model have increased numbers of AVs at post-symptomatic stages [3, 4], suggesting alterations in the autophagy pathway are indeed features of SBMA.

Recent evidence from our lab suggests that transcriptional inhibition of autophagy signaling, rather than activation of autophagy, underlies the observed accumulation of AVs in SBMA [3, 4]. By undertaking an exhaustive analysis of stable cell lines, transgenic mice, and patient IPSC-derived neuronal progenitor cells (NPCs), we uncovered a profound transcriptional inhibition of Transcription Factor E-B (TFEB), a master regulator of autophagy and lysosomal function, in SBMA. We determined that while SBMA cells and motor neurons were competent for autophagy initiation and autophagosome formation, they failed to successfully complete autophagic degradation, due to marked reductions in TFEB target gene expression (Fig. 5.3) [3, 4]. We identified a novel interaction between TFEB and polyQ-AR, suggesting that TFEB dysregulation might account for autophagic flux impairments present in SBMA. Importantly, we restored autophagy flux by over-expressing TFEB in patient-derived NPCs [3, 4], as a proof-of-concept that modulation of TFEB activity could be an important target for therapy development for SBMA. Indeed, treatment of SBMA AR97Q transgenic mice with paneoflorin, a plant extract, partly exerted therapeutic effects on behavioral and pathological

phenotypes by strongly upregulating TFEB expression [67]. Furthermore, we also found evidence of an interaction between normal Q-length AR and TFEB, and detected enhanced TFEB signaling and increased autophagy pathway activity when normal AR is overexpressed [3, 4]. Our data suggest that AR can normally interact with TFEB to promote its function, spatially regulating TFEB activity in response to testosterone. Thus, we have identified a previously unknown AR function on autophagy through interaction with TFEB, and documented a change-of-function upon expansion of the polyQ tract in AR, resulting in TFEB inhibition. As AR normally interacts with many transcription co-regulators and studies of a SBMA fly model indicate that polyQ-AR may promote neurotoxicity by reducing the function of the co-regulators with which it interacts [23], reduced availability of a co-activator protein shared by AR and TFEB may result in decreased TFEB transactivation in SBMA.

Similar to HD, evidence suggests that autophagy is also playing a neuroprotective role in SBMA. Genetic ablation of autophagy in *Drosophila* exacerbates polyQ-AR eye degeneration phenotypes [27] and depletion of p62, an autophagy cargo-adaptor, in AR97Q transgenic mice significantly worsens motor and neurological phenotypes [20]. Conversely, pharmacological activation of autophagy through rapamycin treatment suppresses polyQ-AR eye degeneration, and this effect is dependent on functional autophagy [27]. Treatment of AR97Q mice with 17-allylamino-17-demethoxygeldanamycin (17-AAG) also markedly ameliorated motor impairments by reducing amounts of monomeric and aggregated mutant AR [68]. 17-AAG is a potent Hsp90 inhibitor, and experiments have shown that it can potently enhance autophagic degradation of polyQ-AR [69], although the dependence on autophagy function for SBMA disease rescue by 17-AAG in vivo remains unknown.

It is interesting that autophagy, a cytoplasmic degradation pathway, can counteract polyQ-AR toxicity, which appears to originate from ligand-dependent nuclear accumulation of AR. The explanation most likely lies in the progressive nature of aggregation: degradation of cytoplasmic polyQ-AR oligomers via autophagy would reduce polyQ-AR available for nuclear aggregation and toxicity. In agreement with this, transgenic mice expressing polyQ-AR with a deleted nuclear localization signal (AR dNLS112Q), which results in cytoplasmic retention of AR, show substantially improved motor phenotypes compared to non-mutated full-length polyQ-AR models [47]. Autophagy induction was detected in AR dNLS112Q cultured motor neurons, and inhibition of autophagy augmented testosterone-mediated toxicity in this model [47], further suggesting that targeting of cytosolic polyQ-AR for autophagy degradation could have important beneficial effects in SBMA. Indeed, 17-AAG inhibition of Hsp90 prevents conformational changes in AR necessary to bind androgens, preventing its nuclear translocation and thus facilitating autophagic clearance of polyQ-AR [69]. Depletion of autophagy cargo-marker p62 increases the accumulation of insoluble, aggregated AR in AR97Q mice, strongly suggesting a fraction of polyQ-AR is indeed degraded

through autophagy in vivo [20]. Further studies to increase our understanding of polyQ-AR aggregation dynamics, including the identification of toxic intermediate species, as well as an analysis of the role of p62-recognition of polyQ-aggregates, are needed to clarify the basis for these effects.

5.4.2.3 Autophagy Dysfunction in SBMA

We have recently demonstrated that skeletal muscle plays a primary role in SBMA disease pathogenesis, and is largely responsible for the motor neuron toxicity [3, 4, 70]. Most interestingly, while TFEB activity in SBMA motor neurons and patient-derived NPCs was significantly reduced, analysis of quadriceps muscle samples from symptomatic 14 month-old AR100 transgenic mice yielded an opposite and dramatic up-regulation of TFEB target genes [3, 4], consistent with studies in SBMA knock-in AR113Q mice [71]. This suggests a muscle-specific process of supra-physiological induction of TFEB in diseased SBMA muscle cells. Additionally, recent evidence indicates that HspB8-mediated protein quality control is shifted towards hyperactive autophagy degradation in SBMA muscle [63], suggesting a novel mechanism for polyQ-AR toxicity, wherein polyQ-AR induces a potent autophagy response in muscle cells (Fig. 5.3). Since uncontrolled autophagy is thought to underlie muscle wasting in several models of muscular dystrophy [72], excessive activation of autophagy could contribute to SBMA skeletal muscle degeneration. In agreement with this hypothesis, global reduction of autophagy activity by Beclin-1 haploinsufficiency in SBMA knock-in AR113Q mice increased skeletal muscle fiber size and significantly extended lifespan in this model [73]. Additionally, it is possible that IGF-1 overexpression restores SBMA muscle health via inhibition of FOXO transcription factor, a known activator of muscle autophagy, although this remains to be examined [31].

Interestingly, other regulators of proteostasis also appear to be modulated in a tissue-specific manner in SBMA. HSF-1, a master regulator of the heat-shock response, is reduced in motor neurons and spinal cord of AR97Q mice, but is significantly elevated in SBMA skeletal muscle [57]. The mechanisms accounting for the different tissue responses in TFEB or Hsf-1 dysregulation remain unknown, but are most likely due to cell-type specific alterations in the AR interactome tipping the scales one way or the other. In any case, these results strongly suggest that systemic delivery of autophagy therapies could actually have deleterious effects in SBMA. Understanding the cross-talk between SBMA skeletal muscle and motor neurons, and identifying key players that regulate TFEB activity in a tissue-specific manner will be essential for SBMA translational research. Importantly, however, the non-cell autonomous contribution of skeletal muscle to motor neuron toxicity in SBMA, and the accessibility of skeletal muscle should facilitate delivery of drugs to the affected cell populations.

5.4.3 Other Cellular Pathways and Processes

5.4.3.1 Mitochondrial Dysfunction

Neurons have high energetic demands, requiring a particularly efficient mitochondrial network, Consequently, mitochondrial dysfunction has a major impact on neuronal function and survival. Several findings suggest a key role for mitochondrial dysfunction in SBMA (Fig. 5.3). First, the distribution of mitochondria in neurons is altered in the presence of polyQ-AR, likely due to defects in mitochondrial trafficking [74]. Accumulation of depolarized mitochondria and reactive oxygen species in cell culture models of SBMA also reflects an intrinsic failure of mitochondrial metabolism in SBMA [53, 74], which may stem from transcription interference with PGC-1α, a master regulator of mitochondrial biogenesis and oxidative energy production [53]. Furthermore, analysis of the mitochondrial DNA genome in SBMA patient leukocytes reveals sizable deletions, a marker of mitochondrial impairment commonly associated with aging. Whether this molecular pathology represents late-stage disease changes or is in actuality a primary driver of disease pathogenesis remains unclear. Lastly, polyQ-AR N-terminal fragments can activate a mitochondrial-originating Bax-dependent apoptotic cascade, potentially connecting mitochondrial failure with neuronal demise [29]. Nonetheless, the precise role of mitochondrial dysfunction in SBMA remains unclear. As antioxidants, such as coenzyme Q10, can reduce levels of reactive oxygen species in polyQ-AR expressing cells [53], the potential for therapy development based upon these findings exists, although such treatment strategies are yet to be studied in animal models of SBMA.

5.4.3.2 ER Stress in SBMA

The endoplasmic reticulum (ER) is a major regulator of cellular protein 'quality control', serving as a processing center for the synthesis and folding of nascent membrane and secretory proteins. Various physiological and pathological conditions can impair the folding capacity of the ER, leading to the accumulation of misfolded proteins in the ER lumen, a process known as 'ER stress'. This triggers the unfolded protein response (UPR), an ER-to-nucleus cellular stress response designed to increase the capacity of the ER to fold its client proteins. ER stress has become increasingly important in the understanding of several neurodegenerative proteinopathies, including Huntington's disease, though the role of ER stress in SBMA is still uncertain (Fig. 5.3). Evidence for ER stress in the SBMA pathogenic cascade comes from studies in which N-terminal fragments of polyQ-AR can activate the UPR in vitro [75]. Markers of ER stress are found in motor neurons of presymptomatic SBMA AR100 transgenic mice, implying that ER stress is among the initial insults to motor neuron health in SBMA [76]. ER stress has also been described in skeletal muscle from SBMA AR113Q knock-in mice and SBMA patients [73]. Inhibition of ER stress with salubrinal significantly reduced the activation of ER stress-associated apoptosis by suppressing the activation of caspase-12 in SBMA cell models [76], highlighting drug targeting of ER stress as a potential therapeutic strategy for SBMA.

5.4.3.3 Axonal Vesicle Transport

Of particular importance to motor neurons is the ability to ferry cargo to and from dendrites, with some motor neuron processes extending as far as a meter away, demonstrating that normal axonal transport is likely essential to motor neuron health. Consistent with this view, disruption in axonal motor transport alone is sometimes sufficient to cause motor neuron disease in mice and humans. PolyQ-AR can impair both axonal anterograde and retrograde axonal transport [54, 77] (Fig. 5.3). A marked deficit in retrograde labeling of motor neurons occurs very early in disease in SBMA AR97Q transgenic mice, beginning prior to the neuromuscular phenotype [54]. This axonal transport defect in this SBMA mouse model has been linked to reductions in motor protein dynactin 1. Similar deficits in dynactin-1 in motor neurons have also been detected in SBMA patients [54], but patient material has been ascertained from affected patients or post-mortem, precluding clear interpretation of its pathogenic significance. A 'myogenic' mouse model, which overexpresses AR carrying 22 glutamines (normal repeat size) in skeletal muscle, recapitulates certain disease aspects of SBMA accompanied by striking deficits in retrograde labeling of sciatic nerve motor neurons [78]. These data support the concept of 'non-cell autonomous' disease pathogenesis in SBMA [3, 4], wherein AR-mediated pathology in skeletal muscle leads to neurotoxic effects in the enervating motor neurons. Similar trafficking defects were also reported in motor neurons from SBMA AR113Q knock-in mice [78]. These effects were dependent on androgens, as similar trafficking deficits were detected in symptomatic normal AR-over-expressing myogenic females upon testosterone administration [78]. However, studies of motor neurons from SBMA YAC AR100 transgenic mice challenge these findings, as no abnormalities of axonal transport or expression changes in microtubule-binding and microtubule-associated proteins were found [79]. One possible explanation for these differences could be that SBMA YAC AR100 mice display levels of mutant AR expression roughly equivalent to endogenous mouse AR [7], while the SBMA AR97Q model and the AR22Q myogenic model exhibit very high-level over-expression [5]. As overexpression of normal AR alone can cause neuromuscular disease phenotypes [22], such results obtained from SBMA mouse models that do not recapitulate proper expression levels may reflect cellular processes not occurring in the human disease.

5.5 Cellular Basis of SBMA

SBMA has traditionally been regarded as a motor neuronopathy, with the initial toxic insult originating within motor neurons (or other CNS cells), and then leading to skeletal muscle dysfunction. However, recent work has challenged this paradigm, and many lines of evidence now suggest a less straightforward mechanism in SBMA. First, muscle biopsies of affected SBMA patients consistently show mixed pathology, with both neurogenic and myopathic features [12]. Second,

overexpression of wild-type, non-expanded AR in skeletal muscle in mice is sufficient to generate neuromuscular phenotypes reminiscent of SBMA (including axonopathy and gender bias) [22]. Third, muscle pathology is an early finding in SBMA AR113Q knock-in mice, detectable long before any abnormalities appear in motor neurons [80]. These findings indicate that the myopathic features observed in SBMA patients may not be entirely secondary, and that skeletal muscle could be playing a role in disease pathogenesis. Indeed, in an early onset, progressive glycolytic-to-oxidative fiber type switch in SBMA AR113Q transgenic mice may reflect a selective sensitivity of glycolytic skeletal muscle fibers to polyQ-AR toxicity, prior to any neurodegeneration [81].

Our lab directly evaluated the contribution of skeletal muscle to SBMA disease pathogenesis by dissecting the role of skeletal muscle in an in vivo mouse model. We found that eliminating expression of polyQ-AR exclusively from skeletal muscle, while retaining strong polyQ-AR expression in the spinal cord, completely rescues neuromuscular and systemic disease phenotypes in SBMA transgenic mouse (BAC fxAR121) [3, 4]. These results indicate that skeletal muscle is a principal site for polyQ-AR toxicity, and indeed suggest that targeting muscle may be a viable and successful therapeutic avenue for SBMA. To independently confirm this result, we treated SBMA polyQ-AR mouse models with an antisense-oligonucleotide (ASO) directed against AR mRNA. Using the BAC fxAR121 mouse model described above, we found that peripheral ASO treatment greatly reduced polyQ-AR protein expression levels in skeletal muscle (and other peripheral tissues), but not in spinal cord. This yielded a very significant rescue of weight loss, muscle weakness, motor neuron degeneration, and shortened lifespan in BAC fxAR121 mice [70]. More importantly, this effect could be achieved even if peripheral ASO treatment began after disease onset, suggesting this approach should be applicable to patients, who are generally diagnosed after clinical findings appear.

Targeting skeletal muscle for therapeutic intervention has numerous advantages over targeting the CNS. First, drug delivery to muscle is much more tractable than for the spinal cord. Second, muscle is readily available for tissue sampling during clinical trials, allowing for direct analysis of pharmacological targets and biomarkers. Third (and perhaps most importantly for patients), targeting skeletal muscle could bypass the secondary side effects associated with CNS reduction of AR function, which include loss of libido, lack of focus, and general malaise. This is particularly significant in light of our finding that intraventricular delivery of ASOs, which targets spinal cord but not skeletal muscle expression of polyQ-expanded AR, had no effect on disease onset or progression in SBMA BAC fxAR121 mice [70].

The mechanistic basis for skeletal muscle-mediated polyQ-AR toxicity to motor neurons remains unclear. Skeletal muscle provides trophic support for innervating motor neurons, via expression of key growth factors and electrical impulses that drive motor neuron function. SBMA mice display reduced expression of key neurotrophic factors, including VEGF [7], type II transforming growth factor-beta

receptor (TGF-beta) [82], GDNF and neurotrophin-4 [80]. Thus, polyQ-AR mediated transcriptional dysregulation of growth factors and neuromuscular electrical activity could be directly diminishing the trophic ability of SBMA skeletal muscle. In support of this hypothesis, genes implicated in muscle function, myogenesis, and energy balance are dysregulated in the skeletal muscle of SBMA models [45]. Removal of polyQ-AR from BAC fxAR121 muscle could thus restore its ability to sustain and nourish motor neurons, resulting in disease rescue. Consistent with this hypothesis, muscle-restricted over-expression of IGF-1, a known muscle anabolic factor, improved survival and neuromuscular disease phenotypes in SBMA mice [31].

Despite these findings highlighting the principal role of muscle to SBMA pathogenesis, the inherent vulnerability of motor neurons in SBMA cannot be ignored. Nuclear inclusions in motor neurons and neuronal loss are features of human SBMA disease pathology [13]. Selective expression of polyQ-AR in either motor neurons (NeuroAR) or myocytes (MyoAR) in SBMA transgenic mice supports both neurogenic and myogenic contributions to several aspects of pathology, although the expression levels achieved in this study were too low to allow for proper toxicity to manifest [6]. Recently, ASOs were used to efficiently suppress polyQ-AR in the CNS via a single intracerebroventricular injection in AR97Q transgenic mice. This delayed the onset and progression of motor dysfunction, maintained body weight gain, ameliorated neuropathology, and improved survival in SBMA AR97Q transgenic mice [83]. It is important to note, however, that the therapeutic rescue by this CNS-only ASO delivery was much less striking than that obtained with systemic, non CNS-penetrating, ASO administration in more physiological models (i.e. the BAC fxAR121 transgenic and AR113Q knock-in mice) [70]. However, as timing and dosage of ASO delivery differed between these studies, further work may be required to clarify the contribution of peripheral and central AR suppression for therapeutic intervention in SBMA. Nonetheless, studies published over the last five years have discovered a central role for skeletal muscle in SBMA disease pathogenesis. This realization is provocative and unexpected, and provides a new paradigm for SBMA therapy development.

5.6 Therapeutic Opportunities in SBMA

In recent years, a number of treatment strategies for SBMA have emerged, and some have progressed to clinical trials. Here we describe the current status of certain of these approaches, and how the results from previous clinical trials may inform upcoming trial design and execution.

5.6.1 Post-transcriptional Silencing of PolyQ-AR Expression

SBMA belongs to the polyQ disease category, a family of inherited neurodegenerative disorders. This provides an obvious therapeutic target: the mutant protein itself. Several strategies to reduce mutant AR protein expression are currently at different stages of testing for human delivery, but they share a similar strategy: post-transcriptional gene silencing. Of the approaches being pursued, ASOs have been shown to efficiently "knock-down" peripheral and CNS expression of mutant AR in vivo and results in important therapeutic effects in mouse models of SBMA [70, 83]. These studies are important proof-of-concept that ASO targeting of polyQ-AR expression is indeed feasible, could have potent in vivo effects, and should be explored in SBMA patients.

MicroRNAs (miRNAs) are another tool for target suppression in vivo, and have been shown to efficiently knock-down polyQ-expanded proteins ataxin-1 and ataxin-3 [84, 85]. Importantly, as miRNAs employ endogenous molecular pathways and are smaller in size than ASOs, miRNAs should be safe and well tolerated in human patients, and should be less likely to elicit an immunogenic response. Intramuscular viral delivery of microRNA 196a (miR-196a) to AR97Q mice reduced polyQ-AR expression in vivo, indirectly enhancing the decay of AR mRNA by CELF2, a silencing protein involved in the regulation of AR mRNA stability, and effectively rescuing SBMA disease phenotypes in AR97Q mice [86]. Similar results were obtained by intravenous viral delivery of miR-298, a novel direct suppressor of human AR translation [87]. Interestingly, levels of both miR196a and miR-298 were found to be altered in SBMA mice [86, 87], suggesting that dysfunctional processing of mature miRNAs may contribute to SBMA disease pathogenesis, though this hypothesis remains speculative at present. Nonetheless, these findings indicate that treatments aimed at reducing AR levels are very appealing, and that viral delivery of miRNA could be a therapeutic strategy for SBMA and other polyQ diseases. One key advantage of silencing of AR via miRNAs or ASOs is that this therapy does not reduce circulating testosterone levels, and thus should not produce the side effects associated with androgen-reduction therapy [70, 86].

5.6.2 Modulating Proteostasis Function

Protein misfolding and aggregation are hallmarks of SBMA and other polyQ diseases, as the generation of toxic misfolded species is a key step in the pathogenic cascade. Thus, management of the toxic conformers, either through re-folding or clearance, has great potential as a therapeutic strategy in diseases associated with protein aggregation. Several approaches to reduce protein misfolding in SBMA have been pursued, mostly by targeting members of the heat-shock protein

(Hsp) family. AR is normally stabilized in the cytosol through its interactions with Hsps, and Hsp levels appear reduced in SBMA mouse models [56, 58]. Furthermore, pharmacological induction of the Hsp system via oral administration of geranylgeranylacetone (GGA) ameliorated SBMA phenotypes in AR97Q mice [56], and treatment with the potent Hsp90 inhibitor, 17-allylamino geldanamycin (17-AAG), achieved proteasomal degradation of polyQ-AR and rescued SBMA disease phenotypes in AR97Q transgenic mice without detectable toxicity [68]. A more potent version of 17-AAG, 17-dimethylaminoethylamino-17-demetoxygeldanamycin (17-DMAG), also had beneficial therapeutic effects [88]. Overexpression of Hsp70 is an effective treatment in SBMA AR97Q mice, by also promoting the proteosomal degradation of polyQ-AR [58]. Recently, the plant-derived compound paneoflorin was shown to increase chaperone expression and enhance ubiquitin-proteasome system and autophagy activity in AR97Q mice, mitigating neuromuscular and histopathology phenotypes in SBMA AR97Q mice [67]. Although these compounds were effective in pre-clinical trials in SBMA mice, non-specific induction of Hsp levels can have significant deleterious consequences at an organismal level that preclude their use in SBMA patients. However, a novel co-inducer of the heat-shock response, arimoclomol, likely only targets stressed cells in which the heat-shock response has already been engaged, and was found to be effective in SBMA YAC AR100 mice [89]. Indeed, arimoclomol achieved similar neuroprotective effects in ALS mice, and is now in Phase 2 clinical trials for ALS1 (SOD1) patients, suggesting it may have wider application in motor neuron diseases.

Dysfunction of the autophagy pathway has been identified as a feature of SBMA disease pathogenesis [3, 4, 63, 71]. Rapamycin, a commonly used autophagy-inducer, was found to be neuroprotective in a *Drosophila* model of SBMA, but this effect was dependent on intact autophagy function [27]. However, the availability of nuclear polyQ-AR aggregates to autophagy degradation, a process restricted to the cytosol, is unclear. Recent work with an interesting combination treatment of bicalutamide (which slows down AR nuclear translocation) and trehalose (which induces autophagy) increased the recognition of AR misfolded conformers by the autophagy prior to their migration into the nucleus in in vitro SBMA models [90]. Trehalose has already been shown to have important neuroprotective effects in ALS mice [91], suggesting that this combined treatment strategy could have potential application in SBMA. However, correction of the underlying transcriptional inhibition of autophagy via the polyQ-AR—TFEB interaction may also be necessary to achieve therapy success.

5.6.3 Androgen Deprivation

It is well established that SBMA disease pathology occurs in males, because they have high levels of circulating androgens. Testosterone reduction can also be achieved by gonadotropin analogues, such as leuprorelin, compounds that are part

of the battery of treatments currently available for AR-dependent prostate cancer. Leuprorelin was remarkably protective for lifespan and neuromuscular phenotypes in AR97Q SBMA mice [21]. As such, leuprorelin was enthusiastically advanced to the clinic for several small SBMA clinical trials, but the results of these clinical trials have been mixed. While all trials reported significant decreases in polyQ-AR accumulation in patient biopsies, little to no rescue in clinical outcomes could be detected [82, 92, 93], and side effects of androgen ablation are quite significant. More recently, a randomized, single-site clinical trial with dutasteride, a potent inhibitor of the testosterone-to-DHT conversion step, was completed, but with no differences between the treatment groups [94]. Despite the lack of significant therapeutic benefit, the dramatic effects of androgen deprivation in SBMA mice and strong evidence for androgen-dependence in human patients indicate that further investigation of androgen ablation is warranted. Improving clinical trial design and selecting the most sensitive parameters for such a slowly progressing disease are likely key prerequisites for unmasking the small, but clinically relevant effects, of drug treatments in SBMA, and could uncover therapeutic benefit for androgen ablation in the future. Nonetheless, while leuprorelin is approved and widely used, androgen ablation therapy is associated with significant side effects in humans, including decreased libido, osteoporosis, and fatigue, and these side effects clearly impact a patient's quality of life. For this reason, androgen ablation is not an ideal therapeutic strategy for SBMA, even if eventually found to be an efficacious treatment option.

5.6.4 Modulation of AR Function

Recent studies on SBMA and other polyQ diseases indicate that alteration of the normal function of the disease protein is a key aspect of the pathogenic cascade [3, 4]. Therapeutic strategies aimed at restoring these 'change-of-function' phenotypes are of particular relevance for SBMA, where the biology of AR is very well understood. Building on this model, one approach is to target the AR interactome, an important contributor to polyQ-AR toxicity [23]. Curcumin related compound 5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one (ASC-J9) improved disease phenotypes in SBMA AR97Q mice, likely by disrupting the interaction between AR and its co-regulator ARA70, although the mechanism of ASC-J9 is not well understood [95]. Notably, ASC-J9 treatment ameliorated SBMA phenotypes by decreasing AR97Q aggregation and increasing VEGF164 expression, but with little change in serum testosterone. Moreover, mice treated with ASC-J9 retained normal sexual function and fertility [95]. Recently, another curcumin analogue, ASC-JM17, was found to ameliorate polyQ-AR toxicity in SBMA mice via activation of Nrf1, Nrf2, and Hsf1, thereby increasing the expression of proteasome subunits, antioxidant enzymes, and molecular chaperones [96].

Post-translational modifications are another potential target to modify polyQ-AR activity. Genetic overexpression of IGF-1 in skeletal muscle rescues neuromuscular

and systemic phenotypes in SBMA mice [31], and pharmacological induction of the IGF-1/Akt axis improves motor performance and increases survival in SBMA AR97Q mice, even when treatment is started after disease onset [97]. Interestingly, IGF-1 is a well-established muscle anabolic and motor neuron trophic factor, and can thus have additional benefits in the neuromuscular system independent of AR modifications. Similar activation of Akt-mediated phosphorylation of AR underlies the neuroprotective effects of hepatocyte growth factor (HGF), when used in combination with castration in SBMA AR97Q mice [98]. Furthermore, a clinical trial using clenbuterol, a β2-adrenoreceptor agonist that has an anabolic effect on muscle through the activation of the PI3 K/Akt signaling pathway, yielded some evidence for decreased disease progression [99], but its direct effect on AR phosphorylation was not analyzed.

5.6.5 Future Therapeutic Target

A provocative study recently showed that feeding AR113Q knock-in mice a high-fat diet (HFD) restored molecular phenotypes, related to aberrant muscle fiber type switching, ameliorated muscle pathology, and extended survival [81]. That the intrinsic metabolic changes in SBMA muscle can be modulated by changing nutrient intake is a promising result, but care should be taken before attempting to translate these findings to the clinic. If such profound metabolic changes are indeed a feature in SBMA patients, then high-fat dietary modifications should be carefully determined by a physician and a nutritionist to prevent potentially serious side effects. As SBMA therapeutic approaches target different molecular pathways, combinatorial treatments should be pursued in the future. For example, ASO/miRNA silencing of polyQ-AR together with growth factor supplementation to increase trophic factor release from skeletal muscle, when combined, may yield synergistic benefits to SBMA patients.

5.7 Concluding Remarks

Research into the pathobiology of SBMA has progressed dramatically in the last decade, revealing new insights into the cellular and molecular basis of this disease. Of course, the molecular pathology of SBMA remains complicated, with more than one mechanism likely contributing to SBMA disease pathogenesis. Perhaps the most important advance has been the realization that SBMA, long believed to be a primarily motor neuron disease, is in reality a disease initiated in skeletal muscle [3, 4, 70]. Understanding how mutant AR elicits motor neuron demise will not only reveal important insights into SBMA pathogenesis, with implications for rational therapy development, but will also yield pathways and processes crucial to motor neuron health, with relevance to all motor neuron diseases, including ALS and

SMA. Our current understanding of the cellular basis of SBMA thus signals a major shift in therapy design, as muscle is a readily accessible tissue for compound delivery. Developing promising new treatments for SBMA for use in human patients, however, will only be successful if clinical trial design and outcome measures are modified to permit sensitive detection of clinical improvement, as the signal to noise ratio for such a slowly progressing disease is low.

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References

1. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352 (6330):77–79
2. Bosch-Marce M, Wee CD, Martinez TL, Lipkes CE, Choe DW, Kong L, Van Meerbeke JP, Musaro A, Sumner CJ (2011) Increased IGF-1 in muscle modulates the phenotype of severe SMA mice. Hum Mol Genet 20(9):1844–1853
3. Cortes CJ, Ling SC, Guo LT, Hung G, Tsunemi T, Ly L, Tokunaga S, Lopez E, Sopher BL, Bennett CF, Shelton GD, Cleveland DW, La Spada AR (2014) Muscle expression of mutant androgen receptor accounts for systemic and motor neuron disease phenotypes in spinal and bulbar muscular atrophy. Neuron 82(2):295–307
4. Cortes CJ, Miranda HC, Frankowski H, Batlevi Y, Young JE, Le A, Ivanov N, Sopher BL, Carromeu C, Muotri AR, Garden GA, La Spada AR (2014) Polyglutamine-expanded androgen receptor interferes with TFEB to elicit autophagy defects in SBMA. Nat Neurosci 17(9):1180–1189
5. Katsuno M, Adachi H, Kume A, Li M, Nakagomi Y, Niwa H, Sang C, Kobayashi Y, Doyu M, Sobue G (2002) Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron 35(5):843–854
6. Ramzan F, McPhail M, Rao P, Mo K, Halievski K, Swift-Gallant A, Mendoza-Viveros L, Cheng HY, Monks DA (2015) Distinct etiological roles for myocytes and motor neurons in a mouse model of kennedy's disease/spinobulbar muscular atrophy. J Neurosci 35(16):6444–6451
7. Sopher BL, Thomas PS Jr, LaFevre-Bernt MA, Holm IE, Wilke SA, Ware CB, Jin LW, Libby RT, Ellerby LM, La Spada AR (2004) Androgen receptor YAC transgenic mice recapitulate SBMA motor neuronopathy and implicate VEGF164 in the motor neuron degeneration. Neuron 41(5):687–699
8. Ono S, Geller LN, Lai EV (1974) TfM mutation and masculinization versus feminization of the mouse central nervous system. Cell 3(3):235–242
9. Sato T, Matsumoto T, Yamada T, Watanabe T, Kawano H, Kato S (2003) Late onset of obesity in male androgen receptor-deficient (AR KO) mice. Biochem Biophys Res Commun 300(1):167–171
10. Matsumoto T, Sakari M, Okada M, Yokoyama A, Takahashi S, Kouzmenko A, Kato S (2013) The androgen receptor in health and disease. Annu Rev Physiol 75:201–224
11. Clark PE, Irvine RA, Coetzee GA (2003) The androgen receptor CAG repeat and prostate cancer risk. Methods Mol Med 81:255–266
12. Soraru G, D'Ascenzo C, Polo A, Palmieri A, Baggio L, Vergani L, Gellera C, Moretto G, Pegoraro E, Angelini C (2008) Spinal and bulbar muscular atrophy: skeletal muscle pathology in male patients and heterozygous females. J Neurol Sci 264(1–2):100–105

13. Katsuno M, Tanaka F, Adachi H, Banno H, Suzuki K, Watanabe H, Sobue G (2012) Pathogenesis and therapy of spinal and bulbar muscular atrophy (SBMA). Prog Neurobiol 99:246–256
14. Kobayashi Y, Miwa S, Merry DE, Kume A, Mei L, Doyu M, Sobue G (1998) Caspase-3 cleaves the expanded androgen receptor protein of spinal and bulbar muscular atrophy in a polyglutamine repeat length-dependent manner. Biochem Biophys Res Commun 252(1):145–150
15. Jochum T, Ritz ME, Schuster C, Funderburk SF, Jehle K, Schmitz K, Brinkmann F, Hirtz M, Moss D, Cato AC (2012) Toxic and non-toxic aggregates from the SBMA and normal forms of androgen receptor have distinct oligomeric structures. Biochimica et biophysica acta 1822 (6):1070–1078
16. Adachi H, Katsuno M, Minamiyama M, Waza M, Sang C, Nakagomi Y, Kobayashi Y, Tanaka F, Doyu M, Inukai A, Yoshida M, Hashizume Y, Sobue G (2005) Widespread nuclear and cytoplasmic accumulation of mutant androgen receptor in SBMA patients. Brain J Neurol 128(Pt 3):659–670
17. Li M, Chevalier-Larsen ES, Merry DE, Diamond MI (2007) Soluble androgen receptor oligomers underlie pathology in a mouse model of spinobulbar muscular atrophy. J Biol Chem 282(5):3157–3164
18. Li M, Miwa S, Kobayashi Y, Merry DE, Yamamoto M, Tanaka F, Doyu M, Hashizume Y, Fischbeck KH, Sobue G (1998) Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. Ann Neurol 44(2):249–254
19. La Spada AR, Taylor JP (2010) Repeat expansion disease: progress and puzzles in disease pathogenesis. Nat Rev Genet 11(4):247–258
20. Doi H, Adachi H, Katsuno M, Minamiyama M, Matsumoto S, Kondo N, Miyazaki Y, Iida M, Tohnai G, Qiang Q, Tanaka F, Yanagawa T, Warabi E, Ishii T, Sobue G (2013) p62/SQSTM1 differentially removes the toxic mutant androgen receptor via autophagy and inclusion formation in a spinal and bulbar muscular atrophy mouse model. J Neurosci 33(18):7710–7727
21. Katsuno M, Adachi H, Doyu M, Minamiyama M, Sang C, Kobayashi Y, Inukai A, Sobue G (2003) Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. Nat Med 9(6):768–773
22. Monks DA, Johansen JA, Mo K, Rao P, Eagleson B, Yu Z, Lieberman AP, Breedlove SM, Jordan CL (2007) Overexpression of wild-type androgen receptor in muscle recapitulates polyglutamine disease. Proc Natl Acad Sci U S A 104(46):18259–18264
23. Nedelsky NB, Pennuto M, Smith RB, Palazzolo I, Moore J, Nie Z, Neale G, Taylor JP (2010) Native functions of the androgen receptor are essential to pathogenesis in a Drosophila model of spinobulbar muscular atrophy. Neuron 67(6):936–952
24. Takeyama K, Ito S, Yamamoto A, Tanimoto H, Furutani T, Kanuka H, Miura M, Tabata T, Kato S (2002) Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. Neuron 35(5):855–864
25. Pennuto M, Basso M (2015) In vitro and in vivo modeling of spinal and bulbar muscular atrophy. J Mol Neurosci 58:365–373
26. Chevalier-Larsen ES, O'Brien CJ, Wang H, Jenkins SC, Holder L, Lieberman AP, Merry DE (2004) Castration restores function and neurofilament alterations of aged symptomatic males in a transgenic mouse model of spinal and bulbar muscular atrophy. J Neurosci 24(20):4778–4786
27. Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, Schwartz SL, DiProspero NA, Knight MA, Schuldiner O, Padmanabhan R, Hild M, Berry DL, Garza D, Hubbert CC, Yao TP, Baehrecke EH, Taylor JP (2007) HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 447(7146):859–863
28. Ellerby LM, Hackam AS, Propp SS, Ellerby HM, Rabizadeh S, Cashman NR, Trifiro MA, Pinsky L, Wellington CL, Salvesen GS, Hayden MR, Bredesen DE (1999) Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. J Neurochem 72(1):185–195

29. Young JE, Garden GA, Martinez RA, Tanaka F, Sandoval CM, Smith AC, Sopher BL, Lin A, Fischbeck KH, Ellerby LM, Morrison RS, Taylor JP, La Spada AR (2009) Polyglutamine-expanded androgen receptor truncation fragments activate a Bax-dependent apoptotic cascade mediated by DP5/Hrk. J Neurosci Off J Soc Neurosci 29(7):1987–1997
30. Anbalagan M, Huderson B, Murphy L, Rowan BG (2012) Post-translational modifications of nuclear receptors and human disease. Nucl Recept Signal 10:e001
31. Palazzolo I, Stack C, Kong L, Musaro A, Adachi H, Katsuno M, Sobue G, Taylor JP, Sumner CJ, Fischbeck KH, Pennuto M (2009) Overexpression of IGF-1 in muscle attenuates disease in a mouse model of spinal and bulbar muscular atrophy. Neuron 63(3):316–328
32. Zboray L, Pluciennik A, Curtis D, Liu Y, Berman-Booty LD, Orr C, Kesler CT, Berger T, Gioeli D, Paschal BM, Merry DE (2015) Preventing the Androgen Receptor N/C Interaction Delays Disease Onset in a Mouse Model of SBMA. Cell reports 13(10):2312–2323
33. LaFevre-Bernt MA, Ellerby LM (2003) Kennedy's disease. Phosphorylation of the polyglutamine-expanded form of androgen receptor regulates its cleavage by caspase-3 and enhances cell death. J Biol Chem 278(37):34918–34924
34. Todd TW, Kokubu H, Miranda HC, Cortes CJ, La Spada AR, Lim J (2015) Nemo-like kinase is a novel regulator of spinal and bulbar muscular atrophy. eLife 4:e08493
35. Ju H, Kokubu H, Todd TW, Kahle JJ, Kim S, Richman R, Chirala K, Orr HT, Zoghbi HY, Lim J (2013) Polyglutamine disease toxicity is regulated by Nemo-like kinase in spinocerebellar ataxia type 1. J Neurosci Off J Soc Neurosci 33(22):9328–9336
36. Kaikkonen S, Jaaskelainen T, Karvonen U, Rytinki MM, Makkonen H, Gioeli D, Paschal BM, Palvimo JJ (2009) SUMO-specific protease 1 (SENP1) reverses the hormone-augmented SUMOylation of androgen receptor and modulates gene responses in prostate cancer cells. Mol Endocrinol 23(3):292–307
37. Mukherjee S, Cruz-Rodriguez O, Bolton E, Iniguez-Lluhi JA (2012) The in vivo role of androgen receptor SUMOylation as revealed by androgen insensitivity syndrome and prostate cancer mutations targeting the proline/glycine residues of synergy control motifs. J Biol Chem 287(37):31195–31206
38. Mukherjee S, Thomas M, Dadgar N, Lieberman AP, Iniguez-Lluhi JA (2009) Small ubiquitin-like modifier (SUMO) modification of the androgen receptor attenuates polyglutamine-mediated aggregation. J Biol Chem 284(32):21296–21306
39. Chan HY, Warrick JM, Andriola I, Merry D, Bonini NM (2002) Genetic modulation of polyglutamine toxicity by protein conjugation pathways in Drosophila. Hum Mol Genet 11 (23):2895–2904
40. Chua JP, Reddy SL, Yu Z, Giorgetti E, Montie HL, Mukherjee S, Higgins J, McEachin RC, Robins DM, Merry DE, Iniguez-Lluhi JA, Lieberman AP (2015) Disrupting SUMOylation enhances transcriptional function and ameliorates polyglutamine androgen receptor-mediated disease. J Clin Investig 125(2):831–845
41. Scaramuzzino C, Casci I, Parodi S, Lievens PM, Polanco MJ, Milioto C, Chivet M, Monaghan J, Mishra A, Badders N, Aggarwal T, Grunseich C, Sambataro F, Basso M, Fackelmayer FO, Taylor JP, Pandey UB, Pennuto M (2015) Protein arginine methyltransferase 6 enhances polyglutamine-expanded androgen receptor function and toxicity in spinal and bulbar muscular atrophy. Neuron 85(1):88–100
42. Lieberman AP, Harmison G, Strand AD, Olson JM, Fischbeck KH (2002) Altered transcriptional regulation in cells expressing the expanded polyglutamine androgen receptor. Hum Mol Genet 11(17):1967–1976
43. Montie HL, Pestell RG, Merry DE (2011) SIRT1 modulates aggregation and toxicity through deacetylation of the androgen receptor in cell models of SBMA. J Neurosci 31(48):17425–17436
44. Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, Pinsky L (1993) Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nat Genet 5(2):184–188

45. Mo K, Razak Z, Rao P, Yu Z, Adachi H, Katsuno M, Sobue G, Lieberman AP, Westwood JT, Monks DA (2010) Microarray analysis of gene expression by skeletal muscle of three mouse models of Kennedy disease/spinal bulbar muscular atrophy. PLoS ONE 5(9):e12922
46. Orr CR, Montie HL, Liu Y, Bolzoni E, Jenkins SC, Wilson EM, Joseph JD, McDonnell DP, Merry DE (2010) An interdomain interaction of the androgen receptor is required for its aggregation and toxicity in spinal and bulbar muscular atrophy. J Biol Chem 285(46):35567–35577
47. Montie HL, Cho MS, Holder L, Liu Y, Tsvetkov AS, Finkbeiner S, Merry DE (2009) Cytoplasmic retention of polyglutamine-expanded androgen receptor ameliorates disease via autophagy in a mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 18 (11):1937–1950
48. Thomas PS Jr, Fraley GS, Damian V, Woodke LB, Zapata F, Sopher BL, Plymate SR, La Spada AR (2006) Loss of endogenous androgen receptor protein accelerates motor neuron degeneration and accentuates androgen insensitivity in a mouse model of X-linked spinal and bulbar muscular atrophy. Hum Mol Genet 15(14):2225–2238
49. McCampbell A, Taylor JP, Taye AA, Robitschek J, Li M, Walcott J, Merry D, Chai Y, Paulson H, Sobue G, Fischbeck KH (2000) CREB-binding protein sequestration by expanded polyglutamine. Hum Mol Genet 9(14):2197–2202
50. Minamiyama M, Katsuno M, Adachi H, Waza M, Sang C, Kobayashi Y, Tanaka F, Doyu M, Inukai A, Sobue G (2004) Sodium butyrate ameliorates phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 13(11):1183–1192
51. Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, Dawson TM, Ross CA (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science 291 (5512):2423–2428
52. Katsuno M, Banno H, Suzuki K, Takeuchi Y, Kawashima M, Yabe I, Sasaki H, Aoki M, Morita M, Nakano I, Kanai K, Ito S, Ishikawa K, Mizusawa H, Yamamoto T, Tsuji S, Hasegawa K, Shimohata T, Nishizawa M, Miyajima H, Kanda F, Watanabe Y, Nakashima K, Tsujino A, Yamashita T, Uchino M, Fujimoto Y, Tanaka F, Sobue G (2010) Efficacy and safety of leuprorelin in patients with spinal and bulbar muscular atrophy (JASMITT study): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 9(9):875–884
53. Ranganathan S, Harmison GG, Meyertholen K, Pennuto M, Burnett BG, Fischbeck KH (2009) Mitochondrial abnormalities in spinal and bulbar muscular atrophy. Hum Mol Genet 18(1):27–42
54. Katsuno M, Adachi H, Minamiyama M, Waza M, Tokui K, Banno H, Suzuki K, Onoda Y, Tanaka F, Doyu M, Sobue G (2006) Reversible disruption of dynactin 1-mediated retrograde axonal transport in polyglutamine-induced motor neuron degeneration. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(47):12106–12117
55. Cowan KJ, Diamond MI, Welch WJ (2003) Polyglutamine protein aggregation and toxicity are linked to the cellular stress response. Hum Mol Genet 12(12):1377–1391
56. Katsuno M, Sang C, Adachi H, Minamiyama M, Waza M, Tanaka F, Doyu M, Sobue G (2005) Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc Natl Acad Sci U S A 102(46):16801–16806
57. Kondo N, Katsuno M, Adachi H, Minamiyama M, Doi H, Matsumoto S, Miyazaki Y, Iida M, Tohnai G, Nakatsuji H, Ishigaki S, Fujioka Y, Watanabe H, Tanaka F, Nakai A, Sobue G (2013) Heat shock factor-1 influences pathological lesion distribution of polyglutamine-induced neurodegeneration. Nat Commun 4:1405
58. Adachi H, Katsuno M, Minamiyama M, Sang C, Pagoulatos G, Angelidis C, Kusakabe M, Yoshiki A, Kobayashi Y, Doyu M, Sobue G (2003) Heat shock protein 70 chaperone overexpression ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model by reducing nuclear-localized mutant androgen receptor protein. J Neurosci 23 (6):2203–2211

59. Bailey CK, Andriola IF, Kampinga HH, Merry DE (2002) Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. Hum Mol Genet 11(5):515–523
60. Howarth JL, Kelly S, Keasey MP, Glover CP, Lee YB, Mitrophanous K, Chapple JP, Gallo JM, Cheetham ME, Uney JB (2007) Hsp40 molecules that target to the ubiquitin-proteasome system decrease inclusion formation in models of polyglutamine disease. Mol Ther J Am Soc Gene Ther 15(6):1100–1105
61. Ishihara K, Yamagishi N, Saito Y, Adachi H, Kobayashi Y, Sobue G, Ohtsuka K, Hatayama T (2003) Hsp105alpha suppresses the aggregation of truncated androgen receptor with expanded CAG repeats and cell toxicity. J Biol Chem 278(27):25143–25150
62. Rusmini P, Crippa V, Giorgetti E, Boncoraglio A, Cristofani R, Carra S, Poletti A (2013) Clearance of the mutant androgen receptor in motoneuronal models of spinal and bulbar muscular atrophy. Neurobiol Aging 34(11):2585–2603
63. Rusmini P, Polanco MJ, Cristofani R, Cicardi ME, Meroni M, Galbiati M, Piccolella M, Messi E, Giorgetti E, Lieberman AP, Milioto C, Rocchi A, Aggarwal T, Pennuto M, Crippa V, Poletti A (2015) Aberrant autophagic response in the muscle of a knock-in mouse model of spinal and bulbar muscular atrophy. Sci Rep 5:15174
64. Cortes CJ, La Spada AR (2015) Autophagy in polyglutamine disease: Imposing order on disorder or contributing to the chaos? Mol Cell Neurosci 66(Pt A):53–61
65. Rusmini P, Bolzoni E, Crippa V, Onesto E, Sau D, Galbiati M, Piccolella M, Poletti A (2010) Proteasomal and autophagic degradative activities in spinal and bulbar muscular atrophy. Neurobiol Dis 40(2):361–369
66. Taylor JP, Tanaka F, Robitschek J, Sandoval CM, Taye A, Markovic-Plese S, Fischbeck KH (2003) Aggresomes protect cells by enhancing the degradation of toxic polyglutamine-containing protein. Hum Mol Genet 12(7):749–757
67. Tohnai G, Adachi H, Katsuno M, Doi H, Matsumoto S, Kondo N, Miyazaki Y, Iida M, Nakatsuji H, Qiang Q, Ding Y, Watanabe H, Yamamoto M, Ohtsuka K, Sobue G (2014) Paeoniflorin eliminates a mutant AR via NF-YA-dependent proteolysis in spinal and bulbar muscular atrophy. Hum Mol Genet 23(13):3552–3565
68. Waza M, Adachi H, Katsuno M, Minamiyama M, Sang C, Tanaka F, Inukai A, Doyu M, Sobue G (2005) 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. Nat Med 11(10):1088–1095
69. Rusmini P, Simonini F, Crippa V, Bolzoni E, Onesto E, Cagnin M, Sau D, Ferri N, Poletti A (2011) 17-AAG increases autophagic removal of mutant androgen receptor in spinal and bulbar muscular atrophy. Neurobiol Dis 41(1):83–95
70. Lieberman AP, Yu Z, Murray S, Peralta R, Low A, Guo S, Yu XX, Cortes CJ, Bennett CF, Monia BP, La Spada AR, Hung G (2014) Peripheral androgen receptor gene suppression rescues disease in mouse models of spinal and bulbar muscular atrophy. Cell reports 7 (3):774–784
71. Chua JP, Reddy SL, Merry DE, Adachi H, Katsuno M, Sobue G, Robins DM, Lieberman AP (2014) Transcriptional activation of TFEB/ZKSCAN3 target genes underlies enhanced autophagy in spinobulbar muscular atrophy. Hum Mol Genet 23(5):1376–1386
72. Sandri M, Coletto L, Grumati P, Bonaldo P (2013) Misregulation of autophagy and protein degradation systems in myopathies and muscular dystrophies. J Cell Sci 126(Pt 23):5325–5333
73. Yu Z, Wang AM, Adachi H, Katsuno M, Sobue G, Yue Z, Robins DM, Lieberman AP (2011) Macroautophagy is regulated by the UPR-mediator CHOP and accentuates the phenotype of SBMA mice. PLoS Genet 7(10):e1002321
74. Piccioni F, Pinton P, Simeoni S, Pozzi P, Fascio U, Vismara G, Martini L, Rizzuto R, Poletti A (2002) Androgen receptor with elongated polyglutamine tract forms aggregates that alter axonal trafficking and mitochondrial distribution in motor neuronal processes. FASEB J Off Publ Fed Am Soc Exp Biol 16(11):1418–1420

75. Thomas M, Yu Z, Dadgar N, Varambally S, Yu J, Chinnaiyan AM, Lieberman AP (2005) The unfolded protein response modulates toxicity of the expanded glutamine androgen receptor. J Biol Chem 280(22):21264–21271
76. Montague K, Malik B, Gray AL, La Spada AR, Hanna MG, Szabadkai G, Greensmith L (2014) Endoplasmic reticulum stress in spinal and bulbar muscular atrophy: a potential target for therapy. Brain 137(Pt 7):1894–1906
77. Morfini G, Pigino G, Szebenyi G, You Y, Pollema S, Brady ST (2006) JNK mediates pathogenic effects of polyglutamine-expanded androgen receptor on fast axonal transport. Nat Neurosci 9(7):907–916
78. Kemp MQ, Poort JL, Baqri RM, Lieberman AP, Breedlove SM, Miller KE, Jordan CL (2011) Impaired motoneuronal retrograde transport in two models of SBMA implicates two sites of androgen action. Hum Mol Genet 20(22):4475–4490
79. Malik B, Nirmalananthan N, Bilsland LG, La Spada AR, Hanna MG, Schiavo G, Gallo JM, Greensmith L (2011) Absence of disturbed axonal transport in spinal and bulbar muscular atrophy. Hum Mol Genet 20(9):1776–1786
80. Yu Z, Dadgar N, Albertelli M, Gruis K, Jordan C, Robins DM, Lieberman AP (2006) Androgen-dependent pathology demonstrates myopathic contribution to the Kennedy disease phenotype in a mouse knock-in model. J Clin Invest 116(10):2663–2672
81. Rocchi A, Milioto C, Parodi S, Armirotti A, Borgia D, Pellegrini M, Urciuolo A, Molon S, Morbidoni V, Marabita M, Romanello V, Gatto P, Blaauw B, Bonaldo P, Sambataro F, Robins DM, Lieberman AP, Soraru G, Vergani L, Sandri M, Pennuto M (2016) Glycolytic-to-oxidative fiber-type switch and mTOR signaling activation are early-onset features of SBMA muscle modified by high-fat diet. Acta Neuropathol 132:127–144
82. Katsuno M, Adachi H, Minamiyama M, Waza M, Doi H, Kondo N, Mizoguchi H, Nitta A, Yamada K, Banno H, Suzuki K, Tanaka F, Sobue G (2010) Disrupted transforming growth factor-beta signaling in spinal and bulbar muscular atrophy. J Neurosci 30(16):5702–5712
83. Sahashi K, Katsuno M, Hung G, Adachi H, Kondo N, Nakatsuji H, Tohnai G, Iida M, Bennett CF, Sobue G (2015) Silencing neuronal mutant androgen receptor in a mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 24(21):5985–5994
84. Keiser MS, Boudreau RL, Davidson BL (2014) Broad therapeutic benefit after RNAi expression vector delivery to deep cerebellar nuclei: implications for spinocerebellar ataxia type 1 therapy. Mol Ther J Am Soc Gene Ther 22(3):588–595
85. Rodriguez-Lebron E, Costa Mdo C, Luna-Cancalon K, Peron TM, Fischer S, Boudreau RL, Davidson BL, Paulson HL (2013) Silencing mutant ATXN3 expression resolves molecular phenotypes in SCA3 transgenic mice. Mol Ther J Am Soc Gene Ther 21(10):1909–1918
86. Miyazaki Y, Adachi H, Katsuno M, Minamiyama M, Jiang YM, Huang Z, Doi H, Matsumoto S, Kondo N, Iida M, Tohnai G, Tanaka F, Muramatsu S, Sobue G (2012) Viral delivery of miR-196a ameliorates the SBMA phenotype via the silencing of CELF2. Nat Med 18(7):1136–1141
87. Pourshafie N, Lee PR, Chen KL, Harmison GG, Bott LC, Katsuno M, Sobue G, Burnett BG, Fischbeck KH, Rinaldi C (2016) MiR-298 counteracts mutant androgen receptor toxicity in spinal and bulbar muscular atrophy. Mol Ther J Am Soc Gene Ther 24:937–945
88. Tokui K, Adachi H, Waza M, Katsuno M, Minamiyama M, Doi H, Tanaka K, Hamazaki J, Murata S, Tanaka F, Sobue G (2009) 17-DMAG ameliorates polyglutamine-mediated motor neuron degeneration through well-preserved proteasome function in an SBMA model mouse. Hum Mol Genet 18(5):898–910
89. Malik B, Nirmalananthan N, Gray AL, La Spada AR, Hanna MG, Greensmith L (2013) Co-induction of the heat shock response ameliorates disease progression in a mouse model of human spinal and bulbar muscular atrophy: implications for therapy. Brain J Neurol 136(Pt 3):926–943
90. Giorgetti E, Rusmini P, Crippa V, Cristofani R, Boncoraglio A, Cicardi ME, Galbiati M, Poletti A (2015) Synergic prodegradative activity of Bicalutamide and trehalose on the mutant androgen receptor responsible for spinal and bulbar muscular atrophy. Hum Mol Genet 24 (1):64–75

91. Castillo K, Nassif M, Valenzuela V, Rojas F, Matus S, Mercado G, Court FA, van Zundert B, Hetz C (2013) Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons. Autophagy 9(9):1308–1320
92. Banno H, Adachi H, Katsuno M, Suzuki K, Atsuta N, Watanabe H, Tanaka F, Doyu M, Sobue G (2006) Mutant androgen receptor accumulation in spinal and bulbar muscular atrophy scrotal skin: a pathogenic marker. Ann Neurol 59(3):520–526
93. Banno H, Katsuno M, Suzuki K, Takeuchi Y, Kawashima M, Suga N, Takamori M, Ito M, Nakamura T, Matsuo K, Yamada S, Oki Y, Adachi H, Minamiyama M, Waza M, Atsuta N, Watanabe H, Fujimoto Y, Nakashima T, Tanaka F, Doyu M, Sobue G (2009) Phase 2 trial of leuprorelin in patients with spinal and bulbar muscular atrophy. Ann Neurol 65(2):140–150
94. Fernandez-Rhodes LE, Kokkinis AD, White MJ, Watts CA, Auh S, Jeffries NO, Shrader JA, Lehky TJ, Li L, Ryder JE, Levy EW, Solomon BI, Harris-Love MO, La Pean A, Schindler AB, Chen C, Di Prospero NA, Fischbeck KH (2011) Efficacy and safety of dutasteride in patients with spinal and bulbar muscular atrophy: a randomised placebo-controlled trial. Lancet Neurol 10(2):140–147
95. Yang Z, Chang YJ, Yu IC, Yeh S, Wu CC, Miyamoto H, Merry DE, Sobue G, Chen LM, Chang SS, Chang C (2007) ASC-J9 ameliorates spinal and bulbar muscular atrophy phenotype via degradation of androgen receptor. Nat Med 13(3):348–353
96. Bott LC, Badders NM, Chen KL, Harmison GG, Bautista E, Shih CC, Katsuno M, Sobue G, Taylor JP, Dantuma NP, Fischbeck KH, Rinaldi C (2016) A small-molecule Nrf1 and Nrf2 activator mitigates polyglutamine toxicity in spinal and bulbar muscular atrophy. Hum Mol Genet 25:1979–1989
97. Rinaldi C, Bott LC, Chen KL, Harmison GG, Katsuno M, Sobue G, Pennuto M, Fischbeck KH (2012) Insulinlike growth factor (IGF)-1 administration ameliorates disease manifestations in a mouse model of spinal and bulbar muscular atrophy. Mol Med 18:1261–1268
98. Ding Y, Adachi H, Katsuno M, Huang Z, Jiang YM, Kondo N, Iida M, Tohnai G, Nakatsuji H, Funakoshi H, Nakamura T, Sobue G (2015) Overexpression of hepatocyte growth factor in SBMA model mice has an additive effect on combination therapy with castration. Biochem Biophys Res Commun 468(4):677–683
99. Querin G, D'Ascenzo C, Peterle E, Ermani M, Bello L, Melacini P, Morandi L, Mazzini L, Silani V, Raimondi M, Mandrioli J, Romito S, Angelini C, Pegoraro E, Soraru G (2013) Pilot trial of clenbuterol in spinal and bulbar muscular atrophy. Neurology 80(23):2095–2098

Chapter 6
Spinocerebellar Ataxia Type 1: Molecular Mechanisms of Neurodegeneration and Preclinical Studies

Judit M. Pérez Ortiz and Harry T. Orr

Abstract Spinocerebellar ataxia type 1 (SCA1) is an adult-onset, inherited disease that leads to degeneration of Purkinje cells of the cerebellum and culminates in death 10–30 years after disease onset. SCA1 is caused by a CAG repeat mutation in the *ATXN1* gene, encoding the ATXN1 protein with an abnormally expanded polyglutamine tract. As neurodegeneration progresses, other brain regions become involved and contribute to cognitive deficits as well as problems with speech, swallowing, and control of breathing. The fundamental basis of pathology is an aberration in the normal function of Purkinje cells affecting regulation of gene transcription and RNA splicing. Glutamine-expanded ATXN1 is highly stable and more resistant to degradation. Moreover, phosphorylation at S776 in ATXN1 is a post-translational modification known to influence protein levels. SCA1 remains an untreatable disease managed only by palliative care. Preclinical studies are founded on the principle that mutant protein load is toxic and attenuating ATXN1 protein levels can alleviate disease. Two approaches being pursued are targeting gene expression or protein levels. Viral delivery of miRNAs harnesses the RNAi pathway to destroy ATXN1 mRNA. This approach shows promise in mouse models of disease. At the protein level, kinase inhibitors that block ATXN1-S776 phosphorylation may lead to therapeutic clearance of unphosphorylated ATXN1.

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Spinocerebellar ataxia type 1 (SCA1) is a fatal neurodegenerative disorder with an autosomal dominant inheritance. The genetic basis of SCA1 is a CAG repeat expansion mutation in the coding region of the Ataxin-1 (*ATXN1*) gene [1, 2]. As with other trinucleotide repeat disorders, SCA1 shows genetic anticipation where the unstable repeats expand over generations, and disease onset occurs earlier and is more severe. Unaffected individuals harbor 19–36 CAG repeats, with CAT interruptions in tracts over 21 repeats. SCA1 patients have 43–81 pure CAG repeats [3]. The gene product, ATXN1, is a predominantly nuclear protein that harbors the polyglutamine expansion mutation in its amino-terminal region. While ATXN1 is widely expressed in the brain, cerebellar Purkinje cells present more vulnerability to mutant ATXN1 with their degeneration underlying the ataxic phenotype. As disease progresses, brain stem nuclei become involved and affected individuals succumb due to chronic lung infections and respiratory failure.

6.1 Molecular Mechanisms of Neurodegeneration

Mutant ATXN1 toxicity is not solely dictated by the polyglutamine tract mutation. The C-terminal region of ATXN1 harbors several motifs that are required for the protein to be pathogenic. The nuclear localization signal (NLS) directs ATXN1 translocation into the nucleus. Mice expressing mutant ATXN1[82Q] with a non-functional NLS do not develop disease, which proves ATXN1 exerts its toxicity in the nuclear compartment [4]. Once inside the nucleus, ATXN1 interacts with cellular proteins involved in regulation of gene expression, both at the level of transcription and RNA processing. The ATXN1 AXH domain allows its homodimerization and interaction with regulators of transcription such as the transcriptional repressor Capicua (Cic) [5]. ATXN1 and Cic together exist in large protein complexes where mutant ATXN1 is thought to affect Cic repressor activity [6]. In addition to regulating Cic function, stability of Cic depends on ATXN1 binding. ATXN1 immunodepletion results in loss of Cic and Sca1 null mice have reduced Cic levels.

A knockin SCA1 mouse model that expresses 154Q at the endogenous *Sca1* mouse locus ($Sca1^{154Q/2Q}$) develops an ataxia phenotype as well as other features of the human disease, namely wasting and brainstem degeneration-dependent lethality [7]. $Sca1^{154Q/2Q}$ mice having partial (50%) genetic ablation of *Cic* show improvement in motor coordination, weight, survival, and neuropathology. Microarray analysis revealed that many *Cic* target genes that were repressed in $Sca1^{154Q/2Q}$ were restored to near wild type levels with $Cic^{+/-}$. This finding argues for Atxn1 toxicity acting through a gain of function mechanism, whereby mutant Atxn1 affects the normal function of an Atxn1 binding partner (Cic) and exacerbates its normal gene-repressor function in vivo. This is consistent with previous

in vitro Cic-mediated transcription assay that show Atxn1 enhances Cic repressor activity and this requires Atxn1-Cic interaction [6]. Interestingly, genes normally repressed by Cic were overexpressed in *Sca1*$^{154Q/2Q}$ and partial *Cic* ablation restored their levels. Thus, mutant Atxn1 also results in Cic loss of function, revealing complexity in Atxn1 effects. Partial ablation of *Cic* in *Sca1*$^{154Q/2Q}$ resulted in an improvement in disease, suggesting Atxn1 exerts its toxicity via Cic gain of function and hyper-repression of Cic target genes. Relieving Atxn1-Cic interactions may prove beneficial. Interestingly, nonpathogenic ATXN1[82Q]-S776A does not associate with large protein complexes containing Cic [6]. These results indicate that mutant Atxn1 pathogenicity acts through aberrant interactions with its native binding partners and not via novel interactions.

Another key site at the C-terminus of ATXN1 is the serine at amino acid position 776 (S776). A large body of evidence supports that S776 phosphorylation is critical for ATXN1 toxicity. While SCA1 mice expressing wild type ATXN1[30Q] in Purkinje cells are not affected, ATXN1[30Q]-S776D phosphomimetic mutation drives disease. Furthermore, D776 mutation enhances toxicity in *ATXN1[82Q]-D776* mice [8]. Notably, mice expressing ATXN1[82Q]-S776A phospho-resistant mutation do not develop ataxia and largely resemble wild type mice, despite harboring a mutant polyQ tract [9]. What does S776 phosphorylation do to ATXN1 that changes it so drastically? Two main properties S776 phosphorylation confers to ATXN1 are greater stability and enhanced protein-protein interactions. Abnormally elevated levels of ATXN1, even in the wild type form, are pathogenic when overexpressed in both *Drosophila* and mice, suggesting ATXN1 protein load itself is toxic [10, 11]. The nonpathogenic ATXN1-A776 protein is unstable in vivo and its lowered levels are thought to be an important factor contributing to averting toxicity [12].

One mechanism by which phosphorylated pS776-ATXN1 is stabilized is by interaction with the molecular chaperone 14-3-3. Co-expression of ATXN1[82Q] and d14-3-3ε in Drosophila retina enhances 82Q-induced eye degeneration [13]. In mice, 14-3-3ε haploinsufficiency (*Sca1*$^{154Q/2Q}$*; 14-3-3ε*$^{+/-}$) rescues motor phenotype and PC numbers [14]. In vivo 14-3-3ε haploinsufficiency results in lower Atxn1 levels in the cerebellum [14]; in vitro using a peptide to competitively disrupts 14-3-3/ATXN11 interaction or siRNA-mediated knockdown of 14-3-3 prompts ATXN1-S776 dephosphorylation and increased clearance of ATXN1 [15]. While 14-3-3/pS776-ATXN1 binding protects ATXN1 clearance by blocking dephosphorylation, it seems that the phosphorylation itself is what makes ATXN1 more stable: ATXN1-D776 does not bind 14-3-3 and is as stable as phosphorylated ATXN1, suggesting the phosphorylation alone stabilizes the protein [15]. Further studies exploring 14-3-3ε/pS776-ATXN1 interactions showed that perhaps this interaction is unique to the cerebellum. Although *14-3-3ε*$^{+/-}$ results in lowered Atxn1 (154Q and 2Q) levels in the cerebellum, this was not the case in the brainstem. Whether a different 14-3-3 isoform mediates this stabilizing interaction in the brainstem remains unexplored. Perhaps *14-3-3ε*$^{+/-}$ does not affect Atxn1 levels in the brainstem as it does in the cerebellum due to distinct protein interactions in these different brain regions. Yet, biochemical studies showed a shift in the incorporation

of mutant Atxn1 from large (toxic) to small protein complexes in the cerebellum, but this was not observed in the brainstem. Protein complex composition in the brainstem differs from that in the cerebellum. This may explain why motor deficits related to the cerebellum were rescued in *Sca1*$^{154Q/2Q}$*; 14-3-3ε*$^{+/-}$ mice, even though these mice continued to display brainstem-related deficits, including weight loss, premature death, and respiratory dysfunction. Differences in Atxn1-binding complexes reveal potentially diverging mechanisms by which mutant Atxn1 exerts toxicity in different regions of the brain.

Perhaps the most intriguing interaction modulated by S776 phosphorylation is interaction with splicing factor RBM17 in the nucleus. In Drosophila, RBM17 overexpression worsens retinal degenerative phenotype when co-expressed with mutant ATXN1[82Q], but not with wild type ATXN1[30Q] [16]. Conversely, partial genetic ablation of *dRBM17* (*SPF45*) in transgenic flies expressing mutant ATXN1[82Q] attenuates pathology. Co-immunoprecipitation experiments revealed RBM17 binding is enhanced by two key characteristics that contribute to ATXN1 pathogenicity, namely polyglutamine tract length and S776 phosphorylation. ATXN1[30Q]-D776 also shows enhanced binding to RBM17 and suggests pathogenic effects of this phospho-mimicking protein are due to downstream effects of aberrant RBM17 interaction. ATXN1 and RBM17 interact via their C-terminal domains in large protein complexes, yet these are not the same complexes where ATXN1 interacts with Cic. In fact, RBM17 and Cic complexes compete for ATXN1 binding, as RBM17 knockdown results in greater binding of ATXN1 with Cic. In summary, ATXN1 exists in at least two large protein complexes, likely regulated by S776 phosphorylation. ATXN1's ability to interact with transcription and splicing factors echoes the characteristic ability of factors that regulate co-transcriptional splicing, some of which are affected in other neurodegenerative diseases including ALS [17, 18]. It is possible that ATXN1 regulates gene expression by the spatiotemporal coupling of these two nuclear events. In this model, under normal conditions ATXN1 interacts transiently with at least two macromolecular complexes containing either Cic or RBM17. Glutamine expansion or D776 (i.e. constitutive S776 phosphorylation) create a shift in the normal binding dynamics, favoring a toxic enhanced interaction with RBM17 complex, and disrupting normal equilibrium between gene transcription and splicing (Fig. 6.1). Interesting evidence in favor of a combined gain- and loss- of function mechanism through which ATXN1 acts is via observations of *Sca1*$^{154Q/-}$ mice [16]. Simultaneous deletion of wild type Atxn1[2Q] with mutant Atxn1[154Q] expression (*Sca1*$^{154Q/-}$) produces a mouse that presents with a worse rotarod performance and poorer survival than the already sick *Sca1*$^{154Q/2Q}$. It seems that in the heterozygous state, wild type Atxn1[2Q] expression and function partially compensates for Atxn1[154Q]-induced gain and loss of function toxicity.

In support of this pathogenic model are studies using SCA1 animal models, which consistently implicate aberrations in gene expression as an early part of the disease process. PCR-based cDNA subtractive hybridization first revealed abnormal gene expression in transgenic SCA1 animals [19]. A set of neuronal genes highly expressed by Purkinje cells were downregulated in SCA1 cerebella, as early as P11,

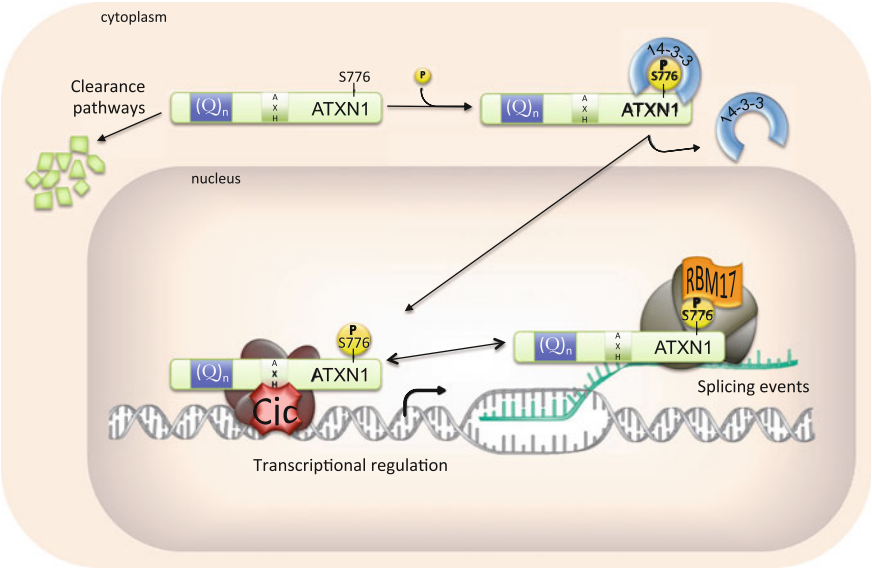

Fig. 6.1 ATXN1 intracellular pathways. ATXN1 is a predominantly nuclear protein that shuffles between the cytoplasm and nucleus. Phosphorylation of ATXN1-S776 in the cytoplasm promotes binding by 14-3-3 at this site, which stabilizes ATXN1. Unphosphorylated ATXN1 is otherwise rapidly cleared. By some yet undefined mechanism, 14-3-3 dissociates and ATXN1 enters the nucleus. Nuclear ATXN1 can incorporate into complexes containing the transcriptional repressor Capicua (Cic) and the splicing factor RBM17 to participate in transcription and splicing events. It is in the nucleus where glutamine-expanded ATXN1 exerts its toxicity

just one day after transgene expression and weeks before histological or behavioral deficits are detectable. These changes were also reflected with immunostaining of SCA1 patient sections. Over the years, studies have focused in groups of genes affected in disease Ca^{2+} handling/homeostasis [19], glutamate signaling [20, 21], dopamine receptor [22], VEGF angiogenic factor [23], and synaptic proteins [24]. Early expression of mutant ATXN1 contributes to disease at least in part by affecting RORα-mediated gene expression important for Purkinje cell maturation and cerebellar development [25]. That disease onset begins by affecting development is a possibility suggested by several SCA1 mouse models [25, 26].

Recent studies show disease onset and progression are distinct processes, best illustrated by transgenic mice expressing ATXN1[30Q]-D776 in Purkinje cells. The S776D mutation confers a phospho-mimicking change that converts nonpathogenic ATXN1[30Q] to a toxic species. *ATXN1[30Q]-D776* mice develop an ataxia that closely resembles that seen in *ATXN1[82Q]* transgenic mice, but their disease does not progress to Purkinje cell death [8]. Recent RNAseq analysis of cerebellar tissue at early, mid, and late stage of disease was performed using *ATXN1[82Q]* mice (with a progressive disease) and *ATXN1[30Q]-D776* mice (having a nonprogressive disease) to uncover pathways that underlie progressive disease as well as those that might prevent disease progression [27]. Weighted Gene Coexpression Network

Analysis (WGCNA) of the RNAseq data across all ages and genotypes identified two modules, designated Magenta and Lt Yellow, as being associated with ataxia. Interestingly, the Magenta module is enriched for genes specifically expressed by Purkinje cells and a significant proportion have upstream Cic binding sites. This is in line with established role of aberrations in Cic target gene expression in SCA1 models discussed above. Moreover, the course of gene changes in the Magenta module track with the progressive phenotypic changes that occur in *ATXN1[82Q]* disease.

Analysis of RNAseq data further revealed cholecystokinin (*Cck*) as being uniquely overexpressed in *ATXN1[30Q]-D776* and downregulated in *ATXN1[82Q]* cerebellar RNA [27]. To test whether *Cck* upregulation protects *ATXN1[30Q]-D776* from disease progression, *ATXN1[30Q]-D776* mice were crossed to $Cck^{-/-}$ mice, producing *ATXN1[30Q]-D776;* $Cck^{-/-}$ mice. Loss of Cck resulted in a progressive disease with molecular layer atrophy and Purkinje cell loss comparable to that seen in *ATXN1[82Q]* model. This raises the possibility that *Cck* overexpression in the *ATXN1 [30Q]-D776* model activates a protective pathway that prevents progression of disease. The activation is thought to be via autocrine activation of Purkinje cell CckR1 since *ATXN1[30Q]-D776;* $CckR1^{-/-}$ (but not *ATXN1[30Q]-D776;* $CckR2^{-/-}$) mice demonstrated a similar progressive pathology.

6.2 Preclinical Studies

To date, two general strategies are being employed to mitigate SCA1 pathogenesis preclinically in animal models. One strategy is to restore expression of a gene product downregulated in SCA1, This was first tested by genetic and pharmacologic treatments of $Sca1^{154Q/2Q}$ mice with VEGF [23]. $Sca1^{154Q/2Q}$ cerebella present with a dramatic downregulation of *Vegfa* mRNA, likely due to increased occupancy by Atxn1 of the *Vegfa* promoter and consequent gene repression, in a histone acetylation dependent manner. VEGF is an angiogenic and neurotrophic factor. In the cerebellum it is expressed by neurons, glia, and endothelial cells [28, 29]. $Sca1^{154Q/2Q}$ mice crossed to transgenic mice that overexpress VEGF from an embryonic stage showed improved rotarod motor performance in adulthood, with apparent full motor recovery by 6 months. This was coupled to only a slight but statistically significant improvement in molecular layer thickness of the cerebellar cortex. To test the therapeutic potential of exogenous VEGF delivery, intracerebroventricular administration of recombinant mouse VEGF was continuously delivered by osmotic pump into the lateral ventricles for two weeks starting at 11 weeks of age. This short period of treatment produced a complete recovery in rotarod motor performance in treated animals. It is unclear whether VEGF benefits come from the effects on microvasculature improving nutrient and oxygen delivery to the brain parenchyma and/or activation of neurotrophic pathways. Regardless, replenishing levels of a gene product downregulated in disease attenuates mutant Atxn1 toxicity.

A more recent study sought to reinstate expression of another cellular component deficient in *Sca1*$^{154Q/2Q}$ cerebella [24]. Mass-spectrometry analysis of *Sca1*$^{154Q/2Q}$ cerebella revealed a reduction in Homer-3 levels in synapses, likely caused by defective mTOR signaling. Blocking mTORC1 signaling depleted Homer-3 levels and worsened *Sca1*$^{154Q/2Q}$ pathology. While enhanced neurotransmission and activation of mTORC1 signaling enriched expression of the Purkinje cell scaffold protein Homer-3 in wild type cerebella, it failed to do so in *Sca1*$^{154Q/2Q}$. Thus, in order to improve Homer-3 synaptic levels, AAV-Homer-3 was injected into the ventricles of neonate brains. Behavioral examination at P40–P200 showed incomplete but significant persistent improvement in rotarod motor performance. Other parameters improved with AAV-Homer-3 gene treatment included Purkinje cell spine density, Purkinje cell numbers, and vesicle pool occupancy. Thus, replacement of Homer-3 levels during development restores synaptic deficits and partially improve motor performance in *Sca1*$^{154Q/2Q}$ mice.

Perhaps the most promising preclinical therapeutic approaches under investigation are predicated on the finding that reducing the amount of mutant protein expressed in the cerebellum can alleviate its toxicity. Conditional SCA1 mice were generated to manipulate *ATXN1[82Q]* expression in Purkinje cells by Doxycycline administration to turn off *ATXN1[82Q]* expression and reduce mutant protein levels. Strikingly, this results in reversal of the motor deficits and histopathology. Moreover, the earlier the gene is turned off, the more pronounced disease reversal [21]. These findings reveal two important points. First, progression of this inherited neurodegenerative disease can be reversed. Early intervention is key to preserve and recover the remaining tissue. If the disease is targeted early enough the cells are still present and able to recover. In fact, later studies have shown that there are differential circuit-specific changes that progress with the course of disease and precede histopathology [30]. Second, there is a therapeutic opportunity in targeting the mutant protein.

Based on the studied outlined above, several targets for development of therapies for SCA1 are being pursued (Fig. 6.2). First, that S776 phosphorylation is linked to ATXN1 pathogenicity makes it an attractive therapeutic target. With this in mind, efforts to identify the kinase to S776 have been under intense investigation. The first candidate kinase identified was Akt. Purified Akt can phosphorylate ATXN1-S776 in vitro, but it seems not to be a relevant kinase in vivo [12, 13]. A mouse model expressing a dominant negative form of Akt failed to lower ATXN1 protein levels in vivo or rescue pathology [12]. Further investigation identified PKA, cAMP protein kinase, as a candidate kinase for ATXN1-S776 in the cerebellum. For example, PKA immunodepletion in mouse cerebellar lysates reduces the ability of the lysate to phosphorylate ATXN1-S776. Also, ATXN1-S776 phosphorylation is blocked by standard PKA inhibitors in cerebellar fractions that express PKA and Atxn1. Recent findings suggest that MSK1 may be another kinase involved in ATXN1-S776 phosphorylation [31]. A high-throughput screen using siRNAs to human kinases was done to examine how this affected the phosphorylation and levels of ATXN1 in human DAOY cells stably expressing mutant mRFP-ATXN1 [82Q]. Fifty final candidates were validated including MSK1, a kinase downstream

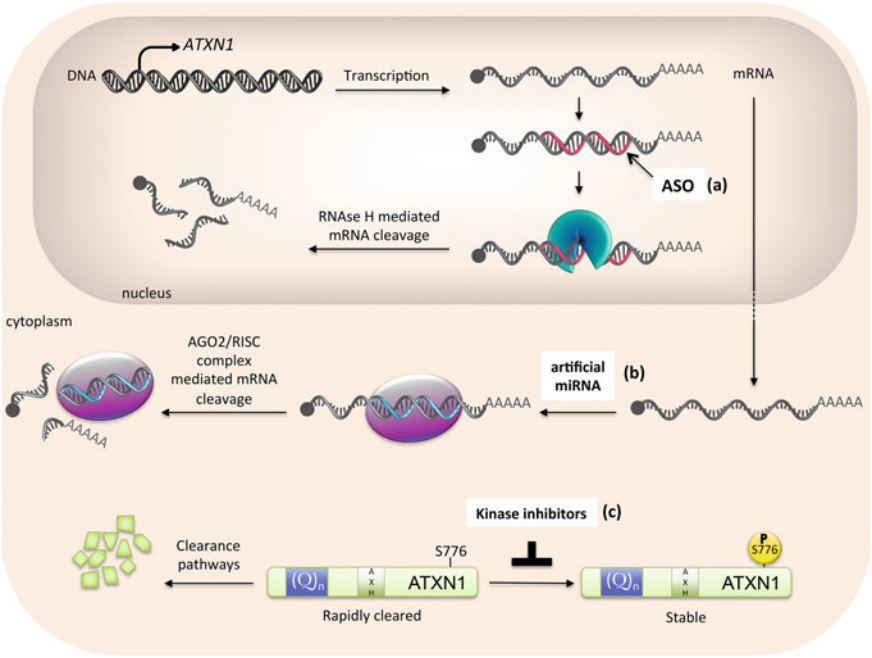

Fig. 6.2 SCA1 Therapeutic Targets. The fundamental strategy underlying these targets is in harnessing pathways that lower ATXN1 levels (wt and mutant). ATXN1 mRNA expression can be targeted two ways. In the nucleus, ASOs complementary to the mRNA promote its cleavage via the RNAse-H pathway (**a**). The RNAi machinery operates in the cytoplasm, where the miRNA is processed, complexes with the target mRNA in the RISC complex, and this leads to mRNA cleavage (**b**). At the protein level, action of small molecule inhibitors that block ATXN1-S776 phosphorylation would promote rapid clearance of unphosphorylated ATXN1 (**c**)

of RAS-MAPK pathway. An SCA1 knock in mouse model heterozygous for Msk1/2 was generated (*Atxn1*$^{154Q/2Q}$; *Msk1*$^{+/-}$ *Msk2*$^{+/-}$) and showed modest improvement in rotarod performance. *Atxn1*$^{154Q/2Q}$; *Msk1*$^{-/-}$ cerebellar lysates showed a mild reduction in Atxn1 protein levels. To date it remains unclear the relative contributions of PKA or MSK1 in regulating ATXN1-S776 phosphorylation and protein stability.

The RNAi pathway is a robust gene silencing mechanism through which mRNAs are cleaved and destroyed in the cytoplasm. In recent years, studies have taken advantage of this powerful tool to reduce mutant Atxn1 expression and test the therapeutic potential of this approach (Fig. 6.2). The first series of experiments focused on viral-mediated delivery of RNAi species targeting transgenic ATXN1 [82Q] selectively overexpressed in murine Purkinje cells. AAV-shRNA injection was targeted to midline cerebellar lobules for expression in Purkinje cells. AAV-shSCA1 delivery at 7 weeks of age achieved complete preservation of molecular layer thickness (examined at 16 weeks of age) and partial improvement in rotarod motor performance (as late as 21 weeks of age) [32]. Advances in RNAi

technology led to development of the more refined use of endogenous miRNA backbones for in vivo expression of mature siRNA sequences [33, 34]. Benefits include improved siRNA processing with lower off-target potential, as well as greater safety profile. Indeed, delivery of AAV-miRNA targeting hATXN1[82Q] in SCA1 transgenic mice (AAV-miSCA1) achieved robust (70%) *hATXN1* mRNA knockdown without toxicity [35]. In these newer studies AAV-miSCA1 injections were targeted to deep cerebellar nuclei (DCN) for uptake and expression by Purkinje cells. With AAV injection at 5 weeks, motor recovery was observed out to 37 weeks of age, including ledge test, hindlimb clasping, and rotarod test for coordination. Histological measures of cerebellar integrity such as molecular layer thickness, total Purkinje cell numbers, and ectopic Purkinje cell numbers, were more modestly improved, consistent with the accumulating notion that full rescue of histopathological changes may not be required for complete recovery of motor performance.

More recently an upgraded AAV-miSCA1 was tested in the $Sca1^{154Q/2Q}$ knockin model to examine its therapeutic effect beyond the Purkinje cell related pathology [36]. As expected, this resulted in Atxn1 knockdown and fully rescued histopathology and motor impairments. A novel finding was that AAV-miSCA1 injection into the cerebellar DCN was able to transduce brainstem nuclei and knock down local Atxn1 levels. One strategic injection site (the DCN) could be sufficient to deliver the AAV-miSCA1 to reach the brain regions most critically affected in SCA1 (the cerebellum and brainstem). It will be important to see whether future studies demonstrate rescue in brainstem related phenotype, notably survival studies. Another important finding in this study is that a single surgical injection was sufficient to produce therapeutic benefit for as long as 40 weeks. Moreover, expression analysis of select genes affected in $Sca1^{154Q/2Q}$ mice showed restoration of their expression levels comparable to wild type. Of note is the preservation of Vegfa levels, previously discussed.

Mutant ATXN1 toxicity is the primary insult in SCA1 disease. Downstream gene changes implicate diverse molecular pathways that contribute to the disease course. It is therefore not surprising that targeting these pathways incompletely rescue pathology. The most efficient therapeutic avenue for SCA1 is to target ATXN1 directly. Harnessing the RNAi pathway to knockdown *ATXN1* gene expression is efficient, without measurable toxicity, and has long lasting therapeutic benefit. Another encouraging technology targeting mRNA degradation are ASOs (anti-sense oligonucleotides), which act via the RNAse-H-dependent pathway in the nucleus (Fig. 6.2). ASOs show therapeutic efficacy in treating disease-causing proteins, including Hungtintin and SOD1, in preclinical studies with clinical trials underway [37, 38]. Therapeutic ASOs could also be tested to target ATXN1 in SCA1 studies in the future.

SCA1 is a fatal trinucleotide repeat neurodegenerative disorder to which there is no cure. This chapter has covered how mouse models and biochemical studies have aided our understanding of the molecular mechanisms underlying this disease. Chiefly, the fundamental basis of pathology is an aberration in the normal function of Purkinje cells affecting regulation of gene transcription and RNA splicing.

Factors that promote ATXN1 stabilization lead to abnormal protein load, toxic protein-protein interactions, and enhanced pathology. Preclinical studies are focused on alleviating protein burden by targeting mRNA levels. Other therapeutic opportunities underway ahead involve kinase inhibitors to block toxic S776 phosphorylation to promote ATXN1 protein clearance.

References

1. Banfi S et al (1993) Mapping and cloning of the critical region for the spinocerebellar ataxia Type 1 gene (SCA1) in a yeast artificial chromosome contig spanning 1.2 Mb. Genomics 18(3):627–635
2. Orr HT et al (1993) Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. Nat Genet 4(3):221–226
3. Chung MY et al (1993) Evidence for a mechanism predisposing to intergenerational CAG repeat instability in spinocerebellar ataxia type I. Nat Genet 5(3):254–258
4. Klement IA et al (1998) Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 95(1):41–53
5. Kim E, Lu HC, Zoghbi HY (2013) Structural basis of protein complex formation and reconfiguration by polyglutamine disease protein ataxin-1 and capicua. Genes Dev 27(6): 590–595
6. Lam YC et al (2006) ATAXIN-1 interacts with the repressor capicua in Its native complex to cause SCA1 neuropathology. Cell 127(7):1335–1347
7. Watase K et al (2002) A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. Neuron 34(6): 905–919
8. Duvick Lisa et al (2010) SCA1-like disease in mice expressing wild-type ataxin-1 with a serine to aspartic acid replacement at residue 776. Neuron 67(6):929–935
9. Emamian ES et al (2003) Serine 776 of Ataxin-1 is critical for polyglutamine-induced disease in SCA1 transgenic mice. Neuron 38(3):375–387
10. Fernandez-Funez P et al (2000) Identification of genes that modify Ataxin-1-induced neurodegeneration. Nature 408(6808):101–106
11. Gennarino VA et al (2015) Pumilio1 haploinsufficiency leads to SCA1-like neurodegeneration by increasing wild-type Ataxin1 levels. Cell 160(6):1087–1098
12. Jorgensen ND et al (2009) Phosphorylation of ATXN1 at Ser776 in the cerebellum. J Neurochem 110(2):675–686
13. Chen HK et al (2003) Interaction of Akt-phosphorylated Ataxin-1 with 14-3-3 mediates neurodegeneration in spinocerebellar ataxia type 1. Cell 113(4):457–468
14. Jafar-nejad P, Ward CS, Richman R, Orr HT, Zoghbi HY (2011) Regional rescue of spinocerebellar ataxia type 1 phenotypes by 14-3-3 ε haploinsufficiency in mice underscores complex pathogenicity in neurodegeneration. PNAS 108(5):2142–2147
15. Lai S, O'Callaghan B, Zoghbi HY, Orr HT (2011) 14-3-3 binding to ataxin-1 (ATXN1) regulates its dephosphorylation at Ser-776 and transport to the nucleus. J Biol Chem 286(40): 34606–34616
16. Lim J et al (2008) Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature 452(7188):713–718
17. Masuda A, Takeda JI, Ohno K (2016) FUS-mediated regulation of alternative RNA processing in neurons: insights from global transcriptome analysis. Wiley Interdiscip Rev RNA 2–5
18. Sánchez-hernández N et al (2016) The in vivo dynamics of TCERG1, a factor that couples transcriptional elongation with splicing. RNA 22(4):571–582

19. Lin X, Antalffy B, Kang D, Orr HT, Zoghbi HY (2000) Polyglutamine expansion down-regulates specific neuronal genes before pathologic changes in SCA1. Nat Neurosci 3(2): 157–163
20. Serra HG et al (2004) Gene profiling links SCA1 pathophysiology to glutamate signaling in purkinje cells of transgenic mice. Hum Mol Genet 13(20):2535–2543
21. Zu Tao et al (2004) Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice. J Neurosci Off J Soc Neurosci 24(40):8853–8861
22. Hearst SM, Lopez ME, Shao Q, Liu Y, Vig PJS (2010) Dopamine D2 receptor signaling modulates mutant ataxin-1 S776 phosphorylation and aggregation. J Neurochem 114(3): 706–716
23. Cvetanovic M, Patel JM, Marti HH, K AR, Opal P (2011) Vascular endothelial growth factor ameliorates the ataxic phenotype in a mouse model of spinocerebellar ataxia type 1. Nat Med 17(11):1445–1447
24. Ruegsegger C et al (2016) Impaired mTORC1-dependent expression of homer-3 influences SCA1 pathophysiology. Neuron 89(1):129–146
25. Serra HG et al (2006) RORa-mediated purkinje cell development determines disease severity in adult SCA1 mice. Cell 127(4):697–708
26. Ebner BA et al (2013) Purkinje cell ataxin-1 modulates climbing fiber synaptic input in developing and adult mouse cerebellum. J Neurosci 33(13):5806–5820
27. Ingram M, et al (2016) Cerebellar transcriptome profiles of ATXN1 transgenic mice reveal SCA1 disease progression and protection pathways. *Neuron* 1194–1207
28. Acker T, Beck H, Plate KH (2001) Cell type specific expression of vascular endothelial growth factor and Angiopoietin-1 and -2 suggests an important role of astrocytes in cerebellar vascularization. Mech Dev 108(1–2):45–57
29. de Almodovar CR et al (2010) Matrix-binding vascular endothelial growth factor (VEGF) isoforms guide granule cell migration in the cerebellum via VEGF receptor Flk1. J Neurosci 30(45):15052–15066
30. Barnes JA et al (2011) Abnormalities in the climbing fiber-purkinje cell circuitry contribute to neuronal dysfunction in ATXN1[82Q] mice. J Neurosci Off J Soc Neurosci 31(36):12778–12789
31. Park J et al (2013) RAS-MAPK-MSK1 pathway modulates ataxin 1 protein levels and toxicity in SCA1. Nature 498(7454):325–331
32. Xia H et al (2004) RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. Nat Med 10(8):816–820
33. Boudreau RL, Martins I, Davidson BL (2009) Artificial microRNAs as siRNA shuttles: improved safety as compared to shRNAs in Vitro and in Vivo. Mol Ther J Am Soc Gene Ther 17(1):169–175
34. McBride JL et al (2008) Artificial miRNAs Mitigate shRNA-mediated toxicity in the brain: implications for the therapeutic development of RNAi. Proc Natl Acad Sci USA 105(15): 5868–5873
35. Keiser MS, Geoghegan JC, Boudreau RL, Lennox KA, Davidson BL (2013) RNAi or overexpression: alternative therapies for spinocerebellar ataxia type 1. Neurobiol Dis 56C:6–13
36. Keiser MS, Boudreau RL, Davidson BL (2014) Broad therapeutic benefit after RNAi expression vector delivery to deep cerebellar nuclei: implications for spinocerebellar ataxia type 1 therapy. Mol Ther 2:588–595
37. Kordasiewicz HB et al (2012) Sustained therapeutic reversal of huntington's disease by transient repression of huntingtin synthesis. Neuron 74(6):1031–1044
38. Miller TM et al (2013) An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. Lancet Neurol 12(5):435–442

Chapter 7
Spinocerebellum Ataxia Type 6: Molecular Mechanisms and Calcium Channel Genetics

Xiaofei Du and Christopher Manuel Gomez

Abstract Spinocerebellar ataxia (SCA) type 6 is an autosomal dominant disease affecting cerebellar degeneration. Clinically, it is characterized by pure cerebellar dysfunction, slowly progressive unsteadiness of gait and stance, slurred speech, and abnormal eye movements with late onset. Pathological findings of SCA6 include a diffuse loss of Purkinje cells, predominantly in the cerebellar vermis. Genetically, SCA6 is caused by expansion of a trinucleotide CAG repeat in the last exon of longest isoform *CACNA1A* gene on chromosome 19p13.1–p13.2. Normal alleles have 4–18 repeats, while alleles causing disease contain 19–33 repeats. Due to presence of a novel internal ribosomal entry site (IRES) with the mRNA, *CACNA1A* encodes two structurally unrelated proteins with distinct functions within an overlapping open reading frame (ORF) of the same mRNA: (1) α1A subunit of P/Q-type voltage gated calcium channel; (2) α1ACT, a newly recognized transcription factor, with polyglutamine repeat at C-terminal end. Understanding the function of α1ACT in physiological and pathological conditions may elucidate the pathogenesis of SCA6. More importantly, the IRES, as the translational control element of α1ACT, provides a potential therapeutic target for the treatment of SCA6.

Keywords SCA6 · Polyglutamine · Purkinje cells · IRES · α1ACT

7.1 Introduction

Spinocerebellar ataxia type 6 (SCA6) was genetically characterized by a large scale genotyping survey of polymorphic CAG repeats and DNA samples from patients with late onset spinocerebellar ataxia [1]. Eight unrelated patients with late onset ataxia had larger CAG repeat alleles in the *CACNA1A* compared to the number of repeats in 475 non-ataxia individuals. Analysis of the repeat length in families of the affected individuals revealed that the expansion segregated with the phenotype

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in every patient. *CACNA1A* encodes the pore-forming subunit α1A, of the P/Q type voltage-gated calcium channel and in several splice variants, the CAG repeat encodes a small tract of glutamine residues in the carboxy terminus (C-terminus) of the α1A subunit in α1A calcium channel subunit. This was the first SCA and first polyglutamine expansion disease attributed to mutations in ion channel gene.

Studies have shown a relevant role of a stable 75 kDa polypeptide consisting of a portion of the intracytoplasmic C-terminal tail (α1ACT) of the α1A subunit in SCA6 pathogenesis. α1ACT contains residues involved in channel inactivation and modulation by intracellular signaling proteins, as well as a tract of polyglutamine residues pathologically expanded in SCA6 and nuclear localization signals [2]. α1ACT is found to be encoded by a second cistron in *CACNA1A* gene and is a functional transcription factor that coordinates the expression of neuronal genes involved in Purkinje cells and cerebellar development [3]. Both the wild type ($\alpha1ACT_{WT}$) and the mutant ($\alpha1ACT_{SCA6}$) forms of the C-terminal peptide can be transported to the Purkinje cell nuclei [4]. $\alpha1ACT_{SCA6}$ is toxic to cultured cells and when expressed transgenically in Purkinje cells [3, 4].

7.2 Clinical Features

SCA6 is characterized by adult onset, slowly progressive cerebellar ataxia, dysarthria, and nystagmus. The range in age of onset is from 19 to 71 years, with a mean age of onset between 43 and 52 years. Although age of onset varies roughly inversely with the size of the repeat expansion, age of onset and clinical picture vary even within the same family. Siblings with the same mutation may differ in age of onset by as much as 12 years, or exhibit, at least initially, an episodic course, despite all having the same size repeat expansion [5, 6]. Lifespan is not shortened.

Initial symptoms in SCA6 are gait unsteadiness, stumbling, and imbalance in about 90% of individuals, while the remainder present with diplopia, oscillopsia, dysarthria, or episodic vertigo several years before the gait disturbance [7]. Symptoms progress slowly, and eventually all persons have gait ataxia, upper limb incoordination, intention tremor, and dysarthria, although severity of these symptoms may range widely in some patients. Diplopia occurs in about 50% of individuals. Others experience visual disturbances related to difficulty fixating on moving objects. Eye findings include horizontal gaze-evoked nystagmus (70–100%) and vertical downbeat nystagmus (65–83%). Other eye movement abnormalities, such as saccadic dysmetria, periodic alternating nystagmus and rebound nystagmus, have also been described [8]. Dysphagia and choking are common [5]. The occurrence of cognitive impairment in SCA6 has been controversial [9].

Progression of ataxia has been best assessed using a standardized semi-quantitative rating scale called Scale for the Assessment and Rating of Ataxia (SARA) [10]. This tool, which has been validated between raters for several forms of SCA, assigns a score of severity for each of eight components including speech,

sitting stability, three upper limb coordination measures, one lower limb coordination motor stance and gait, totaling 0–40. Using this scale patients with SCA6 progress at a rate of approximately 0.8 points per year, compared with 1.5–2 points/year for SCA1-3 [11].

There is also growing clinical evidence showing clinical heterogeneity of SCA6 [12–14]. Other clinical features such as tremors, bradykinesia and postural instability from pure Parkinsonism without cerebellar dysfunction were reported in isolated SCA6 cases [14]. SCA patients rarely present with atypical Parkinsonism and nigrostriatal dysfunction, and idiopathic Parkinsonism suggesting screening for SCA6 mutations in patients with slowly progressive ataxia and Parkinsonism [14–17].

7.3 Epidemiology

Estimates of the overall prevalence of autosomal dominant ataxia vary greatly, from 1–4/10^5 [18] to 50/10^5 [19, 20], and that of SCA6 from 0.02/10^5 to 5/10^5 [20, 21]. The proportion of SCA patients with SCA6 seems to vary by geographical area, presumably relating to founder effects. SCA6, same as SCA1, are found in many populations worldwide [22]. Estimated as a fraction of all kindreds with autosomal dominant spinocerebellar ataxia, rates for SCA6 are 1–2% in Spain [23], France, Italy [24], and South Africa [25]; 3.3% in Mainland China [26], 4% in Brazil [27], 12% in the US, 13% in Germany, 15% in Korea [28], 17% in Australia [29], 24% in Netherlands [30].

SCA6 accounts for approximately half of the cases of pure cerebellar ataxia in Japan [31]. SCA6 was the second most common form in the Netherlands and Japan, after SCA3 [22]. The highest relative prevalence of SCA6 is found in Japan. In Japan, a remarkable regional difference in SCA6 frequency has been observed: 10% of all dominant SCA patients in east Japan; 20–33% of that in the rest region of Japan [32]. A linkage study showed that the pure cerebellar ataxia phenotype was linked with the SCA6 locus in some Japanese families, and these patients were subsequently demonstrated to carry the SCA6 mutation [33]. Haplotype analysis indicates that there are founder chromosomes in Japanese SCA6 patients and a possibly shared haplotype for patients with SCA6 worldwide [20, 34, 35].

7.3.1 Differential Diagnosis

For patients complaining of imbalance as the initial symptom, all autosomal dominant, recessive, and sporadic ataxias should be considered. An obvious dominant or recessive hereditary pattern should direct the workup. In patients with an obvious dominant inheritance pattern, all forms of genetically identified spinocerebellar ataxia as well as non-classified SCAs can have an identical

presentation of pure cerebellar ataxia. The more severe forms of SCA, such as SCA1, SCA2, SCA3, and SCA7, may present in a similar fashion, but commonly develop other symptoms and signs such as ophthalmoplegia, spasticity, and/or primary visual changes. Examination of older relatives, in whom the disease may have progressed further, frequently reveals the additional signs. The presence of late-onset pure cerebellar ataxia with a dominant inheritance pattern and pronounced gaze-evoked nystagmus is strongly suggestive of SCA6 [5, 36]. For patients complaining of dysarthria, vertigo, oscillopsia, or diplopia, numerous other otolaryngological or neuromuscular conditions may be considered, but SCA6 may be suspected based on clinical examination or MRI scan documenting cerebellar atrophy.

SCA6 is not the only disorder caused by mutations in the *CACNA1A* gene, encoding the α1A VGCC subunit. Mutations in the *CACNA1A* gene (other than the CAG expansion noted in SCA6) are associated with at least two other dominantly inherited disorders, episodic ataxia type 2 and familial hemiplegic migraine.

7.3.1.1 Episodic Ataxia Type 2 (EA2)

EA2 is caused by *CACNA1A* mutations that predict protein truncation, abnormal splicing, or, rarely, α1A subunit missense mutations. Like for SCA6, the mode of inheritance is autosomal dominant. EA2 typically starts in childhood or early adolescence and is characterized by attacks of ataxia, vertigo, hand incoordination, and nausea that last minutes to hours to days. Attacks can be associated with dysarthria, diplopia, tinnitus, dystonia, hemiplegia, and headache. Between attacks, individuals may initially be normal but many eventually develop interictal symptoms and signs such as nystagmus and truncal ataxia. After years of episodic ataxia, a condition of interictal ataxia can develop and may be indistinguishable from SCA6 [37, 38].

7.3.1.2 Familial Hemiplegic Migraine (FHM)

FHM is an autosomal dominant condition with an estimated penetrance of 80–90% [39]. Two clinical forms exist: (1) pure FHM (found in 80% of affected families), in which interictal examination is normal in all family members; (2) FHM with permanent cerebellar symptoms (found in 20% of affected families), in which some family members show interictal nystagmus and/or ataxia. Approximately 50% of families with FHM, including those with permanent cerebellar symptoms, have missense mutations in the *CACNA1A* gene [38, 40]. FHM is characterized by an aura of hemiplegia that is always associated with at least one other aura symptom such as hemianopsia, hemisensory deficit, or aphasia. The aura is followed by a moderate to severe headache. The phenotype includes coma and seizures [41], which can be triggered by minor head injury or angiography.

Despite their well-described phenotypes, SCA6, EA2, and FHM demonstrate clinical overlap:

- Individuals with SCA6 can present with episodic ataxia. In one study, up to 33% of individuals with 21 or more CAG repeats in *CACNA1A* had episodic features prominent enough to warrant the diagnosis of EA2 [42].
- In one family with a CAG repeat expansion, some members had episodic ataxia and others had progressive ataxia; in all affected members the abnormal allele had 23 CAG repeats [6].
- In a family with EA2, affected members also had hemiplegia, and one affected member had migraine during episodes of ataxia [43].
- In one family with a *CACNA1A* missense mutation, phenotypes of both SCA6 and FHM were observed [44].

Although the clinical overlap and the idea that SCA6, EA2 and FHM may be considered the same (complex) disease with a large variability of symptoms, the mutation in each case may produce considerably different effects at the level of neuromuscular junction [45]. Thus, neuromuscular junction dysfunction may be seen in EA2 and FHM but not in SCA6. Lastly, some *CACNA1A* mutations causing EA2 or FHM have been associated with eye movement disorder in childhood, cognitive impairment, seizures, and cerebral edema [46– 48].

7.3.2 Clinical Genetics

SCA6 should be suspected in individuals with adult-onset, slowly progressive ataxia, dysarthria, and nystagmus. However, because phenotypic manifestations of SCA6 are not specific, the diagnosis of SCA6 rests on molecular genetic testing. SCA6 is only caused by an expanded CAG repeat in exon 47 of the *CACNA1A* gene. This CAG repeat is polymorphic, being in the range of 4–18 in normal alleles, expanded to 19–33 in individuals with SCA6 [31, 49, 50].

7.3.3 Penetrance

Alleles with 20–33 CAG repeats have full penetrance. Asymptomatic individuals bearing an expansion of $(CAG)_{20}$ or greater are expected to develop symptoms at some time in their life. Heterozygous individuals with 19 repeats are usually asymptomatic although one patient with 19/7 CAG alleles developed ataxia in his 50s [51]. The average disease-causing allele has 22 CAG repeats. Age of onset of disease in SCA6 is inversely correlated with the length of the glutamine repeat expansion. For example, typically, individuals with a glutamine repeat of 20 begin to exhibit symptoms in their 70s, while individuals with a repeat length of 30

glutamines begin to exhibit symptoms as early as 30 years old. However, patients with repeats of 21–23 may have episodic symptoms and subtle cerebellar signs in their mid-20s [5].

7.3.4 Genotype–Phenotype Correlations

7.3.4.1 Heterozygous Individuals

While the age of onset of symptoms correlates inversely with the length of the expanded CAG repeat, the same broad range of onset has been noted for individuals with 22 CAG repeats, the most common disease-associated allele [5, 52]. In few individuals with $(CAG)_{30}$ or $(CAG)_{33}$, onset has been later than in individuals with $(CAG)_{22}$ and $(CAG)_{23}$ [31, 53]. A recent retrospective study has shown even closer correlation of age of onset with the sum of the two allele sizes [54].

7.3.4.2 Homozygous Individuals

Several individuals who are homozygous for an abnormal expansion in the *CACNA1A* gene have been reported [21, 53, 55, 56]. In homozygous individuals, the onset was earlier and symptoms appeared more severe than in individuals who are heterozygous [21, 55]. In one study, the age of onset correlated with the sum of two disease alleles [54]. It is interesting to highlight, however, that the increase in severity of symptoms with homozygosity of the CAG repeat expansion in *CACNA1A* is not as great as that observed in SCA3 (Machado-Joseph disease). One patient homozygous for 19 repeats developed ataxia at age 33. Her parents were normal past 70 years of age [49].

7.3.5 Anticipation

Expansions of *CACNA1A* are not commonly observed in transmission from parent to child, thus, clinical anticipation has not been observed in SCA6 [53]. In fact, between 65 and 75% of the variation of age of onset can be accounted by the length of the CAG repeat units [57]. However, in a few cases, meiotic instability has been reported [50, 53, 55, 58, 59].

7.4 Neuroimaging

SCA6 is the prototype of a pure cerebellar ataxia [1, 60, 61] and, as such, MRI brain scans in SCA6 have suggested essentially pure cerebellar atrophy. In particular, a significant decrease in gray matter in cerebellar lobules and vermis has been

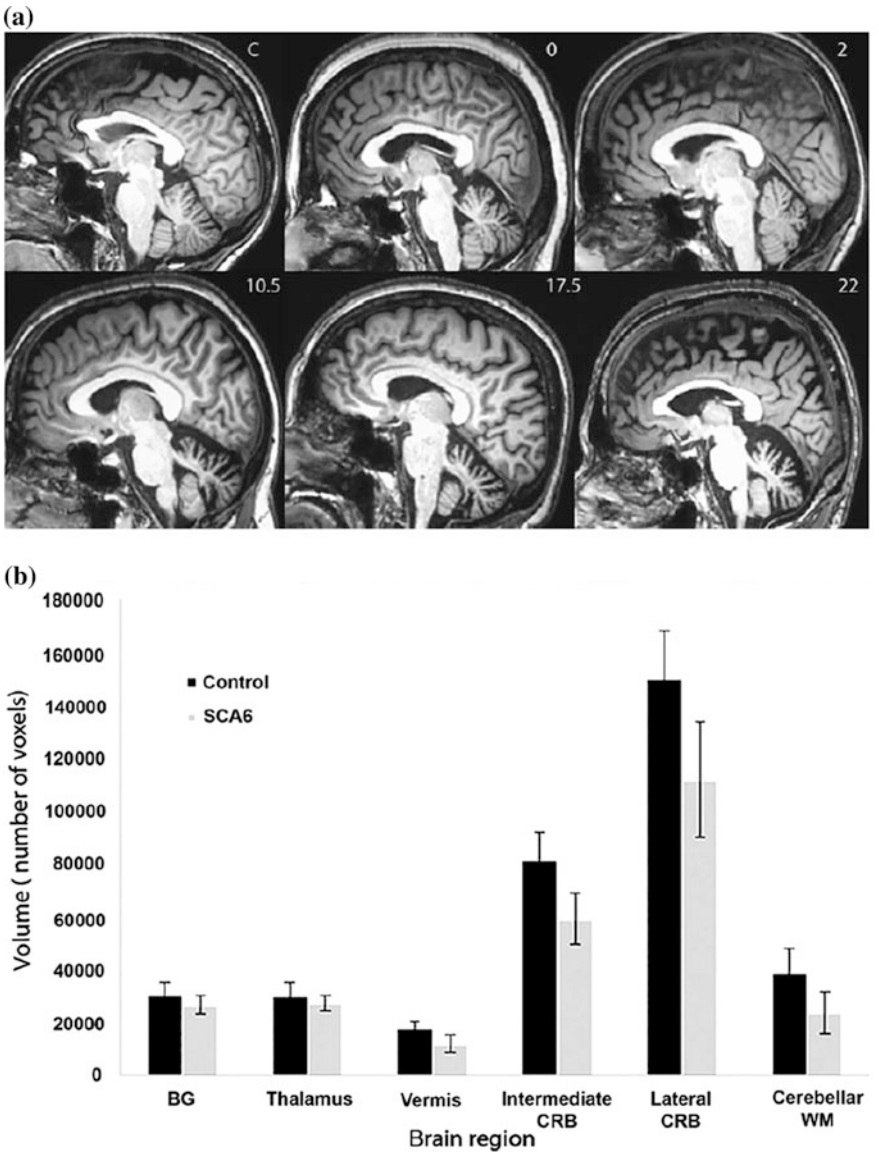

Fig. 7.1 SCA6 Neuroimaging. Volumetric assessment of brain regions in SCA6 and healthy controls. **a** Sagittal view of T1-weighted images in a healthy control (1), a presymptomatic SCA6 case (SARAII = 0, (2)), moderate case (SARAII = 2 (3), SARAII = 10.5 (4), and two severe cases (SARAII = 17.5 (5), and SARAII = 22 (6). Note the increase in cerebellar atrophy along an increase of SARAII scores. **b** Graphical representation of averaged brain region volumes and their standard deviation in healthy controls (black bars) and in SCA6 (gray bars). Abbreviations: BG: basal ganglia; CRB: cerebellum; WM: white matter (permission from Falcon et al. [66])

observed [62]. Brain SPECT studies of regional cerebral blood flow (rCBF) have shown a decrease of rCBF in the vermis and cortex of the cerebellum, in which the decrease in the vermal rCBF was correlated with the severity of the dysarthria, but not with the size of the CAG repeat expansion [63]. In addition, to the changes seen in the structure and function of the cerebellum, limited changes in the inferior olives, the middle cerebellar peduncles, pons, and red nuclei have also been reported [64]. More detailed longitudinal voxel-based volumetric studies have identified progressive atrophy not only of cerebellum but also the thalamus, putamen and pallidum [65]. Consistent with this, an effective connectivity between regions of cerebral cortex, thalamus and cerebellum was at its highest in moderate cases, and disappeared in severe cases, as shown by functional MRI and diffusion tensor imaging (Fig. 7.1) [66]. Studies of cerebral glucose metabolism assessed with PET have shown a decrease in the cerebellum but also in the frontal and prefrontal regions [67], in contrast to an increase seen in the temporal lobe [68]. In addition, a study by Kawai et al. suggested a correlation between a decrease in prefrontal cortex perfusion (evaluated by SPECT) and cognitive decline symptoms, assessed as verbal fluency and visual memory. These measures were not correlated with the age of the subjects or the severity of the ataxic symptoms. Studies probing the content of nigral dopamine in SCA6 using ioflupane (^{123}I) SPECT (*DaTSCAN*) have shown reduced uptake of dopamine in the substantia nigra in some patients with SCA6 [17, 69, 70].

7.5 Neuropathology

Neuropathological studies show striking loss of cerebellar Purkinje cells [5, 71] and profound morphological changes in the few remaining Purkinje cells, such as heterotopic nuclei, unclear cytoplasmic outline, somatic sprouts, dendritic swelling with increased numbers of spine-like protrusions and disorganization of axonal arrangements [61]. In contrast to other SCAs, where cell loss and gliosis in additional regions can be seen secondary to the profound loss of Purkinje cells, very limited degeneration is detected in associated cortical or cerebello-olivary regions in SCA6 [5, 21]. Rather, numerous neurons in the pontine inferior olives, stellate, basket and granule cells in the cerebellum, and red nuclei in the midbrain, seem to be preserved from a retrograde degeneration [72] even though some decrease in the number of neurons in the inferior olivary nuclei may be detected when disease duration is long [73]. In addition, other more recent studies have noted the absence of morphologically intact layer V giant Betz pyramidal cells in the primary motor cortex and widespread degeneration of brainstem nuclei [74]. Although these findings need to be verified they are consistent with the frequent observation of hypereflexia and Babinski signs in SCA6 [5].

7.6 Molecular Genetics

7.6.1 The Gene

7.6.1.1 *CACNA1A* Gene

The *CACNA1A* gene consists of 47 exons that are transcribed into numerous alternatively spliced α1A mRNAs encoding polypeptides ranging from 195 to 270 kDa, and varying in sequence internally and in the C-terminus. These include splice forms that have distinct properties characteristic of P-type and Q-type channels. The discovery of the polymorphic CAG repeat in the 3′ end of the gene, that was expanded in a population of ataxia patients, led to the identification of a novel long splice form of the α1A mRNA encoding a polyglutamine tract in an extended exon 47 [1]. The tract of glutamine residues is polymorphic in the population, with normal alleles ranging from 4 to 18 glutamines in length, and is distal to the C-terminal calmodulin-binding domain and CAG expansion mutations have been demonstrated in SCA6 patients [75, 76]. Disease alleles contain pure tracts of uninterrupted CAG repeats consisting of 19 or more triplet units. The range of pathological alleles in SCA6 patients reported so far varies between 19 and 33 CAG units [31, 49, 77].

As noted above, SCA6 is not the only disorder caused by mutations in the *CACNA1A* gene. Mutations in the *CACNA1A* gene also cause two dominantly inherited episodic disorders in humans: familial hemiplegic migraine (FHM) and episodic ataxia type 2 (EA-2). Their disease mechanisms have both been linked to altered calcium channel function, channelopathies. Patients with FHM suffer from severe headaches, hemiparesis, or hemianesthenia, and occasionally episodic imbalance, with typical onset beginning at ages 10–20 years. In FHM missense mutations at conserved residues in *CACNA1A* can lead to changes in current densities, altered inactivation kinetics, and altered open probability of P/Q-type channels. In EA-2, patients experience episodic imbalance, dysarthria, vertigo, and hand incoordination. *CACNA1A* mutations found in EA-2 are most commonly nonsense or splicing mutations resulting in α1A subunits that are truncated within the repeat domains, or that predict skipped exons causing loss of function when assessed in expression studies. However a growing number of missense mutations have also been associated with EA-2 [78, 79]. Several studies suggest that the truncated subunits exhibit dominant inheritance by exerting a dominant negative effect on the wild-type P/Q current activity [80]. Rare mutations in the *CACNA1A* gene lead to a progressive syndrome of non-episodic ataxia resembling SCA6 (Yue).

7.6.1.2 IRES and *CACNA1A* Gene

In eukaryotic cells, the majority of mRNA translation is cap-dependent, relying on the complex of proteins that assemble at the 7-methyl-guanosine cap at the 5′ end of an mRNA. Another form of translation, termed cap-independent, occurs when the 40S ribosome directly binds mRNA near the start codon at an internal ribosome entry site (IRES) [81]. IRESs are present in the 5′ untranslated region (UTR) of approximately 10–15% of cellular mRNAs, presumably to ensure translation of critical proteins during times of cell stress, cell cycle, or certain developmental stages when cap-dependent protein synthesis may be reduced [82]. A few other genes express mRNAs with IRES present in the open reading frame. In *CACNA1A* a sequence of approximately 1 kb nucleotide within the α1A mRNA forms a predicted complex 2-d hairpin structure using an M-fold-based algorithm [3, 81, 83]. This sequence is located in a highly conserved sequence of 1014 bp upstream the ATG 1960th (nucleotide 6114; GenBank accession number: NM_001127222) in *CACNA1A* mRNA (Fig. 7.2); it exhibits IRES activity in dual luciferase and

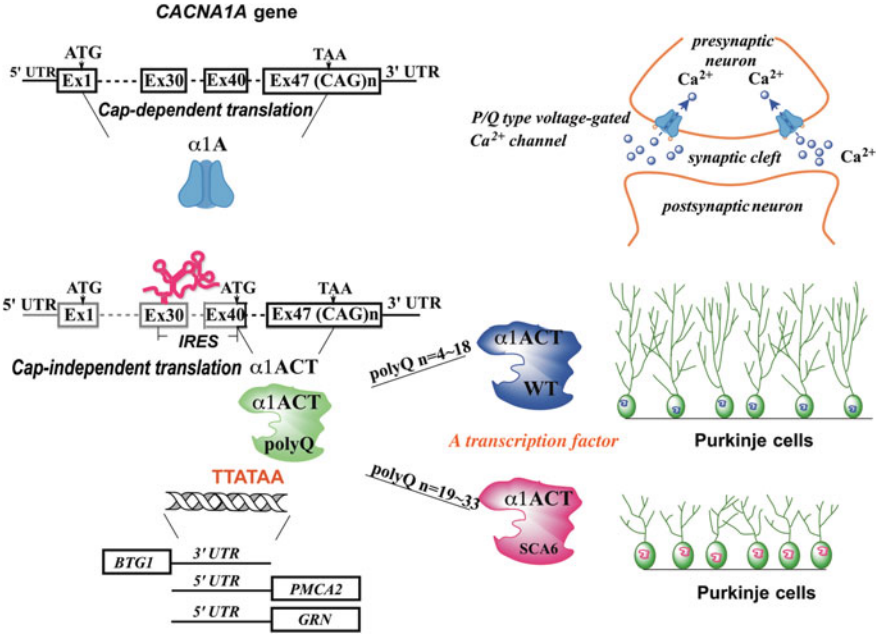

Fig. 7.2 *CACNA1A* gene, IRES, and α1ACT Schematic illustration of expression regulation and function of α1A and α1ACT. The *CACNA1A* gene, encoding the VGCC subunit α1A, is bicistronic. The second gene product, α1ACT, is generated from the α1A transcript by a cellular IRES located within the α1A mRNA. The α1ACT is a transcription factor that regulates expression of several neuronally-expressed genes, promotes neurite outgrowth. The $\alpha1ACT_{SCA6}$ reduces viability of cells and causes cerebellar cortical atrophy in animal model (permission and modified from Du et al. [84])

in vitro translation assays [3]. This *CACNA1A* IRES regulates translation of a secondary protein product of *CACNA1A* gene—a 75 kDa C-terminal "fragment" of α1A (α1ACT) translation. α1ACT containing the polyQ tract forms a stable polypeptide, enriched in nuclear fractions in cerebellum, that acts as a transcription factor for neuronally-expressed genes.

Expression of bi-functional genes, particularly those encoding separate transcription factor proteins in the second cistron, may be a newly-recognized strategy for coordinating gene expression programs tied to individual gene products. This could enable the timely expression of a set of genes coincident with the appearance of key proteins during differentiation.

Most IRESs are thought to function through the binding of accessory translational proteins termed IRES trans-acting factors (ITAFs). These ITAFs interact with RNA sequences or structures in the IRES to nucleate the formation of a translation initiation complex distal to the 5′ cap structure. Some ITAFs are believed to be tissue specific as the activity of some cellular IRESs varies between different cell-types and tissues [82]. In the case of the *CACNA1A* IRES, the activity (reflected by a bicistronic reporter) ranges from 14 to 45, in various cultured cell lines or primary rat granule neurons, with the highest activity seen in neuronal cell lines, PC12 and SY5Y [3, 84]. These latter levels in neuronal cells were 1.8–2-fold greater, respectively, than in HEK 293 cells derived from human kidney cells. This observation suggests that certain neuron-specific cellular factors may influence the activity of the *CACNA1A* IRES. There are different splicing sites in the *CACNA1A* IRES region (Ex30–Ex40), which may be involved in the structure of *CACNA1A* IRES and further affect the generation of α1ACT. A complete understanding of *CACNA1A* IRES control may pave the way for therapies targeted at suppressing the *CACNA1A* IRES function.

7.6.2 The Proteins

7.6.2.1 α1A Subunit

Voltage-gated Ca^{2+} channels (VGCC) of ten classes have a ubiquitous distribution, from muscle cells and neurons to endocrine cells and lymphocytes. Influx of Ca^{2+} through these channels regulates numerous intracellular processes, including contraction, secretion, neurotransmission, and gene expression [85]. VGCC are heteromultimers consisting of a main pore-forming or α1 subunit and auxiliary subunits, α2δ, and β subunits. The VGCC class is determined by the numerous α1 subunits, named α1A, α1B, α1C, α1D, etc., each encoded by a separate gene. α2δ, and the four subtypes of β subunits that are part of each VGCC type, serve to chaperone the subunit to the membrane surface and shape channel kinetics. *CACNA1A* gene encodes the α1A subunit which forms the $Ca_v2.1$ (P/Q-type) channel [86–88]. Depending on different splicing isoforms, α1A subunits are membrane glycoproteins of approximately 2261–2512 amino acids in length in

which primary structure predicts the presence of four homologous repeat domains, each consisting of six transmembrane segments and a pore-forming P loop [89].

P/Q-type calcium channels are high-voltage-activated calcium channels found primarily on neurons and expressed at high levels in granule and Purkinje cells of the cerebellar cortex. Their principal role is believed to be in synaptic transmission [90], synaptic integration, and gene regulation [91]. In terms of synaptic modulation, these channels play a crucial role in membrane excitability, triggering synaptic transmission and neuronal plasticity [92, 93].

In addition to their function in synaptic transmission, P/Q-type voltage-gated calcium channels also have a role in regulating gene expression, both by indirect and by recently identified direct pathways [3, 94]. In particular, Sutton et al. [94] showed that Ca^{2+} fluxes through P/Q-type channels activate expression of a separate set of gene products, including syntaxin 1A, although the pathways were not elucidated (Fig. 7.2).

7.6.2.2 α1ACT

It is now known that the C-terminus of the α1A subunit (α1ACT) is physiologically formed from the mRNA of the full-length subunit and translocated to the nucleus [3, 4]. Immunostains and western blotting studies demonstrate that the 60–70-kDa form is enriched in the nucleus, particularly in P18 mouse cerebellum, while in the cytoplasm or membrane the C-terminus remains part of the full-length α1A subunit (Fig. 7.2).

The IRES located in *CACNA1A* mRNA mediates the expression of the α1ACT fragment. Through an AT-rich element of α1ACT binding site, α1ACT binds and regulates gene expression such as TAF1, BTG1, PMCA2 and GRN in neurons. These genes are abundantly expressed in Purkinje cells, although not uniquely, and they are possibly involved in the neurite outgrowth program. As a transcription factor, the normal α1ACT also enhances the expression of at least three genes, GRN, PMCA2 and BTG1, in PC12 cells and cerebellar tissue, potentiates NGF-mediated neurite outgrowth in PC12 cells, and partially rescues the *CACNA1A* KO mouse phenotype.

7.7 Pathogenesis

While the normal length of the CAG repeat tract at the C-terminus of the α1A subunit of the P/Q-type voltage-gated calcium channel *CACNA1A* ranges between 4 and 18 glutamines, in individuals with SCA6 the tract is expanded to between 19 and 33 glutamines. This channel and the splice form expressing the polyQ tract are expressed abundantly in Purkinje cells of the cerebellum [95]. The α1A subunit is also mutated in several other inherited neurological disorders [89]. Initial studies suggested that SCA6 was a disorder due to P/Q channel dysfunction [96–98], although results were conflicting. Moreover, electrophysiological analysis of $SCA6^{28Q}$ and $SCA6^{30Q}$ knock-in mice failed to find a change in the intrinsic properties of Purkinje cells [99, 100].

On the other hand, the identification of α1ACT as a stable protein bearing the polyQ tract focused attention on this polypeptide as the pathological entity [4, 101, 102]. The C-terminal peptide with an expanded polyQ tract is toxic to tissue culture cells with toxicity being dependent on its nuclear localization. Expressed as an independent transgene protein it causes gait incoordination and cerebellar atrophy in transgenic mice [3]. Because the expanded polyglutamine tract mutation in SCA6 is essentially identical, except for size, to the mutations of numerous other degenerative diseases, such as Huntington's disease, Machado-Joseph disease, and Kennedy's disease, several studies have attempted to demonstrate that SCA6 is a polyglutamine repeat expansion disorder. These results support a pathogenic model for SCA6 where the polyQ-containing C terminus of the α1A transmembrane subunit and its subsequent translocation to the nucleus contributes to disease.

7.7.1 SCA6 as an Ion Channelopathy

The cerebellum is responsible for coordination of movement and maintenance of balance [103, 104]. At the core of the cerebellar computational circuitry are the Purkinje cells (PCs), the sole output of the cerebellar cortex. It is proposed that coordination of movements is achieved by encoding timing signals (for augmentation and inhibition of the appropriate agonist and antagonist muscles) in the rate of firing and pattern of activity of PCs. P/Q-type channels play a dominant role in initiating fast transmitter release at the excitatory parallel fiber (PF) and climbing fiber (CF) synapses onto PCs and presumably at the excitatory synapses PF–inhibitory interneurons and CF–DCN neurons. Inhibitory synaptic transmission at the molecular layer interneurons–PC and PC–PC synapses and at the inhibitory synapses onto granule cells is almost exclusively dependent on P/Q channels [105]. Moreover, P/Q-type channels play a key role in the maintenance of the highly regular, spontaneous intrinsic pacemaking of PCs because they are specifically coupled to the Cav2.1, that control the after hyperpolarization amplitude and the frequency and regularity of tonic firing. After partial block of P/Q, the spontaneous intrinsic firing of PCs in cerebellar slices becomes irregular and the firing frequency increases; a larger block of P/Q channels causes the cells to burst and then go silent [89]. Differential expression of the Cav^{2+}-dependent regulatory proteins and/or of different Cav2.1 splice variants may provide a means of neuron-type-specific regulation of presynaptic P/Q channels and short-term plasticity [106].

The involvement of a functional ion channel subunit (α1A) of a voltage-gated calcium channel in SCA6 favors the hypothesis that SCA6 is an ion channel disorder in which the polyglutamine expansion in the C-terminus of the α1A subunit causes ataxia by altering calcium channel function. This idea is more appealing given the vast amount of data implicating altered calcium signaling in the SCAs as well as the identification of other mutations α1A causing EA-2 ad FHM that clearly affect P/Q channel function. Evidence for alterations in calcium signaling and/or handling is reported for SCA1 [107, 108], SCA2 [109], and SCA3 [110].

In addition, recent data show that SCA15, a non-polyQ SCA, is due to a deletion mutation in the InsP3 receptor 1 gene encoding a receptor that functions in the release of Ca^{2+} from intracellular stores [111].

Studies attempting to implicate altered channel function in SCA6 have yielded conflicting findings. Some reports focusing on voltage dependence of inactivation found that SCA6-associated polyQ expansions had opposing effects on the inactivation of α1A splice forms representing P- or Q-type channels. Specifically, P-type channels (those present in Purkinje cells) bearing SCA6 expansions had a small negative shift in the voltage dependence of inactivation, while in Q-type channels this shift was in the opposite direction [96, 98]. These changes would predict reduced Ca^{2+} entry into Purkinje cells and increased Ca^{2+} entry into granule cells. More recently, expression of SCA6 expansions in cultured cells was associated with increased current density but negligible changes in P/Q gating kinetics [112]. On the other hand, using the Xenopus oocyte expression system, Restituito et al. [97] reported that P/Q-type α1A subunits bearing SCA6 polyQ expansions had a negative shift in the voltage dependence of activation and a delay in inactivation, but only if they were assembled with β4 subunits. Intriguingly, β4 subunits are highly expressed in Purkinje cells. This change favored a gain-of-function mechanism with excessive entry of Ca^{2+} and possible triggering of Ca^{2+}-induced Ca^{2+} release from Purkinje cell internal stores [113–116]. Finally, in two recent studies, transgenic mice expressing α1A subunits with Q28, Q30, or Q84 alleles in the α1A C-terminus generated by homologous recombination had no alteration in P/Q channel kinetics in cerebellar neurons [99, 100]. These studies strongly suggest that the expanded polyQ tract in the C-terminus in SCA6 may act by mechanisms other than perturbation of channel function.

The functional relationship between α1A and α1ACT needs further elucidation, but α1ACT does not eliminate expression of functional α1A subunit, an observation that has important implications for the interpretation of previous electrophysiological studies. It is possible that α1ACT affects kinetic functioning of the full-length channel through α1ACT-regulated signal transduction pathway. Because whole cell patch-clamp techniques, rather than single channel recording have been traditionally used, it is imperative to determine the effect of the polyglutamine-expanded α1A subunit, on normal P/Q-type channel kinetics to SCA6 pathogenesis.

7.7.2 SCA6 as a Consequence of Transcriptional Dysregulation

C-terminal fragments of α1A subunits were detected in protein extracts of purified α1A and α1A-expressing cells nearly two decades ago [117]. While initially seeming to have little importance, these fragments took on greater significance when the SCA6 mutation was identified in the extreme distal C-terminus of the α1A

subunit. Kubodera et al. [101] reported that the C-terminus of the α1A subunit is post-translationally processed from the full-length protein and in HEK cell cultures expressing C-terminal fragments cell death was increased in an allele-dependent manner. C-termini containing Q28 were significantly more toxic than C-termini harboring Q13. In this experiment there was no difference in toxicity of full-length α1A subunits bearing wild-type or expanded polyglutamine tracts [101].

Recent studies have demonstrated that α1ACT with polyglutamine expansion is located in within cerebellar tissue [4]. Of greater significance however, is observation that the endogenous free C-terminal fragment localizes to Purkinje cell nuclei, and that nuclear localization is not affected by polyglutamine length. To understand the molecular basis for the nuclear translocation, three potential nuclear localization signals (NLSs) were eliminated to determine whether they target the C-terminal fragment to the nucleus. In HEK cell cultures, elimination of any of the three NLS reduces nuclear localization of the C-terminus, but elimination of all three NLS drastically reduces C-terminal nuclear localization [4].

As a transcription factor, α1ACT is essential for maintenance of neurite outgrowth through gene-specific signaling pathways. Several groups have demonstrated that in HEK and primary granule cell cultures, expression of the α1ACT, when bearing an expanded polyglutamine tract of 33 glutamines, is toxic to cells [3, 4]. This toxicity is independent of the function of the full-length protein. In addition, like other polyglutamine diseases the toxicity is highly dependent on the nuclear localization of the expanded protein. Compared the properties of the normal α1ACT protein, $\alpha1ACT_{WT}$, the $\alpha1ACT_{SCA6}$ polypeptide has altered binding to the BTG1 enhancer, showing additional DNA-protein complexes in EMSA, and lacks the capacity to medicate expression via BTG1 and GRN luciferase reporters or of the native transcripts of these genes in PC12 cells. $\alpha1ACT_{SCA6}$ also fails to mediate neurite outgrowth when stably expressed in PC12 cells and causes increased cell death. It will be of interest to extend CHIP-based cloning approach using RNA-seq methodology to more completely characterize the normal repertoire of α1ACT-regulated genes, as well as those bound by $\alpha1ACT_{SCA6}$.

Given that the SCA6 expanded C-terminal fragment is the first demonstration that a small CAG expansion of only 28 or 33 repeats can be toxic to cells, these data suggest that SCA6 may have a similar disease mechanism as other polyglutamine diseases. Thus, treatments directed inhibiting *CACNA1A* IRES, nuclear accumulation, or nuclear entry might provide a useful treatment for SCA6, in addition to the other polyglutamine diseases.

7.7.3 SCA6 as a Polyglutamine Disease

The mutational mechanism in SCA6 suggests that the pathogenesis of this disorder may share features in common with polyglutamine diseases (Huntington's disease, dentatorubro-pallidoluysian atrophy, spinobulbar muscular atrophy, SCA1, SCA2, Machado-Joseph disease [SCA3], SCA7, and SCA17). In each of these disorders,

disease arises when a normal stretch of CAGs encoding ~10–40 glutamines is expanded mutationally to 40–100 glutamines. The polyglutamine tracts in these disorders occur within entirely distinct proteins, and the associated disease phenotypes display overlapping, yet clearly distinct, topographic distribution of pathology [118]. Formation of insoluble aggregates, or more direct interference by soluble forms of the mutant protein, with certain vital components of cellular metabolism or nuclear processes has been invoked as pathogenic mechanisms [113]. It remains to be determined whether the accumulation of aggregates contributes to the pathogenesis of or protection from the disease.

While clinically similar to some of the polyglutamine disorders such as SCA1, SCA2, and SCA3 [119, 120], it is quite striking that in SCA6 the disease results from a much smaller expanded polyglutamine tract. Fully penetrant ataxia arises with expansions of only 20–33 glutamines, well within the normal range in other polyQ diseases in which toxicity results at $\geq$ ~40 repeats.

There is growing experimental evidence for the role of the α1A subunit C-terminus as a polyglutamine protein. In postmortem brains studied using an antibody to the C-terminus, non-ubiquitinated accumulations were immunolabeled in the Purkinje cells of an SCA6 patient brain [101].

Recently, using a distinct antibody to the α1ACT, specific immunolabeling of Purkinje cell nuclei in both normal human and mouse and SCA6 cerebellum is seen. Analysis of protein extracts of mouse cerebellum identified a 60–70-kDa α1ACT fragment that was enriched in nuclei. The fragment is also present and enriched in nuclei of cells transfected with α1A expression vectors. Nuclear transport appeared to depend on the presence of evolutionarily conserved nuclear localization signals. Lastly, α1ACT bearing expanded polyglutamine tracts of 33 repeats cause increased cell death compared with wild-type α1ACT, when allowed to translocate to nuclei [4]. These findings suggest that the disease may result from processes more closely related to nuclear accumulation of expanded polyglutamine proteins or fragments as seen in other polyglutamine disorders [121]. Pathogenicity in the context of accumulation of proteins bearing expanded polyQ tracts or insoluble polymers leading to a "toxic gain-of-function" has been proposed to arise from several mechanisms, such as formation of toxic associations with housekeeping genes or transcription factors, interference with the ubiquitin–proteasome pathway or direct DNA damage [70], or the induction of apoptotic pathways [122].

7.8 SCA6 Animal Models

Five spontaneous mouse mutants harboring loss-of-function mutations in the *CACNA1A* gene that lead to reduced P/Q-type density in cerebellar PCs have been described first: tottering (tg), leaner (la), rolling Nagoya (rol), rocker (rkr), and tottering-4j (tg-4j) [123]; Four of the spontaneous *CACNA1A* mutations are missense, leading to substitutions of conserved amino acids in the pore lining or voltage sensor regions, the leaner phenotype is caused by a splice-site mutation producing two

aberrant splice products with altered C-terminal sequences. All homozygous mice show progressive ataxia, ranging from mild in rkr/rkr, tg/tg, and tg-4j/tg4j to severe ataxia and dystonia in la/la. The molecular consequence of one of these mutations, Tg (la), is selective loss of the complete C-terminus of the α1A subunit after either codon 1922 or codon 1967, resulting in seizures, and episodic and progressive cerebellar disease with degeneration of Purkinje cells. Because in this mouse the mutant "tail-less" P/Q-type channels remain largely functional within the cerebellar neurons, this mutant points to a key role for the C-terminus in cerebellar neuronal function and viability, a fact relevant to the human disease SCA6. In the recent several years, more animal models are established and used to study SCA6. Information gained from these studies provide a good starting point for further investigation of the dynamics of gene expression regulation and its role in the pathogenesis of SCA6.

7.8.1 α1A Subunit Knockout (KO) Mouse

To explore the underlying mechanisms of the diverse neuropathological defects generated by α1A subunit mutations, α1A knockout mouse lacking P/Q-type currents has been generated. α1A KO mice show several neurological abnormalities [124]. First, absence seizures, characterized by 3–5 Hz cortical spike-wave discharges associated with behavioral immobility; second, ataxia and dystonia, beginning at about 10 days after birth (P10) and becoming worse with age, if unaided the mice do not survive past weaning; third, selective degeneration of the cerebellum in older mice, with a specific pattern affecting mainly the anterior vermis. Developmental changes in the calcium channel types mediating synaptic transmission are observed at many central synapses, including the inhibitory synapses between Purkinje cells and deep cerebellar nuclei neurons. Interestingly, this occurs at P12–16, the same age at which the ataxic symptoms begins in α1A KO mice. With the finding that P/Q-type calcium channels are absent in cerebellar granule cells in KO mice, this mouse model provided direct evidence that α1A subunit generates both P- and Q-type currents and it its absence causes progressive cerebellar ataxia [106].

A cerebellar phenotype similar to that of la/la and $CaV2.1^{-/-}$ mice (including death of GCs and PCs) has been recently reported for Ca2.1 knockdown mice with 30% of the WT CaV2.1 level, whereas mice with 60 and 55% of the WT Ca2.1 level were normal [125]. It remains unknown why, when reduction of P/Q channel function exceeds the critical threshold, neuronal death in the cerebellum is not homogeneous and occurs in a highly specific pattern.

7.8.2 Purkinje Cell-Specific α1A KO Mouse

To study the specific contribution of α1A calcium channel in Purkinje cells to the development of ataxia that is associated with mutations in the *CACNA1A* gene,

conditional *CACNA1A* mice are crossed with transgenic mice expressing Cre recombinase, driven by the Purkinje cell-specific Pcp2 promoter, to specifically ablate the α1A subunit and thereby P/Q calcium channels in Purkinje cells. Purkinje cell α1A-KO (PCα1A KO) mice had a normal lifespan, clearly distinct from the lethal phenotype seen in α1KO mice. PCα1A KO mice exhibited cerebellar ataxia starting around P12, much earlier than the first signs of progressive Purkinje cell loss, which appears in these mice between P30 and P45. Secondary cell loss was observed in the granular and molecular layers of the cerebellum and the volume of all individual cerebellar nuclei was reduced. In this mouse model with a cell type-specific ablation of P/Q-type $Ca2^{+}$ channels, it is shown that ablation of P/Q-type $Ca2^{+}$ channels restricted to Purkinje cells is sufficient to cause cerebellar ataxia and Purkinje cell degeneration [126].

7.8.3 Postnatal Purkinje Cell-Specific α1A KO Mouse

To visualize P/Q-type $Ca2^{+}$ channels and dissect their role in neuronal networks at distinct developmental stages, a novel conditional *CACNA1A* knock-in mouse was created by inserting the floxed GFP derivative Citrine into the first exon of *CACNA1A*, then crossing it with a postnatally expressing PCP2-Cre line for delayed Purkinje cell (PC) gene deletion within the cerebellum and sparsely in forebrain (purky). PCs in purky mice lacked P/Q-type calcium channel protein and currents within the first month after birth, displayed altered spontaneous firing, and showed impaired neurotransmission. Unexpectedly, adult purky mice exhibited the full spectrum of neurological deficits seen in mice with genomic *CACNA1A* ablation. Results from this mouse model suggest that the ataxia, dyskinesia and absence epilepsy due to inherited disorders of the P/Q-type channel arise from signaling defects beginning in late infancy, revealing an early window of opportunity for therapeutic intervention [127].

7.8.4 SCA6 Knockin (KI) Mouse

To explore the underlying pathogenetic mechanisms of SCA6, two human *CACNA1A* gene knock in mouse models have been generated. Saegusa et al. [99] generated knock-in mouse models that express human Cav2.1 with 28 polyglutamine repeats (disease range) (Q28) and with 13 polyglutamine repeats (normal range) (Q13) by targeted insertion of the fully spliced α1A cDNA. Heterozygous SCA6 Q28 mice did not have abnormal phenotype, although homozygous Q28 as well as homozygous Q13 mice developed ataxia and died by 3 weeks of age. This was attributable to an artifact in the expression of the transgene as all knockin alleles had 7–11% expression levels compared with endogenous *CACNA1A*. Despite the problems with expression studies in these mice P/Q channels could be

recorded in dissociated Purkinje cells from these mice. Surprisingly, the voltage dependence of activation and inactivation and current density were not different between SCA6 Q28, Q13 and control. These results do not support the notion that the alteration of the channel properties underlie the pathogenic mechanism of SCA6.

Watase et al. [100] established another SCA6KI mouse model. In this model, CAG repeat and its immediate flanking sequence derived from human *CACNA1A* were inserted into the *Cana1a* locus using homologous recombination in embryonic stem cells. SCA6KI mice carrying a hyperexpanded (84Q) polyQ developed age-dependent motor impairment. Neither SCA6KI mice with an expanded, Q30, polyQ tract in the range of SCA6 patients, nor those with Q14, control size polyQ tract developed any neurological signs, including in the homozygous state during the life of the mouse. Analysis of splice forms of the *CACNA1A* gene showed that these the *CACNA1A* splice forms were disrupted in these knockin mice, with reduced total expression levels and reduced proportion of the polyQ containing isoforms. In 22-month-old homozygous 84Q mice neuronal inclusions reminiscent of those seen in human SCA6 PCs were found predominantly in the cytoplasm of PCs, but without neurodegenerative changes. These findings are consistent with the hypothesis that SCA6 pathogenesis involves a toxic gain-of-function mechanism associated with accumulation of the expanded polyQ protein similar to that of other polyglutamine diseases. Thus, although both SCA6 knockin studies had unexpected consequences of altered expression levels and splice variants, they provided evidence that the P/Q channels that were formed with expanded polyQ tracts of 29 and 33 did not exhibit abnormal P/Q channel function. Another mouse knockin model of a massive expansion of 118 repeats in the calcium channel machine showed evidence of lysosomal abnormalities, although the relevance of this to the human disease is uncertain [128].

7.8.5 Purkinje Cell-Specific α1ACT Transgenic Mice

To investigate the physiological and pathological function of the α1ACT fragment in PCs, the Pcp2 promoter and Tet-off system [129] were used to generate two double transgenic mouse lines, Pcp2-tTA/TRE-α1ACT (abbreviated, PC-α1ACT), expressing at comparable levels either $\alpha1ACT_{WT}$ (WT = Q4, the smallest α1ACT polyQ seen in humans) or $\alpha1ACT_{SCA6}$ (SCA6 = Q33, the largest α1ACT polyQ seen in SCA6) fragments tagged with an N-terminal myc epitope. These mice appeared to grow and develop normally and live a full life span. $\alpha1ACT_{SCA6}$ mice, however, have mild progressive motor problems that are evident using the computerized treadmill (Digigait). Although there is no obvious sign of cell loss in the cerebellum in $\alpha1ACT_{SCA6}$ mice at approximately 2 years of age, measurement of the molecular layer thickness shows that $\alpha1ACT_{SCA6}$-expressing mice have significant thinning of this layer, compared with age-matched $\alpha1ACT_{WT}$ and WT mice. Lastly, using RT-PCR to examine the expression levels of ChIP-identified

genes TAF, GRN, BTG1 and PMCA2 in cerebellar tissues of 2-year-old PC-α1ACT$_{SCA6}$ mice compared with age-matched WT mice, transcript levels of each of the α1ACT-regulated genes are decreased. These findings are the first to demonstrate clinical and pathological changes in an animal model of SCA6 expressing appropriate-sized pathological alleles within a *CACNA1A* protein [3].

AAV CT knockin mice lines have been generated that exogenously expressed the human CT polypeptides-CT-short (ending at exon 46) and CT-long polyQ (ending at exon 47 and containing 27 polyQ residues)—in mouse cerebellar PCs using viral and transgenic approaches. Although wt allele controls were not included, the findings appear to support the point that, CTs cluster in nuclear inclusions and cytoplasmic aggregates in infected PCs. Postnatal expression of the disease peptide causes SCA6 symptoms, including ataxia and PC degeneration, that are correlated with altered PC firing in vivo. Most importantly, expression of the SCA6 peptide causes a virtually complete loss of (eyeblink conditioning) EBC that is correlated with the loss of cerebellar plasticity (i.e., LTP and LTD) at the parallel fiber (PF)-to-PC synapse. The results suggest that the polyQ carrying the CT fragment of the P/Q-type channel is sufficient to cause SCA6 pathogenesis in mice [130]. On the other hand, a neonatal model of SCA6 has been generated using AAV to deliver α1ACT. These mice have severe ataxia phenotype and disrupted Purkinje cell development [131]. These studies confirm that the α1ACT protein is the toxic moiety, suggesting that a reaspnable treatment strategy would center around suppression of α1ACT expression while sparing α1A expression. In fact, a microRNA targeted to the *CACNA1A* IRES, delivered by AAV, can prevent this neonatal form of SCA6.

7.9 Conclusions

SCA6 is a late onset autosomal dominant, essentially pure, cerebellar ataxia associated with an expanded CAG repeat in the gene, *CACNA1A*. While there is some support for the view that SCA6, like many other *CACNA1A* disorders is a channelopathy, the identification of the transcription factor, α1ACT, encoded by a second cistron in the *CACNA1A* gene, a mechanism involving transcription dysregulation mediated by a polyQ-expanded α1ACT protein has become a more likely scenario in the pathogenesis of SCA6. A better characterization of the physiological function of *CACNA1A* gene and its two proteins, α1A and α1ACT, and the regulation of α1ACT expression will be important to clarify the mechanism of SCA6 pathogenesis and to design therapies.

References

1. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. Nat Genet 15:62–69
2. Catterall WA (2000) From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 26:13–25
3. Du X, Wang J, Zhu H, Rinaldo L, Lamar KM, Palmenberg AC, Hansel C, Gomez CM (2013) Second cistron in CACNA1A gene encodes a transcription factor mediating cerebellar development and SCA6. Cell 154:118–133
4. Kordasiewicz HB, Thompson RM, Clark HB, Gomez CM (2006) C-termini of P/Q-type Ca^{2+} channel alpha1A subunits translocate to nuclei and promote polyglutamine-mediated toxicity. Hum Mol Genet 15:1587–1599
5. Gomez CM, Thompson RM, Gammack JT, Perlman SL, Dobyns WB, Truwit CL, Zee DS, Clark HB, Anderson JH (1997) Spinocerebellar ataxia type 6: gaze-evoked and vertical nystagmus, Purkinje cell degeneration, and variable age of onset. Ann Neurol 42:933–950
6. Jodice C, Mantuano E, Veneziano L, Trettel F, Sabbadini G, Calandriello L, Francia A, Spadaro M, Pierelli F, Salvi F et al (1997) Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. Hum Mol Genet 6:1973–1978
7. Globas C, du Montcel ST, Baliko L, Boesch S, Depondt C, DiDonato S, Durr A, Filla A, Klockgether T, Mariotti C et al (2008) Early symptoms in spinocerebellar ataxia type 1, 2, 3, and 6. Mov Disord 23:2232–2238
8. Hashimoto T, Sasaki O, Yoshida K, Takei Y, Ikeda S (2003) Periodic alternating nystagmus and rebound nystagmus in spinocerebellar ataxia type 6. Mov Disord 18:1201–1204
9. Globas C, Bosch S, Zuhlke C, Daum I, Dichgans J, Burk K (2003) The cerebellum and cognition. Intellectual function in spinocerebellar ataxia type 6 (SCA6). J Neurol 250: 1482–1487
10. Schmitz-Hubsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, Giunti P, Globas C, Infante J, Kang JS et al (2006) Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 66:1717–1720
11. Jacobi H, du Montcel ST, Bauer P, Giunti P, Cook A, Labrum R, Parkinson MH, Durr A, Brice A, Charles P et al (2015) Long-term disease progression in spinocerebellar ataxia types 1, 2, 3, and 6: a longitudinal cohort study. Lancet Neurol 14:1101–1108
12. Fukutake T, Kamitsukasa I, Arai K, Hattori T, Nakajima T (2002) A patient homozygous for the SCA6 gene with retinitis pigmentosa. Clin Genet 61:375–379
13. Tashiro H, Suzuki SO, Hitotsumatsu T, Iwaki T (1999) An autopsy case of spinocerebellar ataxia type 6 with mental symptoms of schizophrenia and dementia. Clin Neuropathol 18:198–204
14. Yun JY, Kim JM, Kim HJ, Kim YE, Jeon BS (2012) SCA6 presenting with young-onset parkinsonism without ataxia. Mov Disord 27:1067–1068
15. Khan NL, Giunti P, Sweeney MG, Scherfler C, Brien MO, Piccini P, Wood NW, Lees AJ (2005) Parkinsonism and nigrostriatal dysfunction are associated with spinocerebellar ataxia type 6 (SCA6). Mov Disord 20:1115–1119
16. Takeshima S, Takeda I, Kobatake K, Yamashita T, Abe K, Kuriyama M (2015) SCA6 presenting parkinsonism without ataxia–A case report. Rinsho Shinkeigaku 55:243–247
17. Xie T, Appelbaum D, Bernard J, Padmanaban M, Pu Y, Gomez C (2016) Evaluation of parkinsonism and striatal dopamine transporter loss in patients with spinocerebellar ataxia type 6. J Neurol 263:2302–2307
18. Manto MU (2005) The wide spectrum of spinocerebellar ataxias (SCAs). Cerebellum 4:2–6
19. Craig K, Keers SM, Archibald K, Curtis A, Chinnery PF (2004) Molecular epidemiology of spinocerebellar ataxia type 6. Ann Neurol 55:752–755

20. Craig K, Takiyama Y, Soong BW, Jardim LB, Saraiva-Pereira ML, Lythgow K, Morino H, Maruyama H, Kawakami H, Chinnery PF (2008) Pathogenic expansions of the SCA6 locus are associated with a common CACNA1A haplotype across the globe: founder effect or predisposing chromosome? Eur J Hum Genet 16:841–847
21. Geschwind DH, Perlman S, Figueroa KP, Karrim J, Baloh RW, Pulst SM (1997) Spinocerebellar ataxia type 6. Frequency of the mutation and genotype-phenotype correlations. Neurology 49:1247–1251
22. Ruano L, Melo C, Silva MC, Coutinho P (2014) The global epidemiology of hereditary ataxia and spastic paraplegia: a systematic review of prevalence studies. Neuroepidemiology 42:174–183
23. Pujana MA, Corral J, Gratacos M, Combarros O, Berciano J, Genis D, Banchs I, Estivill X, Volpini V (1999) Spinocerebellar ataxias in Spanish patients: genetic analysis of familial and sporadic cases. The Ataxia Study Group. Hum Genet 104:516–522
24. Brusco A, Gellera C, Cagnoli C, Saluto A, Castucci A, Michielotto C, Fetoni V, Mariotti C, Migone N, Di Donato S et al (2004) Molecular genetics of hereditary spinocerebellar ataxia: mutation analysis of spinocerebellar ataxia genes and CAG/CTG repeat expansion detection in 225 Italian families. Arch Neurol 61:727–733
25. Bryer A, Krause A, Bill P, Davids V, Bryant D, Butler J, Heckmann J, Ramesar R, Greenberg J (2003) The hereditary adult-onset ataxias in South Africa. J Neurol Sci 216: 47–54
26. Jiang H, Tang B, Xia K, Zhou Y, Xu B, Zhao G, Li H, Shen L, Pan Q, Cai F (2005) Spinocerebellar ataxia type 6 in Mainland China: molecular and clinical features in four families. J Neurol Sci 236:25–29
27. Teive HA, Munhoz RP, Raskin S, Werneck LC (2008) Spinocerebellar ataxia type 6 in Brazil. Arq Neuropsiquiatr 66:691–694
28. Bang OY, Huh K, Lee PH, Kim HJ (2003) Clinical and neuroradiological features of patients with spinocerebellar ataxias from Korean kindreds. Arch Neurol 60:1566–1574
29. Storey E, du Sart D, Shaw JH, Lorentzos P, Kelly L, McKinley Gardner RJ, Forrest SM, Biros I, Nicholson GA (2000) Frequency of spinocerebellar ataxia types 1, 2, 3, 6, and 7 in Australian patients with spinocerebellar ataxia. Am J Med Genet 95:351–357
30. van de Warrenburg BP, Sinke RJ, Verschuuren-Bemelmans CC, Scheffer H, Brunt ER, Ippel PF, Maat-Kievit JA, Dooijes D, Notermans NC, Lindhout D et al (2002) Spinocerebellar ataxias in the Netherlands: prevalence and age at onset variance analysis. Neurology 58:702–708
31. Yabe I, Sasaki H, Matsuura T, Takada A, Wakisaka A, Suzuki Y, Fukazawa T, Hamada T, Oda T, Ohnishi A et al (1998) SCA6 mutation analysis in a large cohort of the Japanese patients with late-onset pure cerebellar ataxia. J Neurol Sci 156:89–95
32. Sasaki H, Yabe I, Tashiro K (2003) The hereditary spinocerebellar ataxias in Japan. Cytogenet Genome Res 100:198–205
33. Ishikawa K, Tanaka H, Saito M, Ohkoshi N, Fujita T, Yoshizawa K, Ikeuchi T, Watanabe M, Hayashi A, Takiyama Y et al (1997) Japanese families with autosomal dominant pure cerebellar ataxia map to chromosome 19p13.1-p13.2 and are strongly associated with mild CAG expansions in the spinocerebellar ataxia type 6 gene in chromosome 19p13.1. Am J Hum Genet 61:336–346
34. Verbeek DS, Piersma SJ, Hennekam EF, Ippel EF, Pearson PL, Sinke RJ (2004) Haplotype study in Dutch SCA3 and SCA6 families: evidence for common founder mutations. Eur J Hum Genet 12:441–446
35. Yabe I, Sasaki H, Yamashita I, Tashiro K, Takei A, Suzuki Y, Kida H, Takiyama Y, Nishizawa M, Hokezu Y et al (2001) Predisposing chromosome for spinocerebellar ataxia type 6 (SCA6) in Japanese. J Med Genet 38:328–333
36. Gomez CM (1993) Spinocerebellar ataxia type 6. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH et al (eds) GeneReviews(R). Seattle, WA
37. Griggs RC, Nutt JG (1995) Episodic ataxias as channelopathies. Ann Neurol 37:285–287

38. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SM, Lamerdin JE, Mohrenweiser HW, Bulman DE, Ferrari M et al (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene CACNL1A4. Cell 87:543–552
39. Montagna P (2000) Molecular genetics of migraine headaches: a review. Cephalalgia 20:3–14
40. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Mohrenweiser H, Litt M, Hofker MH, Haan J, Ferrari MD, Frants RR (1996) A 3-Mb region for the familial hemiplegic migraine locus on 19p13.1-p13.2: exclusion of PRKCSH as a candidate gene. Dutch Migraine Genetic Research Group. Eur J Hum Genet 4:321–328
41. Terwindt GM, Ophoff RA, Haan J, Frants RR, Ferrari MD (1996) Familial hemiplegic migraine: a clinical comparison of families linked and unlinked to chromosome 19.DMG RG. Cephalalgia 16:153–155
42. Geschwind DH, Perlman S, Figueroa CP, Treiman LJ, Pulst SM (1997) The prevalence and wide clinical spectrum of the spinocerebellar ataxia type 2 trinucleotide repeat in patients with autosomal dominant cerebellar ataxia. Am J Hum Genet 60:842–850
43. Jen J, Yue Q, Nelson SF, Yu H, Litt M, Nutt J, Baloh RW (1999) A novel nonsense mutation in CACNA1A causes episodic ataxia and hemiplegia. Neurology 53:34–37
44. Alonso I, Barros J, Tuna A, Coelho J, Sequeiros J, Silveira I, Coutinho P (2003) Phenotypes of spinocerebellar ataxia type 6 and familial hemiplegic migraine caused by a unique CACNA1A missense mutation in patients from a large family. Arch Neurol 60:610–614
45. Schelhaas HJ, Van de Warrenburg BP, Kremer HP, Zwarts MJ (2004) Neuromuscular transmission in SCA6. Ann Neurol 55:451–452
46. Freilinger T, Ackl N, Ebert A, Schmidt C, Rautenstrauss B, Dichgans M, Danek A (2011) A novel mutation in CACNA1A associated with hemiplegic migraine, cerebellar dysfunction and late-onset cognitive decline. J Neurol Sci 300:160–163
47. Garcia Segarra N, Gautschi I, Mittaz-Crettol L, Kallay Zetchi C, Al-Qusairi L, Van Bemmelen MX, Maeder P, Bonafe L, Schild L, Roulet-Perez E (2014) Congenital ataxia and hemiplegic migraine with cerebral edema associated with a novel gain of function mutation in the calcium channel CACNA1A. J Neurol Sci 342:69–78
48. Tantsis EM, Gill D, Griffiths L, Gupta S, Lawson J, Maksemous N, Ouvrier R, Riant F, Smith R, Troedson C et al (2016) Eye movement disorders are an early manifestation of CACNA1A mutations in children. Dev Med Child Neurol 58:639–644
49. Mariotti C, Gellera C, Grisoli M, Mineri R, Castucci A, Di Donato S (2001) Pathogenic effect of an intermediate-size SCA-6 allele (CAG)(19) in a homozygous patient. Neurology 57:1502–1504
50. Shizuka M, Watanabe M, Ikeda Y, Mizushima K, Okamoto K, Shoji M (1998) Molecular analysis of a de novo mutation for spinocerebellar ataxia type 6 and (CAG)n repeat units in normal elder controls. J Neurol Sci 161:85–87
51. Katayama T, Ogura Y, Aizawa H, Kuroda H, Suzuki Y, Kuroda K, Kikuchi K (2000) Nineteen CAG repeats of the SCA6 gene in a Japanese patient presenting with ataxia. J Neurol 247:711–712
52. Schols L, Kruger R, Amoiridis G, Przuntek H, Epplen JT, Riess O (1998) Spinocerebellar ataxia type 6: genotype and phenotype in German kindreds. J Neurol Neurosurg Psychiatry 64:67–73
53. Matsuyama Z, Kawakami H, Maruyama H, Izumi Y, Komure O, Udaka F, Kameyama M, Nishio T, Kuroda Y, Nishimura M et al (1997) Molecular features of the CAG repeats of spinocerebellar ataxia 6 (SCA6). Hum Mol Genet 6:1283–1287
54. Takahashi H, Ishikawa K, Tsutsumi T, Fujigasaki H, Kawata A, Okiyama R, Fujita T, Yoshizawa K, Yamaguchi S, Tomiyasu H et al (2004) A clinical and genetic study in a large cohort of patients with spinocerebellar ataxia type 6. J Hum Genet 49:256–264
55. Ikeuchi T, Takano H, Koide R, Horikawa Y, Honma Y, Onishi Y, Igarashi S, Tanaka H, Nakao N, Sahashi K et al (1997) Spinocerebellar ataxia type 6: CAG repeat expansion in alpha1A voltage-dependent calcium channel gene and clinical variations in Japanese population. Ann Neurol 42:879–884

56. Takiyama Y, Sakoe K, Namekawa M, Soutome M, Esumi E, Ogawa T, Ishikawa K, Mizusawa H, Nakano I, Nishizawa M (1998) A Japanese family with spinocerebellar ataxia type 6 which includes three individuals homozygous for an expanded CAG repeat in the SCA6/CACNL1A4 gene. J Neurol Sci 158:141–147
57. Zoghbi HY (1997) CAG repeats in SCA6. Anticipating new clues. Neurology 49:1196–1199
58. Lindquist SG, Norremolle A, Hjermind LE, Hasholt L, Nielsen JE (2006) Meiotic CAG repeat instability in spinocerebellar ataxia type 6: maternally transmitted elongation in a presumed sporadic case. J Neurol Sci 241:95–98
59. Shimazaki H, Takiyama Y, Sakoe K, Amaike M, Nagaki H, Namekawa M, Sasaki H, Nakano I, Nishizawa M (2001) Meiotic instability of the CAG repeats in the SCA6/CACNA1A gene in two Japanese SCA6 families. J Neurol Sci 185:101–107
60. Mantuano E, Veneziano L, Jodice C, Frontali M (2003) Spinocerebellar ataxia type 6 and episodic ataxia type 2: differences and similarities between two allelic disorders. Cytogenet Genome Res 100:147–153
61. Yang Q, Hashizume Y, Yoshida M, Wang Y, Goto Y, Mitsuma N, Ishikawa K, Mizusawa H (2000) Morphological Purkinje cell changes in spinocerebellar ataxia type 6. Acta Neuropathol 100:371–376
62. Lukas C, Schols L, Bellenberg B, Rub U, Przuntek H, Schmid G, Koster O, Suchan B (2006) Dissociation of grey and white matter reduction in spinocerebellar ataxia type 3 and 6: a voxel-based morphometry study. Neurosci Lett 408:230–235
63. Honjo K, Ohshita T, Kawakami H, Naka H, Imon Y, Maruyama H, Mimori Y, Matsumoto M (2004) Quantitative assessment of cerebral blood flow in genetically confirmed spinocerebellar ataxia type 6. Arch Neurol 61:933–937
64. Murata Y, Kawakami H, Yamaguchi S, Nishimura M, Kohriyama T, Ishizaki F, Matsuyama Z, Mimori Y, Nakamura S (1998) Characteristic magnetic resonance imaging findings in spinocerebellar ataxia 6. Arch Neurol 55:1348–1352
65. Reetz K, Costa AS, Mirzazade S, Lehmann A, Juzek A, Rakowicz M, Boguslawska R, Schols L, Linnemann C, Mariotti C et al (2013) Genotype-specific patterns of atrophy progression are more sensitive than clinical decline in SCA1, SCA3 and SCA6. Brain 136:905–917
66. Falcon MI, Gomez CM, Chen EE, Shereen A, Solodkin A (2015) Early cerebellar network shifting in spinocerebellar ataxia type 6. Cereb Cortex 26:3205–3218
67. Wang PS, Liu RS, Yang BH, Soong BW (2007) Regional patterns of cerebral glucose metabolism in spinocerebellar ataxia type 2, 3 and 6: a voxel-based FDG-positron emission tomography analysis. J Neurol 254:838–845
68. Wullner U, Reimold M, Abele M, Burk K, Minnerop M, Dohmen BM, Machulla HJ, Bares R, Klockgether T (2005) Dopamine transporter positron emission tomography in spinocerebellar ataxias type 1, 2, 3, and 6. Arch Neurol 62:1280–1285
69. Kawai Y, Suenaga M, Watanabe H, Ito M, Kato K, Kato T, Ito K, Tanaka F, Sobue G (2008) Prefrontal hypoperfusion and cognitive dysfunction correlates in spinocerebellar ataxia type 6. J Neurol Sci 271:68–74
70. Ross BP, Braddy AC, McGeary RP, Blanchfield JT, Prokai L, Toth I (2004) Micellar aggregation and membrane partitioning of bile salts, fatty acids, sodium dodecyl sulfate, and sugar-conjugated fatty acids: correlation with hemolytic potency and implications for drug delivery. Mol Pharm 1:233–245
71. Sasaki H, Kojima H, Yabe I, Tashiro K, Hamada T, Sawa H, Hiraga H, Nagashima K (1998) Neuropathological and molecular studies of spinocerebellar ataxia type 6 (SCA6). Acta Neuropathol 95:199–204
72. Koeppen AH (2005) The pathogenesis of spinocerebellar ataxia. Cerebellum 4:62–73
73. Tsuchiya K, Oda T, Yoshida M, Sasaki H, Haga C, Okino H, Tominaga I, Matsui K, Akiyama H, Hashizume Y (2005) Degeneration of the inferior olive in spinocerebellar ataxia 6 may depend on disease duration: report of two autopsy cases and statistical analysis of autopsy cases reported to date. Neuropathology 25:125–135

74. Gierga K, Schelhaas HJ, Brunt ER, Seidel K, Scherzed W, Egensperger R, de Vos RAI, den Dunnen W, Ippel PF, Petrasch-Parwez E et al (2009) Spinocerebellar ataxia type 6 (SCA6): neurodegeneration goes beyond the known brain predilection sites. Neuropathol Appl Neuro 35:515–527
75. Lee A, Scheuer T, Catterall WA (2000) Ca^{2+}/calmodulin-dependent facilitation and inactivation of P/Q-type Ca^{2+} channels. J Neurosci 20:6830–6838
76. Lee A, Wong ST, Gallagher D, Li B, Storm DR, Scheuer T, Catterall WA (1999) Ca^{2+}/calmodulin binds to and modulates P/Q-type calcium channels. Nature 399:155–159
77. Solodkin A, Gomez CM (2012) Spinocerebellar ataxia type 6. Handb Clin Neurol 103: 461–473
78. Jen JC, Graves TD, Hess EJ, Hanna MG, Griggs RC, Baloh RW, CINCH investigators (2007) Primary episodic ataxias: diagnosis, pathogenesis and treatment. Brain 130: 2484–2493
79. Strupp M, Zwergal A, Brandt T (2007) Episodic ataxia type 2. Neurotherapeutics 4:267–273
80. Raike RS, Kordasiewicz HB, Thompson RM, Gomez CM (2007) Dominant-negative suppression of Cav2.1 currents by alpha(1)2.1 truncations requires the conserved interaction domain for beta subunits. Mol Cell Neurosci 34:168–177
81. Palmenberg AC, Sgro JY (1997) Topological organization of picornaviral genomes: statistical prediction of RNA structural signals. Semin Virol 8:231–241
82. Spriggs KA, Stoneley M, Bushell M, Willis AE (2008) Re-programming of translation following cell stress allows IRES-mediated translation to predominate. Biol Cell 100:27–38
83. Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res 31:3406–3415
84. Du X, Semler BL, Gomez CM (2014) Revelations from a bicistronic calcium channel gene. Cell Cycle 13:875–876
85. Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB et al (2000) Nomenclature of voltage-gated sodium channels. Neuron 28:365–368
86. Greenberg DA (1997) Calcium channels in neurological disease. Ann Neurol 42:275–282
87. Hofmann F, Biel M, Flockerzi V (1994) Molecular basis for Ca^{2+} channel diversity. Ann Rev Neurosci 17:399–418
88. Snutch TP, Reiner PB (1992) Ca^{2+} channels: diversity of form and function. Curr Opin Neurobiol 2:247–253
89. Pietrobon D (2002) Calcium channels and channelopathies of the central nervous system. Mol Neurobiol 25:31–50
90. Duenas AM, Goold R, Giunti P (2006) Molecular pathogenesis of spinocerebellar ataxias. Brain 129:1357–1370
91. Miller RJ (1997) Calcium channels prove to be a real headache. Trends Neurosci 20: 189–192
92. Catterall WA, Few AP (2008) Calcium channel regulation and presynaptic plasticity. Neuron 59:882–901
93. Kelly JG, O'Malley K (1993) Calcium antagonists in the elderly. Drugs Aging 3:400–407
94. Sutton KG, McRory JE, Guthrie H, Murphy TH, Snutch TP (1999) P/Q-type calcium channels mediate the activity-dependent feedback of syntaxin-1A. Nature 401:800–804
95. Kulik A, Nakadate K, Hagiwara A, Fukazawa Y, Lujan R, Saito H, Suzuki N, Futatsugi A, Mikoshiba K, Frotscher M et al (2004) Immunocytochemical localization of the alpha 1A subunit of the P/Q-type calcium channel in the rat cerebellum. Eur J Neurosci 19:2169–2178
96. Matsuyama Z, Wakamori M, Mori Y, Kawakami H, Nakamura S, Imoto K (1999) Direct alteration of the P/Q-type Ca^{2+} channel property by polyglutamine expansion in spinocerebellar ataxia 6. J Neurosci 19:RC14
97. Restituito S, Thompson RM, Eliet J, Raike RS, Riedl M, Charnet P, Gomez CM (2000) The polyglutamine expansion in spinocerebellar ataxia type 6 causes a beta subunit-specific enhanced activation of P/Q-type calcium channels in Xenopus oocytes. J Neurosci 20:6394–6403

98. Toru S, Murakoshi T, Ishikawa K, Saegusa H, Fujigasaki H, Uchihara T, Nagayama S, Osanai M, Mizusawa H, Tanabe T (2000) Spinocerebellar ataxia type 6 mutation alters P-type calcium channel function. J Biol Chem 275:10893–10898
99. Saegusa H, Wakamori M, Matsuda Y, Wang J, Mori Y, Zong S, Tanabe T (2007) Properties of human Cav2.1 channel with a spinocerebellar ataxia type 6 mutation expressed in Purkinje cells. Mol Cell Neurosci 34:261–270
100. Watase K, Barrett CF, Miyazaki T, Ishiguro T, Ishikawa K, Hu Y, Unno T, Sun Y, Kasai S, Watanabe M et al (2008) Spinocerebellar ataxia type 6 knockin mice develop a progressive neuronal dysfunction with age-dependent accumulation of mutant CaV2.1 channels. Proc Natl Acad Sci USA 105:11987–11992
101. Kubodera T, Yokota T, Ohwada K, Ishikawa K, Miura H, Matsuoka T, Mizusawa H (2003) Proteolytic cleavage and cellular toxicity of the human alpha1A calcium channel in spinocerebellar ataxia type 6. Neurosci Lett 341:74–78
102. Sakurai T, Westenbroek RE, Rettig J, Hell J, Catterall WA (1996) Biochemical properties and subcellular distribution of the BI and rbA isoforms of alpha 1A subunits of brain calcium channels. J Cell Biol 134:511–528
103. Akbar U, Ashizawa T (2015) Ataxia. Neurol Clin 33:225–248
104. Ilg W, Timmann D (2013) Gait ataxia–specific cerebellar influences and their rehabilitation. Mov Disord 28:1566–1575
105. Kamp MA, Hanggi D, Steiger HJ, Schneider T (2012) Diversity of presynaptic calcium channels displaying different synaptic properties. Rev Neurosci 23:179–190
106. Pietrobon D (2010) CaV2.1 channelopathies. Pflugers Arch 460:375–393
107. Lin X, Antalffy B, Kang D, Orr HT, Zoghbi HY (2000) Polyglutamine expansion down-regulates specific neuronal genes before pathologic changes in SCA1. Nat Neurosci 3:157–163
108. Serra HG, Byam CE, Lande JD, Tousey SK, Zoghbi HY, Orr HT (2004) Gene profiling links SCA1 pathophysiology to glutamate signaling in Purkinje cells of transgenic mice. Hum Mol Genet 13:2535–2543
109. Liu J, Tang TS, Tu H, Nelson O, Herndon E, Huynh DP, Pulst SM, Bezprozvanny I (2009) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 2. J Neurosci 29:9148–9162
110. Chen X, Tang TS, Tu H, Nelson O, Pook M, Hammer R, Nukina N, Bezprozvanny I (2008) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. J Neurosci 28:12713–12724
111. van de Leemput J, Chandran J, Knight MA, Holtzclaw LA, Scholz S, Cookson MR, Houlden H, Gwinn-Hardy K, Fung HC, Lin X et al (2007) Deletion at ITPR1 underlies ataxia in mice and spinocerebellar ataxia 15 in humans. PLoS Genet 3:e108
112. Piedras-Renteria ES, Watase K, Harata N, Zhuchenko O, Zoghbi HY, Lee CC, Tsien RW (2001) Increased expression of alpha 1A Ca2+ channel currents arising from expanded trinucleotide repeats inspinocerebellar ataxia type 6. J Neurosci 21:9185–9193
113. Westenbroek RE, Sakurai T, Elliott EM, Hell JW, Starr TV, Snutch TP, Catterall WA (1995) Immunochemical identification and subcellular distribution of the alpha 1A subunits of brain calcium channels. JNeurosci 15:6403–6418
114. Burgess DL, Jones JM, Meisler MH, Noebels JL (1997) Mutation of the Ca2+ channel beta subunit gene Cchb4 is associated with ataxia and seizures in the lethargic (lh) mouse. Cell 88:385–392
115. Collier ML, Ji G, Wang Y, Kotlikoff MI (2000) Calcium-induced calcium release in smooth muscle: loose coupling between the action potential and calcium release. J Gen Physiol 115:653–662
116. Tully K, Treistman SN (2004) Distinct intracellular calcium profiles following influx through N- versus Ltypecalcium channels: role of Ca2+-induced Ca2+ release. J Neurophysiol 92:135–143
117. Scott VE, Felix R, Arikkath J, Campbell KP (1998) Evidence for a 95 kDa short form of the alpha1A subunit associated with the omega-conotoxin MVIIC receptor of the P/Q-type Ca^{2+} channels. J Neurosci 18:641–647

118. Forman MS, Trojanowski JQ, Lee VM (2004) Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat Med 10:1055–1063
119. Gatchel JR, Zoghbi HY (2005) Diseases of unstable repeat expansion: mechanisms and common principles. Nat Rev Genet 6:743–755
120. Van de Warrenburg BP, Bakker M, Kremer BP, Bloem BR, Allum JH (2005) Trunk sway in patients with spinocerebellar ataxia. Mov Disord 20:1006–1013
121. Nucifora FC Jr, Ellerby LM, Wellington CL, Wood JD, Herring WJ, Sawa A, Hayden MR, Dawson VL, Dawson TM, Ross CA (2003) Nuclear localization of a non-caspase truncation product of atrophin-1, with an expanded polyglutamine repeat, increases cellular toxicity. J Biol Chem 278:13047–13055
122. Lipinski MM, Yuan J (2004) Mechanisms of cell death in polyglutamine expansion diseases. Curr Opin Pharmacol 4:85–90
123. Miki T, Zwingman TA, Wakamori M, Lutz CM, Cook SA, Hosford DA, Herrup K, Fletcher CF, Mori Y, Frankel WN et al (2008) Two novel alleles of tottering with distinct Ca (v)2.1 calcium channel neuropathologies. Neuroscience 155:31–44
124. Jun K, Piedras-Renteria ES, Smith SM, Wheeler DB, Lee SB, Lee TG, Chin H, Adams ME, Scheller RH, Tsien RW et al (1999) Ablation of P/Q-type Ca^{2+} channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the alpha(1A)-subunit. Proc Natl Acad Sci USA 96:15245–15250
125. Saito H, Okada M, Miki T, Wakamori M, Futatsugi A, Mori Y, Mikoshiba K, Suzuki N (2009) Knockdown of Cav2.1 calcium channels is sufficient to induce neurological disorders observed in natural occurring Cacna1a mutants in mice. Biochem Biophys Res Commun 390:1029–1033
126. Todorov B, Kros L, Shyti R, Plak P, Haasdijk ED, Raike RS, Frants RR, Hess EJ, Hoebeek FE, De Zeeuw CI et al (2012) Purkinje cell-specific ablation of Cav2.1 channels is sufficient to cause cerebellar ataxia in mice. Cerebellum 11:246–258
127. Mark MD, Maejima T, Kuckelsberg D, Yoo JW, Hyde RA, Shah V, Gutierrez D, Moreno RL, Kruse W, Noebels JL et al (2011) Delayed postnatal loss of P/Q-type calcium channels recapitulates the absence epilepsy, dyskinesia, and ataxia phenotypes of genomic Cacna1a mutations. J Neurosci 31:4311–4326
128. Unno T, Wakamori M, Koike M, Uchiyama Y, Ishikawa K, Kubota H, Yoshida T, Sasakawa H, Peters C, Mizusawa H et al (2012) Development of Purkinje cell degeneration in a knockin mouse model reveals lysosomal involvement in the pathogenesis of SCA6. Proc Natl Acad Sci USA 109:17693–17698
129. Zu T, Duvick LA, Kaytor MD, Berlinger MS, Zoghbi HY, Clark HB, Orr HT (2004) Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice. J Neurosci 24:8853–8861
130. Mark MD, Krause M, Boele HJ, Kruse W, Pollok S, Kuner T, Dalkara D, Koekkoek S, De Zeeuw CI, Herlitze S (2015) Spinocerebellar ataxia type 6 protein aggregates cause deficits in motor learning and cerebellar plasticity. J Neurosci 35:8882–8895
131. Miyazaki Y, Du X, Muramatsu S, Gomez CM (2016) An miRNA-mediated therapy for SCA6 blocks IRES-driven translation of the CACNA1A second cistron. Sci Transl Med 8:347–394

Chapter 8
Spinocerebellar Ataxia Type 2

Daniel R. Scoles and Stefan M. Pulst

Abstract Spinocerebellar ataxia type 2 (SCA2) is autosomal dominantly inherited and caused by CAG repeat expansion in the *ATXN2* gene. Because the CAG repeat expansion is localized to an encoded region of *ATXN2*, the result is an expanded polyglutamine (polyQ) tract in the ATXN2 protein. SCA2 is characterized by progressive ataxia, and slow saccades. No treatment for SCA2 exists. *ATXN2* mutation causes gains of new or toxic functions for the ATXN2 protein, resulting in abnormally slow Purkinje cell (PC) firing frequency and ultimately PC loss. This chapter describes the characteristics of SCA2 patients briefly, and reviews ATXN2 molecular features and progress toward the identification of a treatment for SCA2.

Keywords Spinocerebellar ataxia type 2 · Ataxin-2 · Neurodegeneration Cerebellum

8.1 SCA2 Clinical Characteristics

While patients with SCA2 possess many of the core clinical characteristics that define the SCAs as a group of neurodegenerative disorders, SCA2 is a clinically distinct. Considered a hallmark characteristic of any SCA, the most noticeable symptom of onset is gait ataxia. In SCA2, onset also frequently, although not always, coincides with muscle cramping. Ataxia onset is then followed by multiple other symptoms characteristic of cerebellar degeneration. For SCA2 these symptoms include appendicular ataxia with instability of stance, dysarthria, and ocular signs including nystagmus, and ocular dysmetria. The signs and symptoms of SCA2 are almost entirely of cerebellar origin, with clearly defined involvement of cerebellar regions and associated cerebellar circuits. However, one predominant ocular feature typical of SCA2, slow or absent saccades, arises from degeneration of

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neurons of the oculomotor brainstem. Dystonia and myoclonus are also frequent in patients with SCA2, as well as neuropathy, muscle spasticity and frontal-executive dysfunction [1–3].

SCA2 phenotype is characterized by gait ataxia in most SCA2 patients, however variant phenotypes have been defined. These variant phenotypes reside outside of the cerebellar spectrum and include L-DOPA responsive parkinsonism and amyotrophic lateral sclerosis (ALS) [3, 4]. Patients with these variant phenotypes present with idiopathic forms of parkinsonism or ALS. While ATXN2-associated parkinsonism and ALS present with no symptoms of cerebellar ataxia, more imaging data are necessary to define whether these variant phenotypes are accompanied with cerebellar atrophy.

8.1.1 SCA2 and ALS

ATXN2 CAG repeat expansions are also associated with ALS-like motor phenotypes. For *ATXN2* CAG repeats in the normal range for SCA2, between 27 and 33 repeats in length, a statistically significant increased risk for ALS has been defined [4, 5]. A meta-analysis of *ATXN2* alleles drawing on worldwide reporting of ALS, however, showed that ALS-risk only increased significantly for CAG repeats ≥ 31. In these patients, the phenotype is indistinguishable from other idiopathic forms of ALS. The causes of the ALS-like phenotypes in patients with ATXN2 expansions are not well described but may associate with the ATXN2 interacting proteins TDP-43 and FUS [4, 6], since mutations in the genes encoding these proteins can cause ALS. ALS and intermediately expanded ATXN2 connects functionally to the action of C9ORF72, since aggregates partially depleted of C9ORF72, including intermediately expanded ATXN2, were neurotoxic due to impaired autophagy [7]. However, it remains to be determined why intermediate expanded ATXN2 increases risk of ALS in the absence of *C9ORF72* mutations. In our meta-analysis an 11-fold increased risk was observed for ATXN2 repeats of 32 [5]. Thus the rarest *ATXN2* alleles represent the highest risk for ALS. However, SCA2 patients with longer *ATXN2* mutations can also present with ALS-like phenotypes [5, 8].

8.2 Discovery of the *ATXN2* Gene

SCA2 was first described in India with the discovery of nine patient families [9]. Nearly two decades later a large population of SCA2 patients was discovered in eastern Cuba [9]. Both discoveries noted that the affected families were characterized by ataxia and other cerebellar signs as well as slow saccades; clinical features that are now known to typify patients with SCA2. In Cuba it was later noted that the prevalence of SCA2 was especially high compared to other regions in the world, attributed to a founder population in the eastern part of the island where 4

out of every 10,000 inhabitants of Holguin province has SCA2 [10, 11]. The existence of the large SCA2 populations in Cuba and elsewhere has aided the identification of the *ATXN2* gene. Varying age of onset (AO) in SCA2 pedigrees helped to establish anticipation in SCA2 of 14.4 ± 7.9 years per generation strongly hinting that the *ATXN2* mutation was likely a repeat expansion mutation [12]. Mapping studies ultimately localized *ATXN2* to Chr 12q24.12 following initial mapping to chromosome 12 [13], and fine mapping to 12q24 [12]. The *ATXN2* gene was identified in 1996 demonstrating the causative mutation as a CAG repeat expansion in the coding region of *ATXN2* resulting in a polyglutamine expansion in the ATXN2 protein [14–16].

8.3 Molecular Genetics of SCA2

Most commonly, the *ATXN2* gene has 22 CAG repeats while ≥ 33 CAG repeats causes SCA2 [17]. SCA2 is characterized by anticipation with strong correlation between age of onset and CAG repeat length (Fig. 8.1). The CAG repeat expansion is dynamic and unstable during meiosis with a strong propensity for expansion.

The *ATXN2* gene consists of 25 exons and spans a total of 147 megabase pairs (147,463 bp). The *ATXN2* transcript is 4699 bp long with relatively small

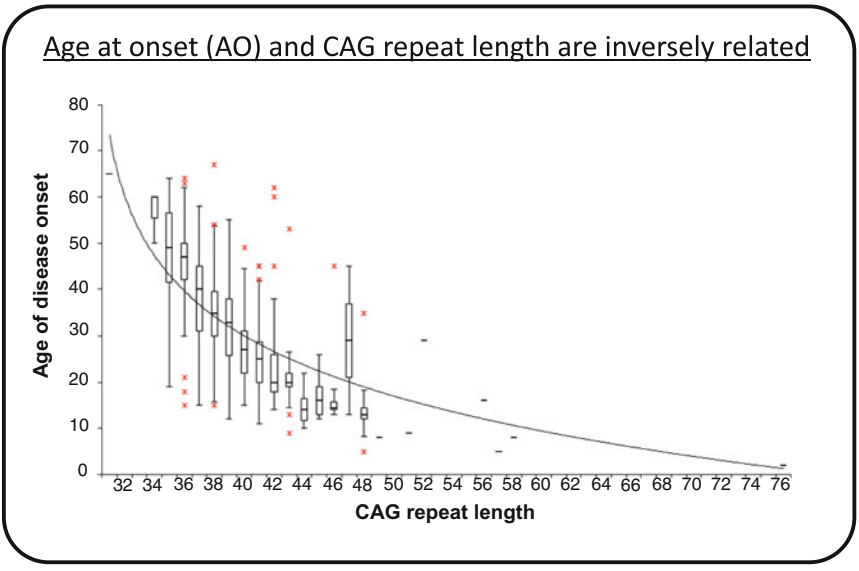

Fig. 8.1 Anticipation in SCA2. SCA2 age of onset is negatively correlated with *ATXN2* CAG repeat length. Note that the variability in AO for any CAG repeat length is partly associated with *CACNA1A* repeat length (but no other polyglutamine disease genes) and likely also to other genetic and environmental factors [10]

untranslated regions (162 bp 5′-UTR, 601 bp 3′-UTR). There are two in-frame start codons at the 5′-end of the sequence with the second one located four codons upstream of the CAG repeat. Transcriptional studies have only partly described which of these are utilized in translation. The predicted molecular weight for ATXN2, when made from the first start codon, is 144 kDa and ATXN2 made from the second start codon is 17 kDa smaller. While western blot analyses typically produce a single ATXN2 protein band consistent for use of the further-most upstream ATG, artificial luciferase tagged ATXN2 promoter constructs lacking the second ATG fail to express proteins [18]. Note that a smaller, approximately 42 kDa, fragment of ATXN2 was observed in brain extracts from SCA2 patients and SCA2 mice [19–21]. Huynh et al. [20] identified a consensus aa sequence for caspase-3 cleavage at ATXN2 aa 396–399 that could explain the origin of this band. ATXN2 is a cytoplasmic protein that also localizes to the trans-Golgi network [22, 23], and is a phosphorylated protein with half-life of ≥ 21 h [22]. *ATXN2* transcription is also regulated by the ETS1 transcription factor [18], and might be altered by CAG repeat expansion since the *ATXN2* CAG repeat is located inside a CpG island [24]. The molecular features of the *ATXN2* gene and the encoded protein are summarized in Table 8.1.

8.3.1 Macromolecules Interacting with ATXN2

The ATXN2 interacting proteins provided clues on the functions controlled by ATXN2. ATXN2 interacts with multiple RNA binding proteins (RBPs) suggesting that ATXN2 has a role in RNA metabolism. ATXN2 also interacts with staufen,

Table 8.1 Molecular features of the ATXN2 gene[a]

Chromosomal position	12q24.12
Number exons	25
Gene length	147,463 bp
Transcript length	4699 bp
3′-UTR length	601 bp
Putative start codons	2
ATG1 use	
5′-UTR length	162 bp
Protein length	1312 aa
Protein size	144 kDa
ATG2 use	
5′-UTR length	642 bp
Protein length	1152 aa
Protein size	127 kDa

[a]Transcript ID ENST00000377617 in Ensembl version ENSG00000204842.14

which controls stress granule formation and itself interacts with RBPs. ATXN2 interactions with IP3R and with the RGS8 mRNA transcript support the ATXN2 roles in calcium homeostasis. ATXN2 also interacts with endophilins and CIN85 indicating a function for ATXN2 in synaptic vesicle endocytosis. The ATXN2 interacting proteins are summarized in Table 8.2, and a graphic representation of the binding sites within ATXN2 is presented in Fig. 8.2.

8.3.2 A2BP1/RBFOX1

A2BP1/RBFOX1 is a regulator of RNA alternative splicing. We discovered A2BP1 as an ATXN2 interacting protein by yeast two-hybrid screening. Secondary two-hybrid assays using protein fragments determined that A2BP1 interacted with the C-terminal half of ATXN2 residues 760–1313 and that the full-length A2BP1 protein interacted stronger than did the C-terminal A2BP1 fragment while an N-terminal A2BP1 fragment did not interact with ATXN2 [25]. A2BP1 labeled in granules present in SCA2 patient dentate neurons and Purkinje neurons. A2BP1 functions in RNA splicing suggested that ATXN2 may regulate alternative splicing

Table 8.2 ATXN2 interacting macromolecules

ATXN2 interactor	ATXN2 binding region[b,c]	ATXN2 domain[d]	Interacting protein function	Citation
A2BP1/ RBFOX1	760–1312	–	RNA binding	Shibata et al. [25]
PABP	816–1312	PAM2	RNA binding	Ralser et al. [26]
DDX6	254–475	Lsm & LsmAD	RNA binding	Nonhoff et al. [27]
TDP-43	FL	–	RNA binding/ALS	Elden et al. [4]
FUS[a]	FL	–	RNA binding/ALS	Farg et al. [6]
Parkin	1–396	–	Ubiquitination	Huynh et al. [28]
Staufen1[a]	FL	–	RNA binding	Paul et al., submitted
IP3R[a]	FL	–	Ca^{2+} signaling	Liu et al. [29]
RGS8 mRNA	FL	–	Ca^{2+} signaling	Dansithong et al. [30]
Endophilin-A1	481–815[e]	SBM2	Vesicle endocytosis	Ralser et al. [31]
Endophilin-A3	481–815[e]	SBM2	Vesicle endocytosis	Ralser et al. [31]
CIN85[a]	FL	–	Vesicle endocytosis	Nonis et al. [32]

[a]Direct interaction with ATXN2 not demonstrated
[b]Smallest ATXN2 amino acid regions experimentally tested for interaction
[c]FL, Full-length ATXN2; narrower interacting regions in ATXN2 not determined
[d]Domain within the ATXN2 binding region required for the interaction
[e]Nonis et al. [32] identified additional flanking binding sites for the endophilins

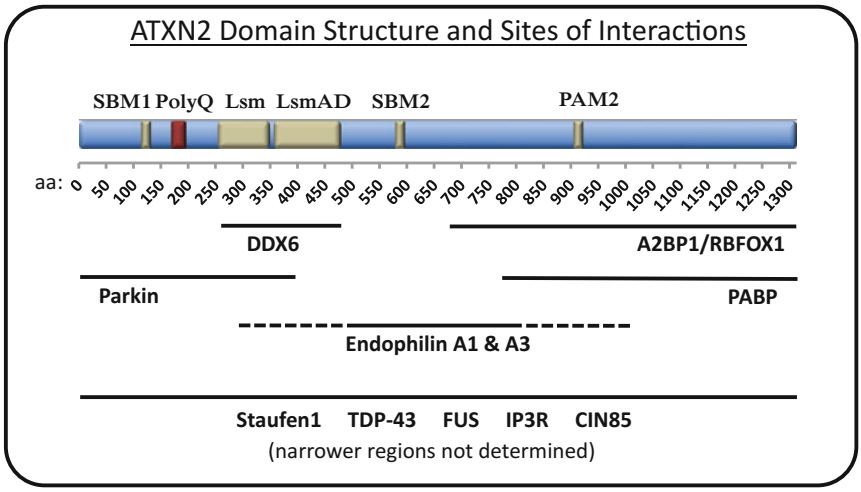

Fig. 8.2 ATXN2 domain structure and sites of interactions. The diagram depicts the amino acids of the ATXN2 protein with locations of known domains indicated. Lines represent the minimal regions experimentally tested for interaction. The domains and their locations are as follows: Polyglutamine tract (PolyQ) (aa 166–187), SRC homology 3 (SH3) domain binding motifs 1 (SBM1) (aa 117–126) and 2 (SBM2) (aa 587–596), Like sm domain (Lsm) (aa 254–345), Lsm associated domain (LsmAD) (aa 353–475), PABP interacting motif 2 (PAM2) (aa 908–925). In addition, ATXN2 has an acidic domain (aa 256–405), a predicted clathrin-mediated sorting signal (aa 414–416), and a predicted site for caspase cleavage (aa 396–399) [20]

in a tissue-specific manner or of a subset of RNAs. A2BP1 was the first RNA binding protein discovered to interact with ATXN2, and in simultaneous work in our research group, this led to our first attention on the Lsm and LsmAD domains located in the N-terminal region of ATXN2 [19] that are common in spliceosomal small nuclear ribonucleoproteins (snRNPs) and function in RNA binding and protein-protein interactions [33].

8.3.3 PABP & DDX6

Poly(A)-binding protein (PABP) interacts with the polyA end of mRNAs in the initiation of protein translation [26]. Other interactions made by PABP are facilitated by the 12 amino acid PABP-interacting motif 2 (PAM2) domain. A survey of multiple PAM2 proteins demonstrated that a PAM2 domain in ATXN2 and a high level of conservation of the PAM2 domain among the proteins [34]. A physical interaction between PABP and ATXN2 was demonstrated by yeast two hybrid testing and co-immunoprecipitation [26]. PABP is a component of mammalian stress granules, and ATXN2 and PABP colocalized in stress granules in heat-shock treated COS1 cells [26]. The study was the first to demonstrate the localization of ATXN2 to stress granules. The same research group further investigated ATXN2 in stress

granules by characterizing its interaction with the DEAD/H-box RNA helicase (DDX6) [35]. DDX6 is a stress granule protein that like PABP is localized to stress granules as well as processing bodies (p-bodies). ATXN2 was shown to directly interact with DDX6 by way of the Lsm and LsmAD domains in ATXN2 by yeast two-hybrid interaction testing [27]. Upon identifying DDX6 as an ATXN2 interacting protein, the investigators further demonstrated that ATXN2 localized to both stress granules and processing bodies (p-bodies). ATXN2 also interacts with polyribosomes which are also known to be regulated by RNA granule formation [35].

Interaction between ATXN2 and PABP appears to connect functionally to the control of translation by ATXN2 involving mTOR signaling. Increased ATXN2 mRNA was observed in SH-SY5Y cells stressed by serum starvation [36]. The authors also showed sequestration of PABP and proteins of the cap-binding complex with ATXN2 in stress granules in mouse embryo fibroblasts (MEFs) stressed with arsenite [36]. A connection between ATXN2 and mTOR signaling was further confirmed by demonstrating increased phosphorylation of S6 and 4EBP1 in MEFs null for *ATXN2*, as well as elevated *ATXN2* mRNA abundance in SH-SY5Y cells treated with the mTOR inhibitor rapamycin but not the PI3-kinase inhibitor LY294002 [36], suggesting the presence of a compensatory feed-back mechanism activating *ATXN2* when mTOR is inhibited.

8.3.4 TDP-43 & FUS

Both TDP-43 and FUS are RBDs that are mutated in amyotrophic lateral sclerosis (ALS). The identification of ATXN2 as an interactor with TDP-43 was presented along with the discovery that moderate expansions in the ATXN2 gene CAG repeat are associated with increased risk for ALS [4]. More on ATXN2 and ALS is discussed below. The interaction between TDP-43 and ATXN2 was demonstrated by yeast two-hybrid interaction testing, and in HEK293 cells by coimmunoprecipitation (co-IP) of overexpressed GFP-TDP-43 fragments with endogenous ATXN2 and by immunofluorescent colocalization. In co-IP tests, including RNAse or including TDP-43 proteins mutated to abolish RNA binding, the TDP-43-ATXN2 interaction was abolished, demonstrating that the interaction is RNA dependent. FUS was also demonstrated to interact with ATXN2 [6]. Both TDP-43 and FUS have been characterized in RNA granules containing ATXN2 [37], suggesting a pathogenic connection for ATXN2 in increased ALS risk is associated with abnormal stress granule function.

8.3.5 Parkin

To investigate a functional connection that might explain why Parkinsonism is sometimes observed in SCA2, Huynh et al. [28] tested ATXN2 as an interacting

protein with Parkin. Parkin directly interacted with the ATXN2 N-terminal domain (residues 1–396) when hemagglutinin (HA)-tagged Parkin was pulled down with an anti-HA antibody in HEK293 cells expressing GFP fused to full-length ATXN2 or N- or C-terminal fragments of ATXN2 [28]. The interaction was verified for ATXN2 proteins with Q22, as well as expanded polyglutamine tracts of Q58 and Q104. Parkin, an E3 ubiquitin ligase, ubiquitinated the full-length ATXN2 more efficiently than ATXN2 N-terminal fragments. ATXN2 ubiquitination by Parkin was more pronounced when ATXN2 was polyglutamine expanded but less efficient with Parkin-C289G mutated. The induced overexpression of Parkin in tetracycline inducible PC12 cells was associated with increased turnover of the ATXN2 protein. ATXN2 and Parkin colocalized in cytoplasmic structures of Purkinje cells from normal (non-SCA2) individuals [28]. The interaction between Parkin and ATXN2 was independently confirmed by coimmunoprecipition of polyglutamine expanded ATXN2 with Parkin from the cerebella of ATXN2-CAG42 knock-in mice [38]. Note that the latter study also demonstrated that the E3 ubiquitin ligase Fbxw8 also coimmunoprecipitated with ATXN2.

8.3.6 Staufen

Recently we demonstrated that ATXN2 interacts with Staufen1. Staufen is a key regulator of stress granule formation. We demonstrated that staufen expression is increased in SCA2 derived patient fibroblasts, lymphoblasts, iPSCs, and in the cerebella of our ATXN2-Q127 transgenic and our ATXN2-Q72 BAC mice. The result of elevated staufen expression in these systems is constitutively present stress granules. The identification of staufen as an interacting protein with ATXN2, whose expression is elevated upon *ATXN2* mutation, demonstrates a functional role for ATXN2 in either staufen mediated decay, stress granule mediated mRNA processing or stress granule mediated dendritic mRNA trafficking for localized expression control.

8.3.7 RGS8 *mRNA & IP3R*

ATXN2 interacts with *RGS8* mRNA and IP3R supporting roles for ATXN2 in calcium homeostasis. We determined that Rgs8 expression is reduced in the cerebella of SCA2 mice by transcriptome analysis, and verified RGS8 reduction in SCA2 patient lymphoblast cells. We also demonstrated that Rgs8 translation is reduced in the presence of mutant ATXN2 using rabbit reticulocyte in vitro translation assays. Thus, reduced Rgs8 could be the result of mRNA degradation, as well as RGS8 mRNA translation inhibition perhaps mediated by sequestration in stress granules. RGS8 inhibition could impact calcium levels in Purkinje cells, since RGS8 is believed to be an inhibitor of mGluR1. The role of mGlur1 in the normal functioning

of Purkinje neuron and motor coordination is well described in a review by Hartmann et al. [39]. In Purkinje cells, mGluRs produce two distinct signals including a local dendritic Ca^{2+} signal and a slow excitatory postsynaptic potential. The dendritic Ca^{2+} signal originates through Ca^{2+} release from the ER mediated by the inositol-triphosphate receptor type 1 (IP3R). The slow excitatory postsynaptic potentials are mediated by Ca^{2+} influx, via the transient receptor potential cation channel 3 (TRPC3) that is gated by diacylglycerol (DAG) and IP3R [39]. IP3R is abnormally activated upon interaction by mutant ATXN2, resulting in abnormal release of Ca^{2+} from intracellular stores [29]. The Bezprozvanny group verified that IP3R specifically interacts with the polyglutamine expanded ATXN2 protein but not the normal ATXN2 protein [29]. The interaction was demonstrated between endogenous ATXN2 and overexpressed GST-IP3R in COS7 cells using a pull-down assay. A second assay using cerebellar homogenates from Pcp2-ATXN2-Q58 mice demonstrated that polyglutamine expanded ATXN2 co-precipitated with greater abundance of radiolabeled IP3 than did wildtype ATXN2, consistent with an interaction between mutant ATXN2 and IP3R [29]. Further experiments on modulating IP3R function for modifying SCA2 mouse phenotypes are described below.

8.3.8 Endophilins and CIN85

Endophilin and CIN85 are proteins that function along with Cbl in endocytosis of cell surface receptor tyrosine kinases [40]. The Huntington disease protein huntingtin, another polyglutamine disease protein, interacts with endophilin A3 resulting in abnormal sequestration of proteins of endocytic vesicle systems [41, 42]. Therefore, to test whether ATXN2 could interact with endophilins, Ralser et al. [31] performed two-hybrid interaction tests that demonstrated ATXN2 direct binding with both endophilin A1 and endophilin A3. The interaction was mediated by the SH3 domain binding motif 2 (SBM2) in ATXN2, and the investigators also showed that ATXN2 failed to interact with endophilin A2. The study also demonstrated competitive binding between ATXN2 and huntingtin for endophilin A3 in the yeast two-hybrid system. Another study investigated ATXN2 interactions with endophilin A1 and endophilin A3 by GST pull-down tests showing that the endophilins interacted with other ATXN2 protein regions not including the SBM2 domain [32]. Extensive cytoplasmic colocalization of ATXN2 with endophilins A1 and A3 was also demonstrated by immunofluorescent labeling of HEK293 and SH-SY5Y cells [31, 32]. Co-immunoprecipitation assays demonstrated ATXN2 exists in complexes containing endophilin A3, CIN85, Cbl, and EGF receptor (EGFR) [32]. Overexpression of ATXN2 in CHO cells inhibited EGF-stimulated EGFR internalization, demonstrating a functional role of ATXN2 in endocytosis [32]. Endocytosis controlled by the Endophilin-CIN85-Cbl complex is mediated by clathrin-coated pits. A putative site for clathrin binding in ATXN2 was described by Huynh et al. [20] at aa 414–416. However Turnbull et al. [22] could demonstrate no co-localization between ATXN2 and clathrin-coated pits or vesicles.

8.3.9 *Other ATXN2 Interaction Studies*

Various studies have demonstrated proteins with which ATXN2 coimmunoprecipitated without formally testing direct interactions. Discussed briefly in the PABP paragraph above, Lastres-Becker et al. [36] demonstrated that ATXN2 coimmuniprecipitated with TIA1, eIF3B, eIF4G, eIF4A1 and S6 from HEK293 cells treated with or without arsenite. Blokhuis et al. [43] characterized the ATXN2 interactome in Neuro2A cells using mass spectrometry with validations performed by coimmunoprecipitation. Key interacting proteins verified in coimmunoprecipitation experiments included Fmrp, Upf1, Caprin1, HuD, Pabpc4, and Dhx9. The investigators also produced interactomes for Fus and Tdp43 and presented interactions shared among these proteins and ATXN2 [43].

Studies of the ATXN2 yeast homolog Pbp1 suggest other proteins likely to interact with the ATXN2 protein. The PAS kinase 1 (Psk1) was shown to interact with the C-terminal half of Pbp1 resulting in Pbp1 phosphorylation proximal to the interaction [44]. Another study of Pbp1 demonstrated interactions with Lsm12 and Pbp4 in addition to the yeast homologs of PABP and DDX6 [45].

8.4 SCA2 Mouse Models

We and others have produced multiple SCA2 mouse models, including transgenic and knockout models [20, 30, 46, 47, 48, 49, 50, 51]. In this section we describe these mice. Note that a recent review comprehensively describes each of these mice [52].

8.4.1 Pcp2-ATXN2 *Transgenic Mice*

We have made two types of Pcp2-ATXN2 transgenic mice, including one with ATXN2-CAG58 (Q58) and another with ATXN2-CAG127 (Q127). Both of these mice have ATXN2 expressed under the control of the Purkinje cell protein 2 (*Pcp2*)*/L7* promoter. These mice are characterized by age-dependent molecular, motor and electrophysiological phenotypes. Rotarod testing demonstrated ATXN2-dose dependent motor phenotype for *ATXN2*-Q58 mice first observed at six months of age, and Purkinje cells in these mice contained cytoplasmic, but not nuclear, inclusion bodies [20]. The ATXN2-Q58 mouse was also used in studies demonstrating dantrolene treatment could restore *ATXN2* mouse motor phenotypes [29]. This is discussed in further detail in the section below, on calcium homeostasis. *ATXN2*-Q127 mice also has Purkinje cells with cytoplasmic inclusion bodies, but with the longer repeat length we have observed the motor phenotype as early as eight weeks of age [46]. The Auburger group has also created an ATXN2-CAG42 knock-in mouse by replacing the single CAG in the mouse Atxn2

gene with an expanded CAG42 repeat [50]. The ATXN2-CAG42 mouse was characterized for how mutant ATXN2 alters PABPC1 solubility and availability for functions in RNA metabolism.

8.4.2 SCA2 BAC Transgenic Mice

SCA2 BAC mice possess the entire 176 kb *ATXN2* gene region including 16 kb upstream sequence and 2.5 kb downstream sequence. Presently we have two SCA2 BAC lines including alleles expressing ATXN2-Q22 normal length and ATXN2-Q72 expanded [30]. The Q22 line has no motor, transcriptomic or neurophysiological phenotype. However, the Q72 line has a progressive onset of its motor phenotype, determined using the accelerating rotarod that is mimicked by progressive reduction of the expression of various neuronal and Purkinje cell specific genes, beginning at 8 weeks of age [30]. More recently, we have identified changes in Purkinje cell firing frequencies in the SCA2-Q72 BAC mice, compared to wildtype littermates, present for mice age 6 and 12 months but not mice 4 months of age (unpublished observation). This demonstrates that the neurophysiological phenotype of the BAC-Q72 mice appears later than for the ATXN2-Q127 mice, mirroring the later motor phenotype observed in the BAC-Q72 mice. The delayed onset of SCA2 phenotypes in the BAC-Q72 mice is due to the lower expression from the native ATXN2 promoter, as well as the shorter Q72 repeat compared to the ATXN2-Q127 mouse. Cerebellar molecular phenotype changes determined by qPCR and RNA-seq were largely similar to those observed in ATXN2-Q127 mice [30, 46].

8.4.3 ATXN2-Q75 Transgenic Mice

ATXN2-Q75 mice are transgenic for the *ATXN2* cDNA under transcriptional control by the native *ATXN2* promoter, and include *ATXN2* with 75 CAG repeats [51]. Ubiquitous transgene expression was observed, and hemizygous mice were ataxic by 12 weeks of age in rotarod tests, corresponding with abnormal Purkinje cell morphology.

8.4.4 Atxn2 *Knockout Mice*

Our group and the Auburger group have both produced *Atxn2* knockout mice and we have demonstrated key characteristics that are common to both, which include viable mice with marked obesity and the lack of any significantly debilitating neuropathology [48, 49]. We have also demonstrated normal Purkinje cell

physiology in *Atxn2* knockout mice [53], but these mice are also characterized by abnormal fear-related behavior [47]. The Auburger group demonstrated that *Atxn2* knockout mice had abnormally low insulin receptor expression in both the cerebellar and the liver and concluded that these molecular changes associate with the onset of obesity [49]. These investigators further evaluated their *A2* knockout mouse employing microarray analysis revealing increased expression of transcription factors but overall lower translation [54]. The lack of neuropathology in these mice supports the concept for developing SCA2 therapeutics that target the total expression of *ATXN2*, such as we are with *ATXN2* compounds and *ATXN2* antisense oligonucleotides (ASOs), described below.

8.5 Transcriptome Analyses

Multiple studies on the cerebellar transcriptomes have been conducted using SCA2 mice. In our initial study we used cerebellar RNAs isolated from wildtype and BAC-ATXN2-Q72 mice at ages 1 day, 3 weeks and 6 weeks. In day 1 mice we observed ~200 transcripts that were significantly dysregulated, and more transcripts were altered in the older animals [30]. Most transcripts were reduced in abundance in SCA2 mice as compared to wildtype mice. We also performed transcriptome analysis using Pcp2-ATXN2-Q127 mice for the purpose of identifying pathways commonly modified in these mice and BAC-ATXN2-Q127 mice [30]. It was these studies that resulted in the identification of significant reductions in the *RGS8* mRNA as described above. Other genes that were significantly reduced in both of these SCA2 mouse models included *Pcp2*, *Fam107b* and others. Pathways that we identified that are altered in both Pcp2-ATXN2-Q127 and BAC-ATXN2-Q127 mice include glutamate signaling, calcium signaling and others [30]. We have also begun to compare the transcriptomes of these mice with that of age-matched knockout mice. Unlike the transcriptomes in the SCA2 mice we observed that few transcripts were altered in the A*txn*2 knockout mice (unpublished observations). Similarly, the Auburger group compared transcriptomes of ATXN2-CAG42 transgenic mice with A*txn*2 knockout mice using a microarray analysis approach, demonstrating overlapping abnormalities in calcium homeostasis pathways [55].

8.6 SCA2 Therapeutics

Despite presence of many ATXN2 interactors, we still lack information on which ATXN2 functions are targetable to prevent SCA2 pathogenesis or to delay SCA2 progression. The concept of two different approaches for developing SCA2 therapeutics discussed here, closely follows that developed in a recent review on precision medicine for the spinocerebellar ataxias [56]. One approach is to develop the

known functions for ATXN2 as therapeutic targets for SCA2, including glutamate signaling, calcium homeostatsis, and RNA metabolism (Fig. 8.3). Another promising approach is to target ATXN2 expression directly, because, like for other SCAs caused by mutations leading to polyglutamine expansions, SCA2 is characterized predominantly by a toxic gain of function. We are making efforts to develop SCA2 therapeutics that target ATXN2 functionally and we are also developing antisense oligonucleotides that lower overall ATXN2 expression.

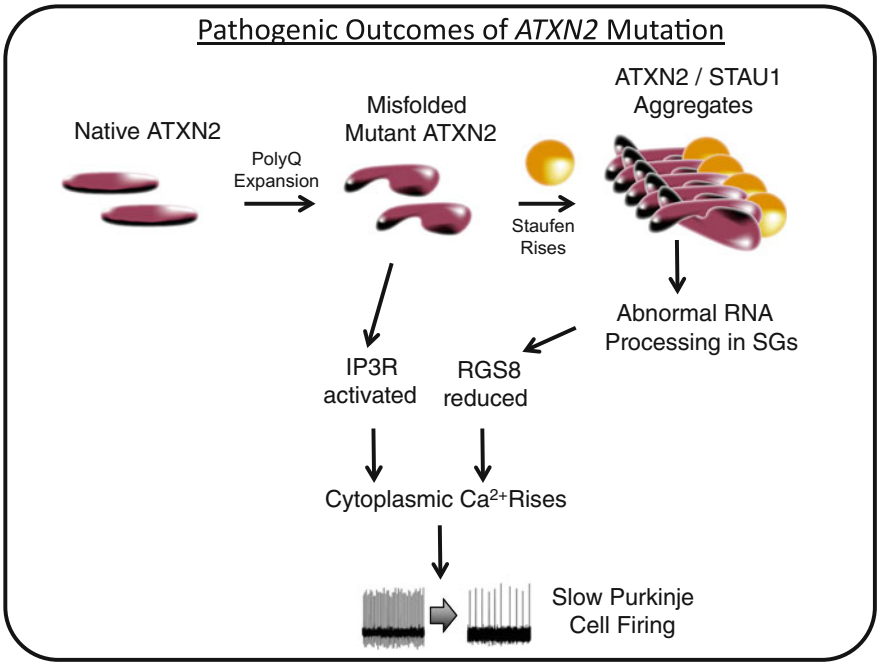

Fig. 8.3 Pathogenic outcomes of ATXN2 mutation. Polyglutamine expansion in ATXN2 results in misfolding and increased staufen1 (STAU1) expression and ATXN2 aggregations leading to stress granule formation and abnormal RNA processing. Misfolded ATXN2 interacts directly with IP3R leading to abnormal IP3R channel activity followed by calcium release from internal stores. ATXN2 also directly interacts with *RGS8* mRNA resulting in reduced RGS8 protein abundance. RGS8 reduction is attributed to decreased RGS8 translation which may be caused by sequestration in stress granules, and to decreased *RGS8* mRNA abundance possibly also related to stress granule functions. The consequence of reduced RGS8 is overactive mGluR1 leading to increased cytoplasmic calcium. The result of is abnormally slow Purkinje cell firing

8.6.1 Therapies Targeting ATXN2-Related SCA2 Pathways

The approach that we have taken for developing therapeutics that target ATXN2-related SCA2 pathways is to target pathways leading to abnormal rise of cytoplasmic Ca^{2+}. These efforts were initiated upon the identification that the mutant, but not the wildtype, ATXN2 protein interacted with the inositol-triphosphate receptor type 1 (ITPR1), also described above [29]. Targeting the Ca^{2+} pathway in SCA2 is in line with the notion that defective Ca^{2+} signaling underlies most neurodegenerative diseases [57]. *ITPR1* mutations or haploinsufficiency are also causative for SCA15/16 [58, 59]. ITPR1 is a calcium channel located on the endoplasmic reticulum membrane controlling the release of intracellular Ca^{2+} stores, and is expressed highly in PCs. Mice harboring ATXN2-Q58 were characterized with increased Ca^{2+} release from the endoplasmic reticulum associated with molecular layer thinning and Purkinje cell loss [20, 29]. Blocking of the functionally coupled ryanodine receptor with dantrolene reduced abnormal calcium release and cell death in culture [29]. Liu et al. [29] also demonstrated that SCA2 motor phenotypes of ATXN2-Q58 mice, as well as improved Purkinje cell survival was delayed by oral treatment of ATXN2-Q58 mice with dantrolene. Tests included the beam walk and accelerating rotarod.

We have also begun to investigate mGlur1 as a therapeutic target for SCA2. As described above, we demonstrated that RGS8 expression is reduced in an age-dependent manner in SCA2 mice. RGS8 is a putative regulator of mGlur1 and its reduced expression is predicted to deregulate mGlur1 [30]. We have now used our Pcp2-ATXN2-Q127 model to replicate the in vitro findings and show that the mGlur1 agonist DHPG enhances firing frequency of Pcp2-ATXN2-Q127 mouse PCs accompanied by abnormally elevated intracellular Ca^{2+} at specific PC firing rates [60]. ATXN2 expression itself may be regulated by intracellular calcium and mGluR1. These data suggest that mGlur1 antagonists could be therapeutic for SCA2.

8.6.2 ATXN2 ASO Therapeutics

Antisense oligonucleotides (ASOs) represent a promising approach for treating SCA2. SCA2 is characterized by a gain of toxic function, thus we hypothesize that lowering ATXN2 expression would be therapeutic for SCA2. Reducing total expression as a therapeutic approach is supported for polyQ diseases by several observations. In SCA2 patients two copies of the mutant *ATXN2* allele can be accompanied by earlier age of onset and more rapid disease progression [61]. The importance of gene dosage is further supported by studies on mice. Doubling of gene dosage in transgenic *ATXN2*-Q58 mice led to earlier onset of abnormal rotarod performance [20]. Studies using tetracycline-regulated promoters in HD, SCA1 or SCA3 mice have demonstrated reversibility of phenotypes even after disease onset

[62–65]. Another study showed that intracerebellar injection of AAV virus encoding shRNA Ataxin-1 reduced transgene expression, improved motor coordination, restored cerebellar morphology and resolved ataxin-1 inclusions in Purkinje neurons (PNs) of SCA1 mice [66]. Recently, ASOs have proven useful for the treatment of spinal muscular atrophy and SOD-ALS [67, 68], and newer phase 1 clinical trials have been initiated using ASOs for the treatment of myotonic dystrophy (DM1) and Huntington disease.

Our ASO approach to therapeutics is being conducted collaboratively with Ionis Pharmaceuticals utilizing modified 2′-MOE-gapmer ASOs. The 2′-MOE-gapmer are 20 bp in length, are phosphorothioate throughout, and have a 2′-O-methoxyethyl group (MOE) on the terminal 5 bps at each end of the oligonucleotide [69]. These modifications prevent degradation by nucleases and the MOE chemical modifications also aid in increasing specificity of target mRNA interaction, supporting target degradation by RNase-H [70].

The SCA2 ASOs that we are developing are for non-allele-specific targeting of *ATXN2* unlike the approach undertaken for Huntington's disease. In Huntington's disease ASOs are made to target SNPs in linkage with CAG repeat expanded alleles [71, 72] because *Htt* knockout in mice disrupts neuronal development [73]. In our approach we permit the ASO to target the mutant *ATXN2* allele as well as the non-mutant allele because knockout of the *Atxn2* gene in mice is well tolerated and associated with no neurodegeneration [47, 48]. However, progress on developing non-allele-specific RNAi therapeutics for HD had favorable outcomes in mice and non-human primates [74, 75].

In collaboration with Ionis Pharmaceuticals we have produced *ATXN2* ASOs that lower human ATXN2 expression in both our Pcp2-ATXN2-Q127 mouse model, as well as our BAC-ATXN2-Q72 mouse model. We have observed as much as 80% ATXN2 mRNA reduction when delivered to mice by introcerebroventricular (ICV) injection to the right lateral ventricle, and we have observed no cytotoxicity indicated by following AIF1 and GFAP expression post injection. We have employed our most promising lead ASO, designated ASO7, in a blinded preclinical treatment trial using Pcp2-ATXN2-Q127 mice (Fig. 8.4). Mice treated at 8 weeks of age were tested at different treatment timepoints, on the accelerating rotarod, demonstrating delayed progression of the SCA2 motor phenotypes in both mouse models. At the endpoint we determined the cerebellar expression of ATXN2, Rgs8 and Pcp2 by qPCR and Western blotting demonstrating prolonged ATXN2 reduction and increases in Rgs8 and Pcp2 expression. Moreover, subsets of mice were tested to determine the effect of the ASO7 treatments on Purkinje cell physiology. We observed that ASO7 treatment could restore the mean PC firing frequency to that unlike observed in age matched mice. The result of our *ATXN2* ASO study was recently published [76]. Finally, we also have ongoing studies to characterize the transcriptomes of SCA2 mice treated with or without *ATXN2* ASO7. Information resulting from the latter work might indicate new pathways that can be exploited for the treatment of ALS associated with *ATXN2*. This is supported by a recent finding that lowering *Atxn2* expression either genetically or by ASO therapy improved survival of TDP-43 ALS mice [77].

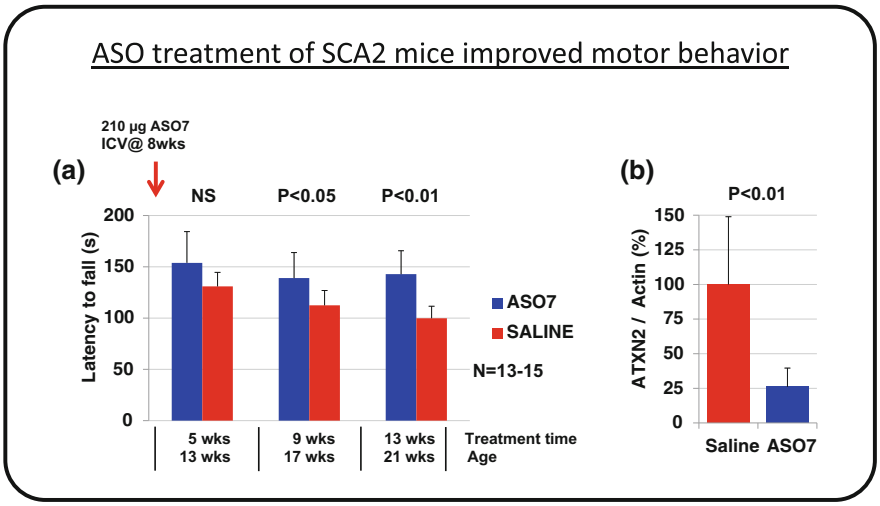

Fig. 8.4 Reduction of *ATXN2* expression improved SCA2 mouse phenotypes. **a** Compared with saline-treated animals, spinocerebellar ataxia type 2 mice (Pcp2-ATXN2-Q127) show significantly reduced progression of motor disability. Mean latency to fall in 3 trials on day 3 of rotarod testing is shown. **b** Cerebellar endogenous mouse and human transgenic ataxin-2 mRNA (*ATXN2*) are reduced by ASO treatment 14 weeks after intracerebroventricular (ICV) injection compared with saline. NS, not significant

8.7 Conclusions

SCA2 is a debilitating disorder for which there is no treatment. Research by numerous teams on SCA2 has resulted in the identification of multiple interacting proteins that have given rise to clues about ATXN2 function. Additionally, multiple SCA2 transgenic mouse models and *Atxn2* knockout models have been studied giving rise to understanding on pathways dysregulated in SCA2 mice. Collectively, these studies have aided understanding on ATXN2 function and have indicated possible pathways that can be targeted in order to delay SCA2 progression. Progress toward developing drugs targeting calcium signaling and related pathways is accumulating. But even with this increased knowledge of ATXN2 function, the opportunity to target *ATXN2* directly using antisense oligonucleotides remains a primary goal of our research group for treating SCA2, garnered by positive preliminary data in SCA2 mice and recent data demonstrating that ASOs are tolerated in humans and effective for SMA and SOD ALS. Preliminary data demonstrating that SCA2 ASOs lower ATXN2 expression in mouse spinal cord also lend hope toward developing *ATXN2* ASOs for SCA2 ALS and perhaps ALS in a more generalized manner.

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Conflict of Interest Statement

SMP is a consultant for Progenitor Life Sciences.

References

1. Geschwind DH, Perlman S, Figueroa CP, Treiman LJ, Pulst SM (1997) The prevalence and wide clinical spectrum of the spinocerebellar ataxia type 2 trinucleotide repeat in patients with autosomal dominant cerebellar ataxia. Am J Hum Genet 60:842–850
2. Ashizawa T, Figueroa KP, Perlman SL, Gomez CM, Wilmot GR, Schmahmann JD et al (2013) Clinical characteristics of patients with spinocerebellar ataxias 1, 2, 3 and 6 in the US; a prospective observational study. Orphanet J Rare Dis 8:177
3. Gwinn-Hardy K, Chen JY, Liu HC, Liu TY, Boss M, Seltzer W et al (2000) Spinocerebellar ataxia type 2 with parkinsonism in ethnic Chinese. Neurology 55:800–805
4. Elden AC, Kim HJ, Hart MP, Chen-Plotkin AS, Johnson BS, Fang X et al (2010) Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466:1069–1075
5. Neuenschwander AG, Thai KK, Figueroa KP, Pulst SM (2014) Amyotrophic lateral sclerosis risk for spinocerebellar ataxia type 2 ATXN2 CAG repeat alleles: a meta-analysis. JAMA Neurol 71:1529–1534
6. Farg MA, Soo KY, Warraich ST, Sundaramoorthy V, Blair IP, Atkin JD (2013) Ataxin-2 interacts with FUS and intermediate-length polyglutamine expansions enhance FUS-related pathology in amyotrophic lateral sclerosis. Hum Mol Genet 22:717–728
7. Sellier C, Campanari ML, Julie Corbier C, Gaucherot A, Kolb-Cheynel I, Oulad-Abdelghani M et al (2016) Loss of C9ORF72 impairs autophagy and synergizes with polyQ Ataxin-2 to induce motor neuron dysfunction and cell death. EMBO J
8. Fischbeck KH, Pulst SM (2011) Amyotrophic lateral sclerosis and spinocerebellar ataxia 2. Neurology 76:2050–2051
9. Wadia NH, Swami RK (1971) A new form of heredo-familial spinocerebellar degeneration with slow eye movements (nine families). Brain 94:359–374
10. Pulst SM, Santos N, Wang D, Yang H, Huynh D, Velazquez L et al (2005) Spinocerebellar ataxia type 2: polyQ repeat variation in the CACNA1A calcium channel modifies age of onset. Brain 128:2297–2303
11. Velazquez-Perez L, Rodriguez-Labrada R, Canales-Ochoa N, Montero JM, Sanchez-Cruz G, Aguilera-Rodriguez R et al (2014) Progression of early features of spinocerebellar ataxia type 2 in individuals at risk: a longitudinal study. Lancet Neurol 13:482–489
12. Pulst SM, Nechiporuk A, Starkman S (1993) Anticipation in spinocerebellar ataxia type 2. Nat Genet 5:8–10
13. Gispert S, Twells R, Orozco G, Brice A, Weber J, Heredero L et al (1993) Chromosomal assignment of the second locus for autosomal dominant cerebellar ataxia (SCA2) to chromosome 12q23-24.1. Nat Genet 4:295–299

14. Pulst SM, Nechiporuk A, Nechiporuk T, Gispert S, Chen XN, Lopes-Cendes I et al (1996) Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet 14:269–276
15. Sanpei K, Takano H, Igarashi S, Sato T, Oyake M, Sasaki H et al (1996) Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. Nat Genet 14:277–284
16. Imbert G, Saudou F, Yvert G, Devys D, Trottier Y, Garnier JM et al (1996) Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. Nat Genet 14:285–291
17. Fernandez M, McClain ME, Martinez RA, Snow K, Lipe H, Ravits J et al (2000) Late-onset SCA2: 33 CAG repeats are sufficient to cause disease. Neurology 55:569–572
18. Scoles DR, Pflieger LT, Thai KK, Hansen ST, Dansithong W, Pulst SM (2012) ETS1 regulates the expression of ATXN2. Hum Mol Genet 21:5048–5065
19. Huynh DP, Del Bigio MR, Ho DH, Pulst SM (1999) Expression of ataxin-2 in brains from normal individuals and patients with Alzheimer's disease and spinocerebellar ataxia 2. Ann Neurol 45:232–241
20. Huynh DP, Figueroa K, Hoang N, Pulst SM (2000) Nuclear localization or inclusion body formation of ataxin-2 are not necessary for SCA2 pathogenesis in mouse or human. Nat Genet 26:44–50
21. Koyano S, Uchihara T, Fujigasaki H, Nakamura A, Yagishita S, Iwabuchi K (1999) Neuronal intranuclear inclusions in spinocerebellar ataxia type 2: triple-labeling immunofluorescent study. Neurosci Lett 273:117–120
22. Turnbull VJ, Storey E, Tarlac V, Walsh R, Stefani D, Clark R et al (2004) Different ataxin-2 antibodies display different immunoreactive profiles. Brain Res 1027:103–116
23. Huynh DP, Yang HT, Vakharia H, Nguyen D, Pulst SM (2003) Expansion of the polyQ repeat in ataxin-2 alters its Golgi localization, disrupts the Golgi complex and causes cell death. Hum Mol Genet 12:1485–1496
24. Aguiar J, Santurlidis S, Nowok J, Alexander C, Rudnicki D, Gispert S et al (1999) Identification of the physiological promoter for spinocerebellar ataxia 2 gene reveals a CpG island for promoter activity situated into the exon 1 of this gene and provides data about the origin of the nonmethylated state of these types of islands. Biochem Biophys Res Commun 254:315–318
25. Shibata H, Huynh DP, Pulst SM (2000) A novel protein with RNA-binding motifs interacts with ataxin-2. Hum Mol Genet 9:1303–1313
26. Ralser M, Albrecht M, Nonhoff U, Lengauer T, Lehrach H, Krobitsch S (2005) An integrative approach to gain insights into the cellular function of human ataxin-2. J Mol Biol 346:203–214
27. Nonhoff U, Ralser M, Welzel F, Piccini I, Balzereit D, Yaspo ML et al (2007) Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-bodies and stress granules. Mol Biol Cell 18:1385–1396
28. Huynh DP, Nguyen DT, Pulst-Korenberg JB, Brice A, Pulst SM (2007) Parkin is an E3 ubiquitin-ligase for normal and mutant ataxin-2 and prevents ataxin-2-induced cell death. Exp Neurol 203:531–541
29. Liu J, Tang TS, Tu H, Nelson O, Herndon E, Huynh DP et al (2009) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 2. J Neurosci 29:9148–9162
30. Dansithong W, Paul S, Figueroa KP, Rinehart MD, Wiest S, Pflieger LT et al (2015) Ataxin-2 regulates RGS8 translation in a new BAC-SCA2 transgenic mouse model. PLoS Genet 11: e1005182
31. Ralser M, Nonhoff U, Albrecht M, Lengauer T, Wanker EE, Lehrach H et al (2005) Ataxin-2 and huntingtin interact with endophilin-A complexes to function in plastin-associated pathways. Hum Mol Genet 14:2893–2909
32. Nonis D, Schmidt MH, van de Loo S, Eich F, Dikic I, Nowock J et al (2008) Ataxin-2 associates with the endocytosis complex and affects EGF receptor trafficking. Cell Signal 20:1725–1739

33. Hermann H, Fabrizio P, Raker VA, Foulaki K, Hornig H, Brahms H et al (1995) snRNP Sm proteins share two evolutionarily conserved sequence motifs which are involved in Sm protein-protein interactions. EMBO J 14:2076–2088
34. Albrecht M, Lengauer T (2004) Survey on the PABC recognition motif PAM2. Biochem Biophys Res Commun 316:129–138
35. Satterfield TF, Pallanck LJ (2006) Ataxin-2 and its Drosophila homolog, ATX2, physically assemble with polyribosomes. Hum Mol Genet 15:2523–2532
36. Lastres-Becker I, Nonis D, Eich F, Klinkenberg M, Gorospe M, Kotter P et al (2016) Mammalian ataxin-2 modulates translation control at the pre-initiation complex via PI3 K/mTOR and is induced by starvation. Biochim Biophys Acta 1862:1558–1569
37. Nihei Y, Ito D, Suzuki N (2012) Roles of ataxin-2 in pathological cascades mediated by TAR DNA-binding protein 43 (TDP-43) and Fused in Sarcoma (FUS). J Biol Chem 287:41310–41323
38. Halbach MV, Stehning T, Damrath E, Jendrach M, Sen NE, Basak AN et al (2015) Both ubiquitin ligases FBXW8 and PARK2 are sequestrated into insolubility by ATXN2 PolyQ expansions, but only FBXW8 expression is dysregulated. PLoS ONE 10:e0121089
39. Hartmann J, Henning HA, Konnerth A (2011) mGluR1/TRPC3-mediated synaptic transmission and calcium signaling in mammalian central neurons. Cold Spring Harb Perspect Biol 3
40. Soubeyran P, Kowanetz K, Szymkiewicz I, Langdon WY, Dikic I (2002) Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. Nature 416:183–187
41. Sittler A, Walter S, Wedemeyer N, Hasenbank R, Scherzinger E, Eickhoff H et al (1998) SH3GL3 associates with the Huntingtin exon 1 protein and promotes the formation of polygln-containing protein aggregates. Mol Cell 2:427–436
42. Qin ZH, Wang Y, Sapp E, Cuiffo B, Wanker E, Hayden MR et al (2004) Huntingtin bodies sequester vesicle-associated proteins by a polyproline-dependent interaction. J Neurosci 24:269–281
43. Blokhuis AM, Koppers M, Groen EJ, van den Heuvel DM, Dini Modigliani S, Anink JJ et al (2016) Comparative interactomics analysis of different ALS-associated proteins identifies converging molecular pathways. Acta Neuropathol 132:175–196
44. DeMille D, Badal BD, Evans JB, Mathis AD, Anderson JF, Grose JH (2015) PAS kinase is activated by direct SNF1-dependent phosphorylation and mediates inhibition of TORC1 through the phosphorylation and activation of Pbp1. Mol Biol Cell 26:569–582
45. Swisher KD, Parker R (2010) Localization to, and effects of Pbp1, Pbp4, Lsm12, Dhh1, and Pab1 on stress granules in Saccharomyces cerevisiae. PLoS ONE 5:e10006
46. Hansen ST, Meera P, Otis TS, Pulst SM (2013) Changes in Purkinje cell firing and gene expression precede behavioral pathology in a mouse model of SCA2. Hum Mol Genet 22:271–283
47. Huynh DP, Maalouf M, Silva AJ, Schweizer FE, Pulst SM (2009) Dissociated fear and spatial learning in mice with deficiency of ataxin-2. PLoS ONE 4:e6235
48. Kiehl TR, Nechiporuk A, Figueroa KP, Keating MT, Huynh DP, Pulst SM (2006) Generation and characterization of Sca2 (ataxin-2) knockout mice. Biochem Biophys Res Commun 339:17–24
49. Lastres-Becker I, Brodesser S, Lutjohann D, Azizov M, Buchmann J, Hintermann E et al (2008) Insulin receptor and lipid metabolism pathology in ataxin-2 knock-out mice. Hum Mol Genet 17:1465–1481
50. Damrath E, Heck MV, Gispert S, Azizov M, Nowock J, Seifried C et al (2012) ATXN2-CAG42 sequesters PABPC1 into insolubility and induces FBXW8 in cerebellum of old ataxic knock-in mice. PLoS Genet 8:e1002920
51. Aguiar J, Fernandez J, Aguilar A, Mendoza Y, Vazquez M, Suarez J et al (2006) Ubiquitous expression of human SCA2 gene under the regulation of the SCA2 self promoter cause specific Purkinje cell degeneration in transgenic mice. Neurosci Lett 392:202–206

52. Alves-Cruzeiro JM, Mendonca L, Pereira de Almeida L, Nobrega C (2016) Motor dysfunctions and neuropathology in mouse models of spinocerebellar ataxia type 2: a comprehensive review. Front Neurosci 10:572
53. Pflieger LT, Dansithong W, Paul S, Scoles DR, Figueroa KP, Meera P, Otis TS, Facelli JC, Pulst SM (2017) Gene co-expression network analysis for identifying modules and functionally enriched pathways in SCA2. Hum Mol Genet 26(16):3069–3080
54. Fittschen M, Lastres-Becker I, Halbach MV, Damrath E, Gispert S, Azizov M et al (2015) Genetic ablation of ataxin-2 increases several global translation factors in their transcript abundance but decreases translation rate. Neurogenetics 16:181–192
55. Halbach MV, Gispert S, Stehning T, Damrath E, Walter M, Auburger G (2016) ATXN2 Knockout and CAG42-Knock-in cerebellum shows similarly dysregulated expression in calcium homeostasis pathway. Cerebellum
56. Bushart DD, Murphy GG, Shakkottai VG (2016) Precision medicine in spinocerebellar ataxias: treatment based on common mechanisms of disease. Ann Transl Med 4:25
57. Bezprozvanny IB (2010) Calcium signaling and neurodegeneration. Acta Naturae 2:72–82
58. van de Leemput J, Chandran J, Knight MA, Holtzclaw LA, Scholz S, Cookson MR et al (2007) Deletion at ITPR1 underlies ataxia in mice and spinocerebellar ataxia 15 in humans. PLoS Genet 3:e108
59. Iwaki A, Kawano Y, Miura S, Shibata H, Matsuse D, Li W et al (2008) Heterozygous deletion of ITPR1, but not SUMF1, in spinocerebellar ataxia type 16. J Med Genet 45:32–35
60. Meera P, Pulst S, Otis T (2017) A positive feedback loop linking enhanced mGluR function and basal calcium in spinocerebellar ataxia type 2. Elife 6. pii:e26377
61. Ragothaman M, Muthane U (2008) Homozygous SCA 2 mutations changes phenotype and hastens progression. Mov Disord 23:770–771
62. Yamamoto A, Lucas JJ, Hen R (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 101:57–66
63. Boy J, Schmidt T, Wolburg H, Mack A, Nuber S, Bottcher M et al (2009) Reversibility of symptoms in a conditional mouse model of spinocerebellar ataxia type 3. Hum Mol Genet 18:4282–4295
64. Zu T, Duvick LA, Kaytor MD, Berlinger MS, Zoghbi HY, Clark HB et al (2004) Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice. J Neurosci 24:8853–8861
65. Oz G, Vollmers ML, Nelson CD, Shanley R, Eberly LE, Orr HT et al (2011) In vivo monitoring of recovery from neurodegeneration in conditional transgenic SCA1 mice. Exp Neurol 232:290–298
66. Xia H, Mao Q, Eliason SL, Harper SQ, Martins IH, Orr HT et al (2004) RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. Nat Med 10:816–820
67. Hache M, Swoboda KJ, Sethna N, Farrow-Gillespie A, Khandji A, Xia S et al (2016) Intrathecal injections in children with spinal muscular atrophy: nusinersen clinical trial experience. J Child Neurol
68. Miller TM, Pestronk A, David W, Rothstein J, Simpson E, Appel SH et al (2013) An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study. Lancet Neurol 12:435–442
69. Rigo F, Seth PP, Bennett CF (2014) Antisense oligonucleotide-based therapies for diseases caused by pre-mRNA processing defects. Adv Exp Med Biol 825:303–352
70. Bennett CF, Swayze EE (2010) RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Ann Rev Pharmacol Toxicol 50:259–293
71. Skotte NH, Southwell AL, Ostergaard ME, Carroll JB, Warby SC, Doty CN et al (2014) Allele-specific suppression of mutant Huntingtin using antisense oligonucleotides: providing a therapeutic option for all Huntington disease patients. PLoS ONE 9:e107434
72. Ostergaard ME, Southwell AL, Kordasiewicz H, Watt AT, Skotte NH, Doty CN et al (2013) Rational design of antisense oligonucleotides targeting single nucleotide polymorphisms for

potent and allele selective suppression of mutant Huntingtin in the CNS. Nucleic Acids Res 41:9634–9650
73. White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, Joyner AL et al (1997) Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat Genet 17:404–410
74. McBride JL, Pitzer MR, Boudreau RL, Dufour B, Hobbs T, Ojeda SR et al (2011) Preclinical safety of RNAi-mediated HTT suppression in the rhesus macaque as a potential therapy for Huntington's disease. Mol Ther 19:2152–2162
75. Boudreau RL, McBride JL, Martins I, Shen S, Xing Y, Carter BJ et al (2009) Nonallele-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. Mol Ther 17:1053–1063
76. Scoles DR, Meera P, Schneider MD, Paul S, Dansithong W, Figueroa KP, Hung G, Rigo F, Bennett CF, Otis TS, Pulst SM (2017) Antisense oligonucleotide therapy for spinocerebellar ataxia type 2. Nature 544(7650):362–366
77. Becker LA, Huang B, Bieri G, Ma R, Knowles DA, Jafar-Nejad P, Messing J, Kim HJ, Soriano A, Auburger G, Pulst SM, Taylor JP, Rigo F, Gitler AD (2017) Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. Nature 544(7650):367–371

Chapter 9
Molecular Mechanisms and Therapeutic Strategies in Spinocerebellar Ataxia Type 7

Alice Karam and Yvon Trottier

Abstract Spinocerebellar Ataxia type 7 (SCA7, OMIM # 164500) is an autosomal dominant neurodegenerative disorder characterized by adult onset of progressive cerebellar ataxia and blindness. SCA7 is part of the large family of autosomal dominant cerebellar ataxias (ADCAs), and was estimated to account for 1–11.7% of ADCAs in diverse populations. The frequency of SCA7 is higher where local founder effects were observed as in Scandinavia, Korea, South Africa and Mexico. SCA7 is pathomechanistically related to the group of CAG/polyglutamine (polyQ) expansion disorders, which includes other SCAs (1–3, 6 and 17), Huntington's disease, spinal bulbar muscular atrophy and dentatorubro pallidoluysian atrophy. Two distinctive characteristics of SCA7 are the strong anticipation by which earlier onset and more severe symptoms are observed in successive generations of affected families, and the loss of visual acuity due to cone-rod dystrophy of the retina. The pathology is caused by an unstable CAG repeat expansion coding for a polyQ stretch in Ataxin-7 (ATXN7). PolyQ expansion in ATXN7 confers toxic properties and leads to selective neuronal degeneration in the cerebellum, the brain stem and the retina. Herein, we summarize the genetic, clinical and pathological features of SCA7 and review our current knowledge of pathomechanisms and preclinical studies.

Keywords Spinocerebellar ataxia 7 · Polyglutamine expansion · Retinopathy Cerebellar degeneration · Aggregation

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9.1 Genetic, Clinical and Pathological Description

The causative mutation of SCA7 was identified in 1995 [1] and the ATXN7 gene was isolated in 1998 [2] and was shown to contain a polymorphic CAG repeat. The wild-type alleles of ATXN7 have between 4 and 36 CAG repeats, while SCA7 alleles have typically beyond 36 CAGs and can even reach >460 repeats [3]. Among CAG/polyQ disorders, SCA7 CAG repeats show the highest tendency to expand upon transmission, explaining the strong anticipation observed in families (mean 19 ± 13 years) [2, 4, 5]. The length of mutant CAG repeats is inversely correlated with the age of onset and the disease duration. The majority of SCA7 alleles ranges between 36 and 55 CAG repeats and are responsible for the classical adult-onset form, which progresses over several decades until death [4]. Repeats >70 CAG typically result in juvenile-onset forms with accelerated disease course. The repeat length also influences the symptoms at onset: large repeat expansions are typically associated with early onset and cause visual loss before cerebellar ataxia, while shorter expansions with later onset cause ataxia symptoms before visual loss [2, 6, 7]. Intriguingly, extremely large CAG expansions (>100 CAG) cause infantile forms with multisystem disorders such as failure to thrive, hypotonia, myoclonic seizures and noncentral nervous systems dysfunctions like congestive heart failure, patent ductus arteriosus, renal failure, and muscle atrophy, and lead to death within few years or months [3, 6, 8–12].

SCA7 progressive cerebellar ataxia is manifested by the inability to coordinate balance, gait, and speech. Additional neurological deficits include slow eye movement, opthalmoplegia, dysphagia, as well as pyramidal signs [2, 6]. Variable levels of cerebellar and pontine atrophy are observed by magnetic resonance imaging [2, 4, 13–16]. Neuropathologically, the neuronal loss is substantial in the Purkinje cell layer, in the dentate nuclei, in the inferior olivary nuclei and in basis pontis, which is associated with the atrophy of spinocerebellar and pyramidal tracts [4, 15, 17, 18]. Atrophy or loss of myelin is observed in the cerebellar white matter and extra cerebellar associated structures [16, 18]. Visual impairment in SCA7 first affects cone photoreceptors and then progresses toward a cone-rod dystrophy and complete blindness [10, 19–21]. Fundoscopy examination shows atrophic macula with granular pigmentation, pale areas with pigmentary atrophy and poor vasculature [4, 20]. Post-mortem retinal sections reveal almost complete loss of photoreceptors and substantial loss of the bipolar and ganglion neurons, associated with a severe thinning of the nuclear and plexiform layers especially in the foveal and parafoveal regions [4, 17, 22]. In addition, damages in the Bruch's membrane, retinal pigmentary epithelium and hypomyelinisation of the optic nerve were also reported [10, 15, 17, 20].

9.2 ATXN7 Protein

The ATXN7 gene encodes two protein isoforms, ATXN7a and ATXN7b, that harbor a polymorphic polyQ stretch in the amino-terminus, three nuclear localization signals (NLS) and one nuclear export signal (NES) (Fig. 9.1a). Besides the NLS and NES, the different C-termini of ATXN7a and ATXN7b are likely to control their subcellular localization, as ATXN7a appears predominantly in the nucleus and ATXN7b in the cytoplasm [23]. However, the extent to which the two isoforms contribute to SCA7 pathogenesis remains unclear, as most studies have been done so far with ATXN7a, which was identified first. ATXN7a and ATXN7b have three conserved domains that are shared with three paralogs, ATXN7L1, L2 and L3: a typical C2H2 zinc-finger (ZnF) motif, an atypical Cys-X9–10–Cys-X5–Cys-X2-His motif, now known as SCA7 domain, and a third domain absent in ATXN7L3 (Fig. 9.1a) [24]. ATXN7 is also regulated by

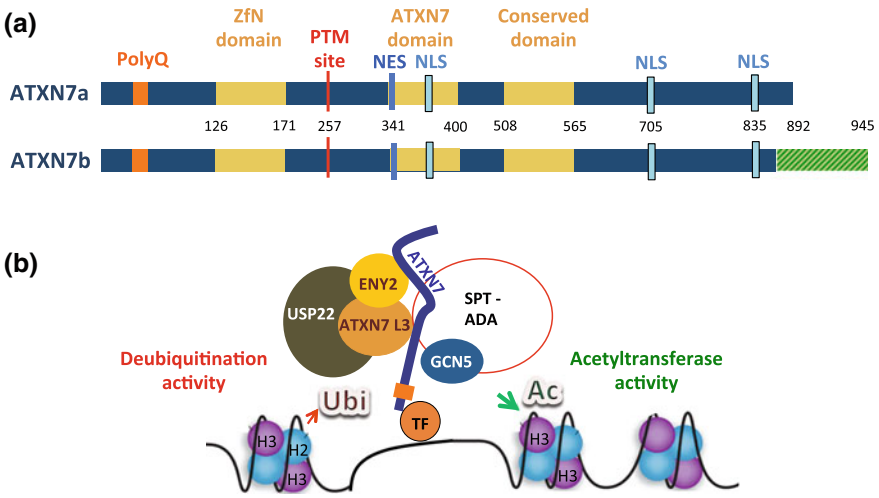

Fig. 9.1 Structural composition of ATXN7 and functional interactions with SAGA components. a Are depicted the sequences of ATXN7a and ATXN7b isoforms with 892 and 945 amino acids, respectively, corresponding to a wild-type allele with 10 CAG-encoding glutamine units. Wild-type alleles contain from 4 to 36 glutamine repeats, whereas mutant pathogenic alleles have beyond 36 glutamines and can even reach >460 residues. The conserved domains are indicated as yellow boxes: a typical C_2H_2 zinc-finger (ZnF) motif, an atypical Cys-X9–10–Cys-X5–Cys-X2-His motif known as ATXN7 domain and a third conserved domain. ATXN7 isoforms have three nuclear localization signals (NLS), one nuclear export signal (NES) and one site (lysine 257) for post-translational modifications including acetylation and SUMOylation, but differ at their carboxy-terminal end. **b** ATXN7 appears to be a molecular scaffold of SAGA. ATXN7 belongs to the deubiquitination module (DUBm), together with the ubiquitin protease USP22, ATXN7L3, and ENY2, and mediates the interaction with GCN5 and the SAGA core complex (SPT-ADA; other subuits are not indicated). ATXN7 can also interact with transcriptional factors such as CRX. SAGA complex harbors both histone acetylation (dependent of GCN5) and deubiquitination (dependent of USP22) activities on histones H3 and H2B, respectively

SUMOylation and acetylation [25, 26]. ATXN7 mRNA and protein are widely expressed in neural and non-neural tissues [27–33]. There is no apparent correlation between cellular or subcellular localization and the vulnerability of neurons to degeneration in SCA7.

ATXN7 and its yeast ortholog sgf73 are core components of SAGA complexes (Spt-Ada-Gcn5 Acetyltransferase) involved in chromatin remodeling (also known in human as the TBP-free TAF-containing complex (TFTC) and the SPT3-TAF9–GCN5 complex (STAGA) [24, 34–36]. SAGA complexes harbor both histone acetylation (dependent of GCN5) and deubiquitination (dependent of USP22) activities, located in distinct functional modules (Fig. 9.1b). ATXN7 belongs to the deubiquitination module (DUBm) together with the human ubiquitin protease USP22, ATXN7L3, and ENY2. Histone acetylation is known to increase decompaction of chromatin and the accessibility of gene promoters to transcription factors, while deubiquitination of monoubiquitinated H2B (H2Bub) is required for optimal transcriptional initiation/elongation [37]. Bonnet et al. [38] recently unveiled a role of SAGA in general RNA polymerase II recruitment and transcription. SAGA is recruited to all active genes to acetylate H3K9 on promoters and to deubiquitinate H2Bub on gene bodies in yeast and human cells. On the contrary to the ATXN7 nuclear function, the role of ATXN7 in the cytoplasm is yet unclear. When overexpressed, ATXN7 associates with and stabilizes microtubules [39]. Yeast two-hybrid screen showed that ATXN7 interacts with several cytoplasmic proteins associated with the vesicular system and centrosomes [40]. In fly and zebrafish, inactivation of ATXN7 causes defect in retina and brain development [41, 42]. In zebrafish, ATXN7 seems to be required for full differentiation of photoreceptors and Purkinje neurons, suggesting that partial loss of function of wild type ATXN7 may account for the selective degeneration in SCA7. The physiological role of ATXN7 in tissue development and homeostasis thus deserves further investigation.

9.3 Pathomechanisms Underlying SCA7 Neurodegeneration

9.3.1 Commonalities and Differences Between PolyQ Disorders

SCA7 and other polyQ disorders share a number of common features. They are adult-onset and progressive neurodegenerative diseases. The dominant inheritance and genetic experimentations indicate that polyQ expansion confers toxic properties to mutant proteins. The toxicity increases with the expanded polyQ length and the age of disease onset and the severity of symptoms are function of the polyQ length. There is an apparent polyQ length threshold above which the disease becomes fully penetrant. Finally, a hallmark of polyQ diseases is the intracellular accumulation of amyloid-like aggregates containing protein fragments bearing the polyQ expansion

[43]. However, polyQ disorders differ on many aspects. While the mutant proteins bear a similar polyQ tract, they do not share any other domain and have different cellular functions. The polyQ proteins are ubiquitously expressed, however, neuronal degeneration affects specific and different brain regions, leading to disease specific symptoms. Therefore, particularities of each disease must come from the protein context into which the polyQ expansion is embedded.

Our current understanding of SCA7 pathogenesis relies on biochemical, molecular and cellular studies and on the characterization of model systems developed in yeast, fly and mouse. Hereafter, we discuss the major characteristics of mutant ATXN7 (mATXN7) toxicity, which might underlie the unique features of SCA7 pathogenesis.

9.3.2 mATXN7 Misfolding, Accumulation and Toxicity

One major consequence of the polyQ expansion mutation is the intensive intracellular accumulation of mATXN7 in disease tissues. Studies in SCA7 mouse models showed the time-dependent accumulation of mutant, but not wild type ATXN7 in neuronal nuclei [44, 45]. mATXN7 accumulation is faster in neurons targeted by the disease than in spared neurons. Ultimately, protein accumulation leads to the formation of mATXN7 aggregates, observed as nuclear inclusions (NIs) by immunohistochemistry. In post-mortem brains, NIs are widely distributed in degenerated and non-degenerated tissues [18]. However, in SCA7 mice, NIs form faster in vulnerable tissues such as retina and cerebellum, although their detection occurs after the onset of functional defects [44]. The role of NIs in the pathogenesis of polyQ disorders is actively debated. It is now thought that small oligomeric or multimeric forms of the misfolded mutant protein, not visible by immunohistochemistry, are the most toxic species. These species are indeed readily detected at very early stages in SCA7 mice using biochemical approach [44]. PolyQ expansion appears to stabilize mATXN7, which could happen because of a slower turnover, by a propensity to stably oligomerise or both (Fig. 9.2).

One important step in mATXN7 accumulation and toxicity is the proteolysis. Indeed, an amino-terminal fragment of about 55 kDa containing the polyQ expansion is detected in protein samples from SCA7 mice and from SCA7 patients [46], and appears to be the major component of NIs in SCA7 mouse [47]. A similar fragment can be released by caspase-7 cleavage in vitro and in vivo and was shown to be more cytotoxic than the full-length mATXN7 [48]. Interestingly, transgenic mice expressing a mATXN7 form that contain a mutation at the caspase-7 cleavage site show reduced neurodegeneration, improved visual and motor performance and prolonged lifespan [49]. These results suggest that the caspase-7 cleavage is a major step in the pathogenesis. The presumed size of the mATXN7 fragment is short enough for passive diffusion through nuclear pore complexes, but is retained in the nucleus [48]. It harbors the polyQ expansion and the ZnF domain, but not the ATXN7 domain. With this composition, the mATXN7 fragment may alter the

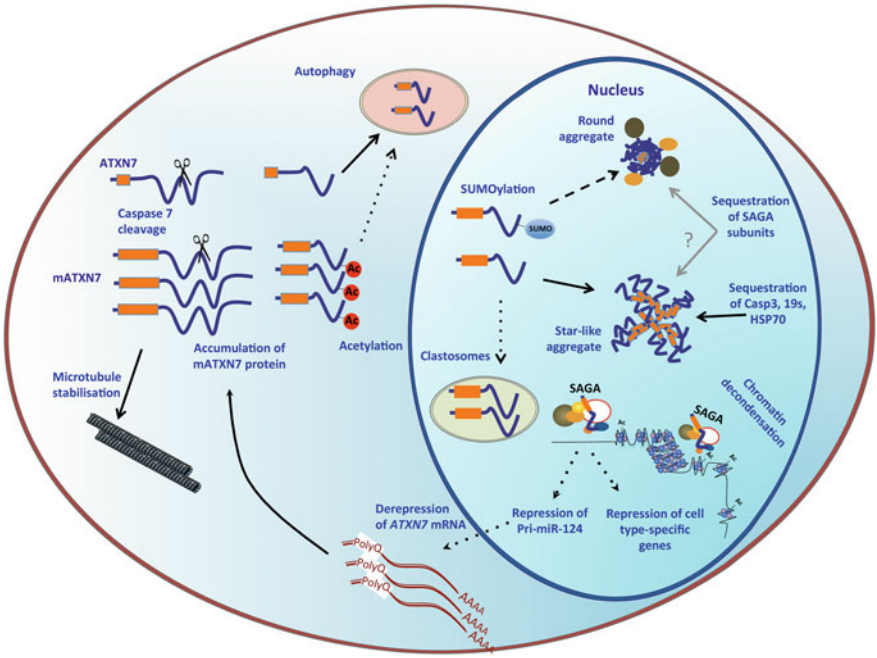

Fig. 9.2 Pathogenic events underlying SCA7 pathogenesis and potential therapeutic targets. Mutant ATXN7 (mATXN7) accumulates and is cleaved by caspase-7 to release amino-terminal fragments (Nter). Acetylation of Nter-mATXN7 at K257 prevents its degradation by autophagy in the cytoplasm. In the nucleus, proteosomal activity of clastosomes, which normally degrades mATXN7, is overwhelmed, leading to mATXN7 accumulation. Aggregation of SUMoylated mATXN7 leads to the formation of «round» aggregates, while the non-SUMOylated mATXN7 forms «star-like» aggregate, which sequestered the activated caspase-3, 19 s proteasome subunit, and HSP70. Components of SAGA (GCN5, ATXN7L3 and USP22) are also sequestered in aggregates. Transcriptional alterations resulting from SAGA dysfunction and perturbation of transcription regulators such as CBP, p53, RORalpha1, lead to down-regulation of cell type-specific genes (such as photoreceptor- and oligodendrocyte-specific genes) and of Pri-miR-124. The low level of miR-124 causes the accumulation of ATXN7 mRNA, which in turn leads to increased level mATXN7. The involvement of non-coding RNA *lnc-SCA7* in the regulation of ATXN7 mRNA level is not depicted here. Therapeutic opportunities include: (i) blocking proteolysis with caspase inhibitors; (ii) preventing acetylation by overexpressing deacetylase; (iii) preventing nuclear accumulation by inducing clastosome formation with interferon beta; (iv) inhibiting the formation of toxic aggregates; (v) upregulating transcription by inhibiting histone deacetylation or ubiquitination or by treatment with neurotrophy factors such as HGF and with the antibiotic Ceftriaxone; (vi) preventing ATXN7 mRNA accumulation or translation using RNA inhibition; (vii) preventing mATXN7 binding to microtubules

function of SAGA complex, either by replacing the full-length protein in the complex or by aggregation and sequestration of SAGA components.

Further analysis indicated that the accumulation of the mATXN7 fragment is associated with an increased acetylation at lysine-257 (K257) located close to the caspase-7 cleavage site [50]. In the absence of lysine acetylation, the fragment is

degraded by autophagy. These results suggest that aberrant K257 acetylation prevents the clearance of the fragment by autophagy, and thereby slows down its turnover. Interestingly, the same K257 residue was shown to be a specific site for SUMOylation [26]. SUMO modification not only decreases the propensity of mATXN7 to aggregate, but also influences the shape and the protein composition of aggregates. With SUMO, aggregates are homogenous, round and strongly stained with SUMO antibodies, while in the absence of SUMO, aggregates are heterogeneous, have a star-like shape and are immunostained for chaperones, proteasomes and activated caspase-3, suggesting that this type of aggregates are associated with cells that undergo cytotoxic. In conclusion, proteolysis and post-translational modifications are involved in the accumulation of ATXN7. Whether acetylation and SUMOylation are in competition to modify K257 in order to trigger specific mATXN7 fate or whether they act successively on K257 through rounds of deacetylation/deSUMOylation remain to be determined.

In the brain of SCA7 patients, mATXN7 aggregates often colocalize with nuclear bodies formed by the promyelocytic leukemia (PML) protein [51, 52]. A subset of PML bodies formed by PML IV isoform and known as clastosomes, contain components of ubiquitin-proteasome system and chaperones and were suggested to be a site for protein degradation in the nucleus. Interestingly, PML IV-positive clastosomes actively recruit soluble mATXN7, but not the wild type form, for degradation through the proteasome [53]. Moreover, interferon beta, which induces PML IV expression and clastosome formation, enhances the clearance of the mATXN7 and increases the survival of rat primary Purkinje neurons [53, 54]. In patients, endogenous clastosomes might prevent the accumulation of mATXN7 for several decades before onset of aggregation. Over time, the degradative activity of clastosomes might be overwhelmed by the aggregation process.

9.3.3 Transcriptional Alterations

Studies performed on cellular and mouse models of SCA7 have identified transcriptional alterations as an early pathogenic event associated with neuronal dysfunction [44, 55–58]. Transcriptome analysis of SCA7 mouse retina revealed an early and progressive down-regulation of most photoreceptor-specific genes [55], while expression profile of SCA7 mouse cerebellum showed down-regulation of genes involved in the maintenance and function of neuronal dendrites and CNS myelin sheath [56].

The possibility that transcriptional alterations in SCA7 could result from dysfunction of SAGA acetylation and deubiquitination has been explored in several studies, specially because mATXN7 had been shown to properly incorporate in SAGA [24, 34, 59]. The outcome of these studies differs given the model system investigated. In yeast and HEK2937 kidney cells, mATXN7-containing SAGA lacks critical subunits and leads to the reduction of GCN5 acetylation activity and gene transcription [34, 59]. In agreement with GCN5 dysfunction, promoters of

photoreceptor-specific genes were shown to have histone H3 hypoacetylation, which would explain their decreased expression in SCA7 mouse retina [34]. However, at variance with the above studies, mATXN7-containing SAGA purified from SCA7 mouse retina was correctly assembled and had normal acetylation activities [57]. In this study, promoters of photoreceptor-specific genes were found hyperacetylated, but the presence of RNA Pol II on promoters was strongly reduced, which would explain the low level of photoreceptor-specific mRNA transcripts [57]. The discrepancy between these studies is yet unclear and might dependent on the use of two different SCA7 mouse models [Prp SCA7-c92Q and R7E (see Table 9.1)] or on the analysis of different stages of retinal degeneration.

Table 9.1 Mouse models of SCA7 retinal degeneration

Models	Design[a]	Retina pathology[b]	References
R7E (R7N)	Human rhodopsin (rods) ATXN7a cDNA 90Q (10Q)	(i) Reduction of rod then cone ERG activity (ii) Thinning of retina layer, loss of photoreceptor OS and polarity, enlargement of nucleus, dark degeneretion, apoptosis, proliferation, gliosis (iii) Expression profiles showed repression of photoreceptor-specific genes and re-activation of developmental gene; chromatin decondensation and H3 hyperacetylation; activation of cellular stress response signaling (iv) Onset at 3–5 weeks	[24, 47, 55, 57, 68, 72, 73]
Prp SCA7-c92Q (Prp SCA7-c24Q)	Murine prion (brain except for PC) ATXN7a cDNA 92Q (24Q)	(i) Reduction of cone then rod ERG activity (ii) Thinning of retina layer, apoptosis, gliosis (iii) Repression of photoreceptor-specific genes; H3 hypocetylation (iv) Onset at 11 weeks	[34, 58]
$SCA7^{266Q/5Q}$ (WT mice)	Mouse ATXN7 mouse ATXN7 266Q(5Q)	(i) Reduction of cone then rod ERG activity (ii) Thinning of retina layer, loss of photoreceptor OS, enlargement of nucleus, apoptosis, gliosis (iii) Repression of photoreceptor-specific genes; chromatin decondensation (iv) Onset at 5 weeks	[44, 57]
$SCA7^{100Q/100Q}$ (WT mice)	Mouse ATXN7 mouse ATXN7 100Q(5Q)	(i) Reduction of rod ERG activity (ii) Thinning of retina layer, loss of photoreceptor OS, dark degeneration (iii) Reduced expression of photoreceptor-specific genes (iv) Onset at 6 weeks(transcription anomalies)	[61][c]

BAC bacterial artificial chromosome; *PC* Purkinje cells; *OS* outer segments
[a]promoter/targeted cells/cDNA or gene/repeat length
[b](i) dysfunction; (ii) neuropathology; (iii) molecular altertions; (iv) onset
[c]YT personal observations

Interestingly, histone hyperacetylation in R7E mouse retina correlates massive chromatin decondensation of photoreceptor nuclei, which are enlarged compared to wild type [57]. It is thus possible that the overall perturbation of chromatin organization accounts for major changes in the expression of photoreceptor-specific genes. Besides hyperacetylation, chromatin decondensation in R7E retina might also result from an abnormal low expression of histone H1, which is involved in chromatin compaction [60]. The importance of GCN5 in SCA7 pathogenesis was recently addressed using a mouse genetic approach [61]. One allele inactivation of GCN5 accelerates retina degeneration in SCA7 mice, but does not worsen the transcriptional repression of photoreceptor-specific genes, suggesting that GCN5 might have non-transcriptional function in the retina. Furthermore, total loss of GCN5 in Purkinje cells leads to milder ataxia than SCA7 mouse ataxia. This suggests that GCN5 could participate to some degree to SCA7 cerebellar ataxia. Potential dysfunction of DUBm activity of SAGA has also been investigated in SCA7 cellular and mouse models. Monoubiquitination of H2B is globally increased in cultured cells expressing mATXN7 and in the cerebellum of SCA7 mice, but in the latter correlation with transcriptional alterations has not been established [62–64]. Two components of DUBm, ATXN7L3 and USP22, are sequestered in mATXN7 aggregates, which might lead to DUBm dysfunction and hence an increased H2Bub [63, 64]. Although the current data would support that SAGA dysfunction accounts for SCA7 transcriptional dysregulations, it remains to determine how the dysfunction of a general co-activator complex like SAGA, which is involved in the expression of all RNA Pol II-regulated genes, would only affect specific subsets of genes in SCA7 affected tissues.

Interestingly, a recent study suggests that the increased expression of mATXN7 would be an indirect consequence of mATXN7-containing SAGA dysfunction [65]. In fact, SAGA regulates the microRNA miR-124, which in turn controls the abundance of ATXN7 transcripts and of a non-coding RNA lnc-SCA7. The level of lnc-SCA7 also seems to control the level of ATXN7 transcripts by a mechanism yet unclear. Dysfunction of SAGA in SCA7 leads to post-transcriptional derepression of ATXN7 transcripts, due to the reduced level of miR-124 and the increased level of lnc-SCA7. Given that miR-124 is highly expressed in the cerebellum and retina and that the levels of lnc-SCA7 and ATXN7 are tightly correlated, the cross-talk between these two noncoding RNAs in the post-transcriptional regulation of ATXN7 transcripts is thought to account for the tissue specificity of SCA7.

Besides SAGA dysfunction, other mechanisms are proposed to contribute to chromatin modifications and transcriptional alterations in SCA7. mATXN7 aggregates sequester CBP [45], a histone acetyltransferase, and impair CBP-mediated and RORalpha1-mediated transcription in cultured neurons [66]. Most interesting, a study in PC12 cells made a link between metabolic defect in SCA7 and transcriptional alterations [67]. Abnormal mitochondria were observed in SCA7 mouse retina, [68] and reduced electron transport chain activity and metabolic acidosis were reported in muscle biopsy of patients [69]. In PC12 cells expressing mATXN7, p53 is sequestered in aggregates and its transcriptional activity is reduced, leading to dysregulation of metabolic proteins, such as TIGAR, AIF and

NOX1 [67]. These alterations result in a reduced respiratory capacity, associated with an increased reliance on glycolysis for energy production and a subsequent reduction of ATP in SCA7 cells. Investigation of these transcriptional and metabolic pathways in SCA7 mice is thus warranted, in particular because loss of AIF in mice results in primarily neurodegeneration of cerebellar and retinal neurons.

9.4 Insights from Pathophysiological Studies of SCA7 Mouse Models

Different transgenic and knock-in mouse models have been generated during the past years and have provided important insights into the nature of SCA7 neurodegeneration (Tables 9.1 and 9.2).

9.4.1 Retinopathy

In SCA7 models, the retina develops normally before showing a progressive reduction of electroretinograph activity, thinning of the retina and repression of photoreceptor-specific genes [44, 47, 58]. Early on, these transcriptional alterations were attributed to the dysfunction of CRX (cone-rod homeobox protein), a key transcription factor of photoreceptor genes. This is because CRX was previously shown to require interaction with ATXN7 and SAGA for its transactivation activity on photoreceptor gene promoters, and because mATXN7 was shown to suppress the transactivation activity in SCA7 retina [58, 70]. Later on, analysis of SCA7$^{266Q/5Q}$ KI and R7E mouse retina showed that transcriptional alterations were not restricted to CRX target genes [44, 55]. In particular, the expression profile of R7E retina unveiled the dysregulation of transcriptional programs controlling the maintenance of mature photoreceptors, thus showing on the one hand the down-regulation of the photoreceptors specific transcription factors CRX, NRL (neural retina leucine zipper protein), and Nr2E3 (Nuclear Receptor Subfamily 2, Group E, Member 3) as well as most of their target genes, and on the other hand the re-activation of OPTX2, STAT3 and HES5 that normally inhibit the differentiation of precursor neurons into mature photoreceptors during development [55]. And indeed, SCA7 photoreceptors progressively lose their outer segments and cell polarity, and relapse to round cell shape [68]. Thus, SCA7 retinopathy primarily results from the progressive regression of mature photoreceptor to an ill-defined state, which occurs long before cell demise (Fig. 9.3a). This atypical scheme of slow degeneration contrasts with most photoreceptor degenerative processes reported in mice, where alterations of outer segment integrity rapidly leads to cell death [71]. Yet the initial trigger leading to SCA7 photoreceptor degeneration remains to be determined.

Table 9.2 Mouse models of SCA7 cerebellar degeneration

Models	Design[a]	Cerebellar pathology[b]	References
P7E (P7N)	Pcp-2 (Purkinje) hATXN7a cDNA 90Q (10Q)	(i) Reduction of rotarod function (ii) Purkinje reduced dentritic (iii) Sequestration of chaperones and proteasome subunits in aggregates (iv) Onset at 11 months	[45, 47]
B7E2 (B7N)	PDGF-ß (ubiquitous) ATXN7a cDNA 128Q (10Q)	(i) Ataxic phenotypes (ii) Purkinje reduced dentritic arborization (iii) Sequestration of transcription factors and co-regulators in aggregates (iv) Onset at 3–5 months	[45]
Prp SCA7-c92Q (Prp SCA7-c24Q)	Murine prion (brain except PC) ATXN7a cDNA 92Q (24Q)	(i) Reduction of rotarod function (ii) Purkinje neuron shrinkage, reduced dentritic arborization and dark degeneration (no cell loss); thickened Bergmann glia radial processes, reduced glutamate uptake (iii) Reduced GLAST expression and -dependent glutamate uptake (iv) Onset at 13–15 weeks	[46, 74]
Gfa2-SCA7-92Q (Gfa2-SCA7-10Q)	Human Gfa2 (Bergmann glia) ATXN7a cDNA 92Q (10Q)	(i) Reduction of rotarod function (ii) Purkinje neuron shrinkage, reduced dentritic arborization and dark degeneration; thickened Bergmann glia radial processes, reduced glutamate uptake (iii) Reduced GLAST expression and GLAST-dependent glutamate uptake (normal GLT1 expression) (iv) Onset at 9–12 months	[74]
PrP-floxed-SCA7-92Q BAC	BAC murin prion (whole brain) floxed ATXN7a cDNA 92Q	(i) Reduction of rotarod function; reduced stride length on footprint (ii) Purkinje reduced dentritic arborization; reduced molecular layer thickness; late thickened Bergmann glia radial processes (iii) Reduced EAAT4 glutamate transporter expression (normal GLAST expression) (iv) Onset at 21 weeks	[76, 84, 85]
Ataxin-7-Q52 (WT mice)	PDGF-ß ATXN7 cDNA 52Q	(i) Reduction of rotarod function; decreased locomoter activity; ataxic wobbling gait (ii) Purkinje neuron shrinkage and reduced dentritic arborization (no cell loss); loss of inferior olive neurons	[56]

(continued)

Table 9.2 (continued)

Models	Design[a]	Cerebellar pathology[b]	References
		(iii) Expression profiles showed reduced expression of oligodendrocyte myelin specific genes and deregulation of many other pathways; p53 activation of Bax and Puma (iv) Onset at 9 months	
$SCA7^{266Q/5Q}$ (WT mice)	Mouse ATXN7 mouse ATXN7 266Q (5Q)	(i) Reduction of performance on rotarod, beam walking test balance and fine paw coordinatio)n and locotronic test (motor coordination); reduced survival (ii) Purkinje neuron shrinkage (no reduced dentritic arborization and no cell loss); gliosis (iii) Reduced GLAST and GLT1 expression; increased Interferon beta (iv) Onset at 5 weeks	[44, 54, 75]
$SCA7^{100Q/100Q}$ (WT mice)	Mouse ATXN7 mouse ATXN7 100Q (5Q)	(i) Reduction of performance on rotarod, footprint anomalies, reduced survival (11 months) (ii) Purkinje neuron shrinkage (no cell loss); gliosis	[61]

BAC bacterial artificial chromosome; *PC* Purkinje cells; *OS* outer segments
[a]promoter/targeted cells/cDNA or gene/repeat length
[b](i) dysfunction; (ii) neuropathology; (iii) molecular alterations; (iv) onset

Degenerating photoreceptors in SCA7 retina ultimately die through a mechanism reminiscent of dark neuronal cell death [68]. Dark degeneration also occurs in SCA7 mouse cerebellum and was reported in several mouse models of polyQ disorders. Interestingly, apoptosis was also observed in R7E mouse retina, but only occurs for a short time window during early disease stages. Concomitant with the apoptotic wave, stealthy cells expressing proliferation markers were observed, which afterwards express photoreceptor specific genes, suggesting that new photoreceptors might be produced to replace the dead ones at early disease stages. From these observations, it appears that R7E photoreceptors go through different cell fates as a response to mATXN7 toxicity (e.g. apoptosis, cell reshaping, dark degeneration, proliferation, etc.) [68]. Different cellular responses may be triggered by different mATXN7 toxic species, since the relative amount of full-length mATXN7, proteolytic fragments, soluble and insoluble aggregates varies considerably from early to late disease stages and might influence the way individual photoreceptors respond to these different proteotoxic products [68]. The overall proteotoxic stress in R7E retina induces a stress response involving the Jnk/c-Jun signaling pathway, which in turn accounts for Nrl repression [72, 73]. It was shown that inhibition of c-Jun activation delays retinal degeneration in R7E mice.

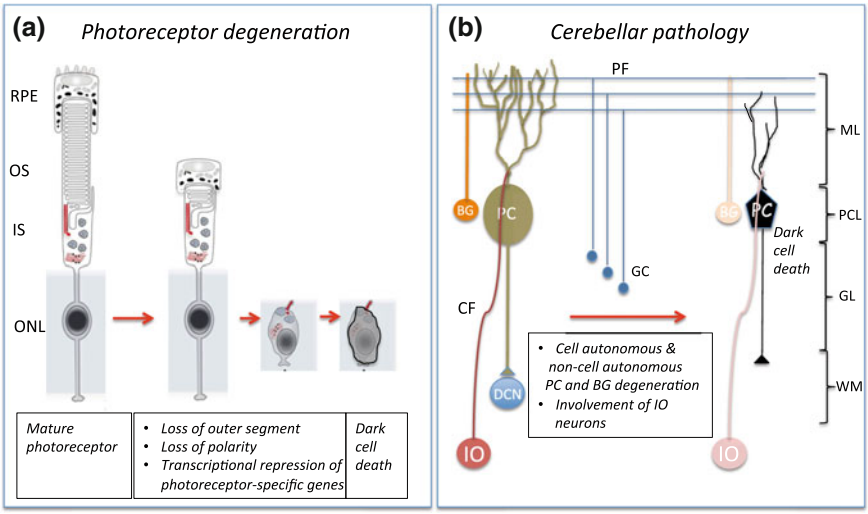

Fig. 9.3 Schematic of the major degenerative pathways of photoreceptors (a) and Purkinje neurons (b) in SCA7 mice. ONL, outer nuclear layer; IS, inner segment; OS, outer segment; RPE, retinal pigmented epithelium; PC, Purkinje cell; BG, Bergmann glia; IO, inferior olive; GC, granular cell; DCN, deep cerebellar nuclei; CF, climbing fiber; PF, parallel fiber; GL, granular layer; ML, molecular layer; PCL, Purkinje cell layer; WM, white matter. Other cerebellar cell types are not depicted

9.4.2 Cerebellar Pathology

Analyses of the PrP-SCA7-c92Q mouse model have highlighted the importance of cell–cell interactions in the cerebellar pathology [46]. These mice develop motor defects and show dark degenerating Purkinje neurons. Interestingly, Purkinje cell pathology occurs despite the fact the MoPrP promoter drives the expression of mATXN7 in all cerebellar neurons, except for Purkinje cells, suggesting that they are affected via a non cell-autonomous mechanism. In this model, Bergmann glia cells, which also express mATXN7, display pathological signs as well [74]. Given that Bergmann glia are regulators of glutamate levels in the surrounding environment of Purkinje cells and that dark degeneration often results from excitotoxicity, new transgenic mice were generated to express mATXN7 only in Bergmann glia cells to assess whether the pathology would affect Purkinje cells as well. Indeed, Gfa2-SCA7-92Q mice also show Purkinje cell degeneration and motor dysfunctions. Moreover, in this model, like in PrP-SCA7-c92Q mice, Bergmann glia cells express low levels of the glia-specific glutamate transporter GLAST, and hence have a decreased glutamate uptake function, supporting the hypothesis that glutamate accumulation leads to excitotoxicity and Purkinje dark degeneration [74]. However, compared to PrP-SCA7-c92Q mice, Gfa2-SCA7-92Q mice develop a late onset and milder ataxia, suggesting that other dysfunctional neurons may account for PC degeneration in PrP-SCA7-c92Q mice. Several pathological features of PrP-SCA7-c92Q were replicated in SCA7$^{266Q/5Q}$ KI mice, including decreased

motor functions, shrunken Purkinje cells and reduced expression of GLAST as well as GLT-1 (also named EAAT2 or SLC1A2), another glia-specific glutamate transporter [75].

The contribution of different cell types and their interaction to the cerebellar pathology was further addressed using a new set of engineered mice in which mATXN7 cDNA was flanked by loxP sites at the start site of translation in the murine PrP gene in a bacterial artificial chromosome (PrP-floxed-SCA7-92Q BAC) [76]. When crossed with mice expressing Cre recombinase under Bergmann glia promoter (Gfa2) or under promoter specific to Purkinje and inferior olive neurons (Pcp2), mATXN7 was deleted specifically in these cell types. Deletion of mATXN7 from Bergmann glia has mild beneficial effects and does not prevent Bergmann glia pathology. In contrast, deletion of mATXN7 from Purkinje and inferior olive neurons improves motor performance and histopathology as well as prevents Bergmann glia pathology. Finally, deletion of mATXN7 in the three cell types is more effective to prevent the pathology. These observations led to two conclusions. First, Bergmann glia pathology is in large part non-cell autonomous in SCA7. Second, it is likely that the dysfunction of inferior olive neurons accounts for the SCA7 motor dysfunction. This is because it was observed that the cerebellar pathology in P7E mice, which express mATXN7 only in Purkinje cells, is less severe than in PrP-SCA7-c92Q mice, which express mATXN7 in all cerebellar neurons, except for Purkinje cells. Together, these results further highlight a complex cell–cell interaction between Bergmann glia, Purkinje and inferior olive neurons in the development of SCA7 cerebellar dysfunction (Fig. 9.3b).

The expression profile of the cerebellum of Ataxin-7-Q52 transgenic mice, which also display motor dysfunction and Purkinje cell pathology, revealed gene deregulations affecting different pathways including synaptic transmission, axonal transport, glial functions and neuronal differentiation [56]. Perhaps the most interesting finding is the down regulation of a set of myelin-associated proteins (CNP, MAG, MBP, MOG, MOBP and PLP1) and of their regulators, the transcription factor Olig1 and transferrin [56]. This is consistent with the loose and poorly compacted myelin sheaths observed in the cerebellar white matter of these mice, and with the myelin pallor and loss of myelinated fibers reported in the cerebellar white matter of SCA7 patients [18]. Defect in white matter in SCA7 might also result from excitotoxic mechanisms, as relation between excitotoxicity and structural and functional damage to the white matter was observed in injury models [77]. Reminiscent to the loss of photoreceptor maturation in SCA7 mouse retina, mATXN7 toxicity might compromise genetic programs controlling oligodendrocyte maturation and myelin sheath integrity and function.

9.5 Opportunities for Therapeutic Development

Cellular and mouse models have provided several directions for therapeutical strategies (Fig. 9.2). Given the vulnerability of Purkinje cells to excitotoxicity-mediated dark degeneration and the reduced expression of GLAST and GLT-1, any

strategy to diminish glutamate levels in the cerebellum deserves consideration for preclinical assays with SCA7 mice. One of them is the β-lactamic antibiotic ceftriaxone, which induces GLT-1 expression via NF-kappaB and hence promotes glutamate clearance [78]. Interestingly, administration of ceftriaxone in the SCA28 murine model ($Afg3l2^{+/-}$) protects Purkinje cells from excitotoxicity-mediated dark degeneration [79]. The ceftriaxone-induced GLT-1 expression was long lasting and effective enough to prevent the onset of ataxia in pre-symptomatic and to stop the progression in post-symptomatic mice. In addition to its protective effect on Purkinje cells, the reduction of glutamate levels in the cerebellum might as well be beneficial for the function and maintenance of oligodendrocytes [77]. Ceftriaxone is a promising compound for SCA7 as well as for other SCAs showing excitotoxic-mediated Purkinje cell degeneration.

Another potential therapeutic strategy would be to provide factors with neurotrophic effects in the cerebellum and retina. In particular, the genetic programs of mature photoreceptors and oligodendrocytes are altered and represent specific targets for therapeutic intervention. Although a variety of strategies exist to enhance the protection of these cells [80, 81], the identification of initial triggers that compromise the genetic maturation programs deserves further attention to orientate the therapeutic development. Interestingly, hepatocyte growth factor (HGF) plays a neurotrophic role in the cerebellum during development and in adults [82]. Overexpression of HGF was shown to provide beneficial effect in ALS mice by maintaining GLT-1 levels [83]. Overexpression of HGF in $SCA7^{266Q/5Q}$ KI mice restores GLT-1 and GLAST levels, protects Purkinje cells from shrinkage and reduces motor dysfunction [75]. HGF is currently under consideration for therapeutic development of a number of human pathologies including brain injury, which will contribute to evaluate its efficacy and safety.

One of the most significant therapeutic target is the intracellular accumulation of mATXN7, which strongly correlates with the initiation and progression of SCA7. This has encouraged the implementation of several strategies to prevent mATXN7 accumulation and aggregation, to increase clearance or to interfere with protein synthesis. For instance, the strategy consisting in caspase-7 cleavage inhibition through pharmacological approach or genetic intervention is promising, as proteolysis is an early step in protein accumulation [49]. Interferon beta, which fosters the clearance of mATXN7 over the wild type form through the induction of PML-clastosomes, protects cultured rat primary Purkinje neurons [53, 54]. Interferon beta has been investigated in preclinical assay in $SCA7^{266Q/5Q}$ KI mice and treatment of asymptomatic mice significantly decreases mATXN7 aggregation and improves motor functions [54]. The treatment was not efficient enough to protect against weight loss and premature death, likely because this mouse model has a very severe disease course. Nevertheless, since interferon beta has been used for many years in the treatment of multiple sclerosis, it may hold promise as a potential treatment to delay motor symptoms in SCA7 patients.

One emerging strategy to prevent the expression of toxic polyQ proteins makes use of RNA interference (RNAi), a natural process of gene silencing mediated by small RNAs. RNAi is widely used for biological applications and is now being

harnessed to silence mRNAs encoding pathogenic proteins for therapy. As with any therapeutics, the clinical usefulness of RNAi will depend on its efficacy and safety. To this end, several issues were addressed in preclinical assays using mouse models. Furrer et al. [84] asked to which level mATXN7 must be suppressed in PrP-floxed-SCA7-92Q BAC mice to rescue the phenotype. The results indicate that a reduction of 50% even after the onset of motor phenotype, can prevent disease progression and achieve important amelioration of motor function, cerebellar neuropathology and mATXN7 aggregation. Another important issue concerns potential deleterious effects that could result from concomitant wild-type mRNA suppression. Lessons from zebrafish and fly indicated that partial inhibition of wild-type ATXN7 could affect the differentiation of photoreceptor and Purkinje neurons [41, 42]. Ramachandran et al. [85] utilized adeno-associated viral vectors to introduce miRNA in the deep cerebellar nucleus of Prp SCA7-92Q BAC mice and to test non allele specific silencing where both wild type mouse and mutated human ATXN7 were reduced by about 35–50%. The authors found a significant improvement of motor functions and cerebellar neuropathology, and reexpression of genes abnormally reduced in untreated mice. The non allele specific silencing appeared well tolerated and can be added to the list of similar strategies successfully developed in other polyQ disease models. Nevertheless, selective inhibition of the mutant transcript that would leave the wild-type one intact would be safer and is in theory feasible by exploiting differences between transcripts down to a single base pair; the RNAi sequence would have a complete homology to the mutant transcript and a single nucleotide mismatch with the wild type. In populations with strong founder effect such as South African, a common SNP linked to the SCA7 mutation was identified in 50% of SCA7 patients [86]. Using short-hairpin RNA targeting this polymorphism, allele-specific mATXN7 suppression was achieved in patients' cells [87]. Together, these studies provide the first proofs of efficacy of RNAi strategy to prevent mATXN7 expression. However, those are the initial stages of development and other challenges such as the clinical relevance of off-targets and inflammatory responses, the longevity of RNAi effect in the treatment of chronic neurodegenerative pathology, the brain or retina delivery approach, etc., need to be met. The substantial progress in using gene silencing for treating skin and retinal diseases, for instance, holds promise to bring RNAi technologies in clinic for SCA7 and other polyQ disorders.

9.6 Final Remarks

While biochemical approaches and the characterization of cellular and animal models of SCA7 have greatly advanced our understanding of disease pathogenesis in SCA7, much more needs to be learned before we get a solid comprehension of the pathogenic mechanisms underlying neuronal specific dysfunction and neuronal cell loss. While some of the therapeutic strategies against SCA7 are promising, as they can take first steps into clinical trials, further fundamental investigations are

required to propose new molecular targets for SCA7. Since SCA7 shares many common pathological features with other degenerative disorders affecting the cerebellum and the retina, identification of therapeutics in SCA7 or in one of these diseases is likely to be cross-beneficial.

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References

1. Trottier Y, Lutz Y, Stevanin G, Imbert G, Devys D, Cancel G, Saudou F, Weber C, David G, Tora L et al (1995) Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. Nature 378:403–406
2. David G, Durr A, Stevanin G, Cancel G, Abbas N, Benomar A, Belal S, Lebre AS, Abada-Bendib M, Grid D, Holmberg M, Yahyaoui M, Hentati F, Chkili T, Agid Y, Brice A (1998) Molecular and clinical correlations in autosomal dominant cerebellar ataxia with progressive macular dystrophy (SCA7). Hum Mol Genet 7:165–170
3. van de Warrenburg BP, Frenken CW, Ausems MG, Kleefstra T, Sinke RJ, Knoers NV, Kremer HP (2001) Striking anticipation in spinocerebellar ataxia type 7: the infantile phenotype. J Neurol 248:911–914
4. Michalik A, Martin JJ, Van Broeckhoven C (2004) Spinocerebellar ataxia type 7 associated with pigmentary retinal dystrophy. Eur J Hum Genet 12:2–15
5. Monckton DG, Cayuela ML, Gould FK, Brock GJ, Silva R, Ashizawa T (1999) Very large (CAG) n DNA repeat expansions in the sperm of two spinocerebellar ataxia type 7 males. Hum Mol Genet 8:2473–2478
6. Giunti P, Stevanin G, Worth PF, David G, Brice A, Wood NW (1999) Molecular and clinical study of 18 families with ADCA type II: evidence for genetic heterogeneity and de novo mutation. Am J Hum Genet 64:1594–1603
7. Johansson J, Forsgren L, Sandgren O, Brice A, Holmgren G, Holmberg M (1998) Expanded CAG repeats in Swedish spinocerebellar ataxia type 7 (SCA7) patients: effect of CAG repeat length on the clinical manifestation. Hum Mol Genet 7:171–176
8. Ansorge O, Giunti P, Michalik A, Van Broeckhoven C, Harding B, Wood N, Scaravilli F (2004) Ataxin-7 aggregation and ubiquitination in infantile SCA7 with 180 CAG repeats. Ann Neurol 56:448–452
9. Benton CS, de Silva R, Rutledge SL, Bohlega S, Ashizawa T, Zoghbi HY (1998) Molecular and clinical studies in SCA-7 define a broad clinical spectrum and the infantile phenotype. Neurology 51:1081–1086
10. Enevoldson TP, Sanders MD, Harding AE (1994) Autosomal dominant cerebellar ataxia with pigmentary macular dystrophy. A clinical and genetic study of eight families. Brain J Neurol 117(3):445–460
11. Trang H, Stanley SY, Thorner P, Faghfoury H, Schulze A, Hawkins C, Pearson CE, Yoon G (2014) Massive CAG repeat expansion and somatic instability in maternally transmitted infantile spinocerebellar ataxia type 7. JAMA Neurol

12. Whitney A, Lim M, Kanabar D, Lin JP (2007) Massive SCA7 expansion detected in a 7-month-old male with hypotonia, cardiomegaly, and renal compromise. Dev Med Child Neurol 49:140–143
13. Alcauter S, Barrios FA, Diaz R, Fernandez-Ruiz J (2011) Gray and white matter alterations in spinocerebellar ataxia type 7: an in vivo DTI and VBM study. Neuroimage 55:1–7
14. Bang OY, Lee PH, Kim SY, Kim HJ, Huh K (2004) Pontine atrophy precedes cerebellar degeneration in spinocerebellar ataxia 7: MRI-based volumetric analysis. J Neurol Neurosurg Psychiatry 75:1452–1456
15. Horton LC, Frosch MP, Vangel MG, Weigel-DiFranco C, Berson EL, Schmahmann JD (2013) Spinocerebellar ataxia type 7: clinical course, phenotype-genotype correlations, and neuropathology. Cerebellum 12:176–193
16. Martin J, Van Regemorter N, Del-Favero J, Lofgren A, Van Broeckhoven C (1999) Spinocerebellar ataxia type 7 (SCA7)—correlations between phenotype and genotype in one large Belgian family. J Neurol Sci 168:37–46
17. Rub U, Brunt ER, Seidel K, Gierga K, Mooy CM, Kettner M, Van Broeckhoven C, Bechmann I, La Spada AR, Schols L, den Dunnen W, de Vos RA, Deller T (2008) Spinocerebellar ataxia type 7 (SCA7): widespread brain damage in an adult-onset patient with progressive visual impairments in comparison with an adult-onset patient without visual impairments. Neuropathol Appl Neurobiol 34:155–168
18. Rub U, Brunt ER, Gierga K, Seidel K, Schultz C, Schols L, Auburger G, Heinsen H, Ippel PF, Glimmerveen WF, Wittebol-Post D, Arai K, Deller T, de Vos RA (2005) Spinocerebellar ataxia type 7 (SCA7): first report of a systematic neuropathological study of the brain of a patient with a very short expanded CAG-repeat. Brain Pathol 15:287–295
19. Benomar A, Leguern E, Durr A, Ouhabi H, Stevanin G, Yahyaoui M, Chkili T, Agid Y, Brice A (1994) Autosomal-dominant cerebellar-ataxia with retinal degeneration (ADCA type-II) is genetically different from ADCA type-I. Ann Neurol 35:439–444
20. Gouw LG, Digre KB, Harris CP, Haines JH, Ptacek LJ (1994) Autosomal-dominant cerebellar-ataxia with retinal degeneration—clinical, neuropathologic, and genetic-analysis of a large kindred. Neurology 44:1441–1447
21. Martin JJ, Van Regemorter N, Krols L, Brucher JM, de Barsy T, Szliwowski H, Evrard P, Ceuterick C, Tassignon MJ, Smet-Dieleman H et al (1994) On an autosomal dominant form of retinal-cerebellar degeneration: an autopsy study of five patients in one family. Acta Neuropathol (Berl) 88:277–286
22. Aleman TS, Cideciyan AV, Volpe NJ, Stevanin G, Brice A, Jacobson SG (2002) Spinocerebellar ataxia type 7 (SCA7) shows a cone-rod dystrophy phenotype. Exp Eye Res 74:737–745
23. Einum DD, Clark AM, Townsend JJ, Ptacek LJ, Fu YH (2003) A novel central nervous system-enriched spinocerebellar ataxia type 7 gene product. Arch Neurol 60:97–103
24. Helmlinger D, Hardy S, Sasorith S, Klein F, Robert F, Weber C, Miguet L, Potier N, Van-Dorsselaer A, Wurtz JM, Mandel JL, Tora L, Devys D (2004) Ataxin-7 is a subunit of GCN5 histone acetyltransferase-containing complexes. Hum Mol Genet 13:1257–1265
25. Duncan CE, An MC, Papanikolaou T, Rugani C, Vitelli C, Ellerby LM (2013) Histone deacetylase-3 interacts with ataxin-7 and is altered in a spinocerebellar ataxia type 7 mouse model. Mol Neurodegener 8:42
26. Janer A, Werner A, Takahashi-Fujigasaki J, Daret A, Fujigasaki H, Takada K, Duyckaerts C, Brice A, Dejean A, Sittler A (2010) SUMOylation attenuates the aggregation propensity and cellular toxicity of the polyglutamine expanded ataxin-7. Hum Mol Genet 19:181–195
27. Cancel G, Duyckaerts C, Holmberg M, Zander C, Yvert G, Lebre AS, Ruberg M, Faucheux B, Agid Y, Hirsch E, Brice A (2000) Distribution of ataxin-7 in normal human brain and retina. Brain J Neurol 123(12):2519–2530
28. Einum DD, Townsend JJ, Ptacek LJ, Fu YH (2001) Ataxin-7 expression analysis in controls and spinocerebellar ataxia type 7 patients. Neurogenetics 3:83–90
29. Einum DD, Gouw L, MAtilla A, Townsend J, Fu YH, Ptacek LJ (1999) Expression analysis of ataxin-7 reveals restricted localization of an alternate isoform. Am J Hum Genet 65:A104–A104

30. Jonasson J, Strom AL, Hart P, Brannstrom T, Forsgren L, Holmberg M (2002) Expression of ataxin-7 in CNS and non-CNS tissue of normal and SCA7 individuals. Acta Neuropathol 104:29–37
31. Lindenberg KS, Yvert G, Muller K, Landwehrmeyer GB (2000) Expression analysis of ataxin-7 mRNA and protein in human brain: evidence for a widespread distribution and focal protein accumulation. Brain Pathol 10:385–394
32. Mauger C, Del-Favero J, Ceuterick C, Lubke U, van Broeckhoven C, Martin J (1999) Identification and localization of ataxin-7 in brain and retina of a patient with cerebellar ataxia type II using anti-peptide antibody. Brain Res Mol Brain Res 74:35–43
33. Strom AL, Jonasson J, Hart P, Brannstrom T, Forsgren L, Holmberg M (2002) Cloning and expression analysis of the murine homolog of the spinocerebellar ataxia type 7 (SCA7) gene. Gene 285:91–99
34. Palhan VB, Chen S, Peng GH, Tjernberg A, Gamper AM, Fan Y, Chait BT, La Spada AR, Roeder RG (2005) Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration. Proc Natl Acad Sci USA 102:8472–8477
35. Sanders SL, Jennings J, Canutescu A, Link AJ, Weil PA (2002) Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TFIID by multidimensional mass spectrometry. Mol Cell Biol 22:4723–4738
36. Scheel H, Tomiuk S, Hofmann K (2003) Elucidation of ataxin-3 and ataxin-7 function by integrative bioinformatics. Hum Mol Genet 12:2845–2852
37. Wyce A, Henry KW, Berger SL (2004) H2B ubiquitylation and de-ubiquitylation in gene activation. Novartis Found Symp 259:63–73; discussion 73–67, 163–169
38. Bonnet J, Wang CY, Baptista T, Vincent SD, Hsiao WC, Stierle M, Kao CF, Tora L, Devys D (2014) The SAGA coactivator complex acts on the whole transcribed genome and is required for RNA polymerase II transcription. Genes Dev 28:1999–2012
39. Nakamura Y, Tagawa K, Oka T, Sasabe T, Ito H, Shiwaku H, La Spada AR, Okazawa H (2012) Ataxin-7 associates with microtubules and stabilizes the cytoskeletal network. Hum Mol Genet 21:1099–1110
40. Kahle JJ, Gulbahce N, Shaw CA, Lim J, Hill DE, Barabasi AL, Zoghbi HY (2011) Comparison of an expanded ataxia interactome with patient medical records reveals a relationship between macular degeneration and ataxia. Hum Mol Genet 20:510–527
41. Mohan RD, Dialynas G, Weake VM, Liu J, Martin-Brown S, Florens L, Washburn MP, Workman JL, Abmayr SM (2014) Loss of Drosophila ataxin-7, a SAGA subunit, reduces H2B ubiquitination and leads to neural and retinal degeneration. Genes Dev 28:259–272
42. Yanicostas C, Barbieri E, Hibi M, Brice A, Stevanin G, Soussi-Yanicostas N (2012) Requirement for zebrafish ataxin-7 in differentiation of photoreceptors and cerebellar neurons. PLoS ONE 7:e50705
43. Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23:217–247
44. Yoo SY, Pennesi ME, Weeber EJ, Xu B, Atkinson R, Chen S, Armstrong DL, Wu SM, Sweatt JD, Zoghbi HY (2003) SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant ataxin-7 in neurons and abnormalities in short-term plasticity. Neuron 37:383–401
45. Yvert G, Lindenberg KS, Devys D, Helmlinger D, Landwehrmeyer GB, Mandel JL (2001) SCA7 mouse models show selective stabilization of mutant ataxin-7 and similar cellular responses in different neuronal cell types. Hum Mol Genet 10:1679–1692
46. Garden GA, Libby RT, Fu YH, Kinoshita Y, Huang J, Possin DE, Smith AC, Martinez RA, Fine GC, Grote SK, Ware CB, Einum DD, Morrison RS, Ptacek LJ, Sopher BL, La Spada AR (2002) Polyglutamine-expanded ataxin-7 promotes non-cell-autonomous purkinje cell degeneration and displays proteolytic cleavage in ataxic transgenic mice. J Neurosci Official J Soc Neurosci 22:4897–4905
47. Yvert G, Lindenberg KS, Picaud S, Landwehrmeyer GB, Sahel JA, Mandel JL (2000) Expanded polyglutamines induce neurodegeneration and trans-neuronal alterations in cerebellum and retina of SCA7 transgenic mice. Hum Mol Genet 9:2491–2506

48. Young JE, Gouw L, Propp S, Sopher BL, Taylor J, Lin A, Hermel E, Logvinova A, Chen SF, Chen S, Bredesen DE, Truant R, Ptacek LJ, La Spada AR, Ellerby LM (2007) Proteolytic cleavage of ataxin-7 by caspase-7 modulates cellular toxicity and transcriptional dysregulation. J Biol Chem 282:30150–30160
49. Guyenet SJ, Mookerjee SS, Lin A, Custer SK, Chen SF, Sopher BL, La Spada AR, Ellerby LM (2015) Proteolytic cleavage of ataxin-7 promotes SCA7 retinal degeneration and neurological dysfunction. Hum Mol Genet 24:3908–3917
50. Mookerjee S, Papanikolaou T, Guyenet SJ, Sampath V, Lin A, Vitelli C, DeGiacomo F, Sopher BL, Chen SF, La Spada AR, Ellerby LM (2009) Posttranslational modification of ataxin-7 at lysine 257 prevents autophagy-mediated turnover of an N-terminal caspase-7 cleavage fragment. J Neurosci Official J Soc Neurosci 29:15134–15144
51. Takahashi J, Fujigasaki H, Zander C, El Hachimi KH, Stevanin G, Durr A, Lebre AS, Yvert G, Trottier Y, de The H, Hauw JJ, Duyckaerts C, Brice A (2002) Two populations of neuronal intranuclear inclusions in SCA7 differ in size and promyelocytic leukaemia protein content. Brain J Neurol 125:1534–1543
52. Takahashi J, Fujigasaki H, Iwabuchi K, Bruni AC, Uchihara T, El Hachimi KH, Stevanin G, Durr A, Lebre AS, Trottier Y, de The H, Tanaka J, Hauw JJ, Duyckaerts C, Brice A (2003) PML nuclear bodies and neuronal intranuclear inclusion in polyglutamine diseases. Neurobiol Dis 13:230–237
53. Janer A, Martin E, Muriel MP, Latouche M, Fujigasaki H, Ruberg M, Brice A, Trottier Y, Sittler A (2006) PML clastosomes prevent nuclear accumulation of mutant ataxin-7 and other polyglutamine proteins. J Cell Biol 174:65–76
54. Chort A, Alves S, Marinello M, Dufresnois B, Dornbierer JG, Tesson C, Latouche M, Baker DP, Barkats M, El Hachimi KH, Ruberg M, Janer A, Stevanin G, Brice A, Sittler A (2013) Interferon beta induces clearance of mutant ataxin 7 and improves locomotion in SCA7 knock-in mice. Brain J Neurol
55. Abou-Sleymane G, Chalmel F, Helmlinger D, Lardenois A, Thibault C, Weber C, Merienne K, Mandel JL, Poch O, Devys D, Trottier Y (2006) Polyglutamine expansion causes neurodegeneration by altering the neuronal differentiation program. Hum Mol Genet 15:691–703
56. Chou AH, Chen CY, Chen SY, Chen WJ, Chen YL, Weng YS, Wang HL (2010) Polyglutamine-expanded ataxin-7 causes cerebellar dysfunction by inducing transcriptional dysregulation. Neurochem Int 56:329–339
57. Helmlinger D, Hardy S, Abou-Sleymane G, Eberlin A, Bowman AB, Gansmuller A, Picaud S, Zoghbi HY, Trottier Y, Tora L, Devys D (2006) Polyglutamine-expanded ataxin-7 mediates aberrant recruitment of TFTC-type complexes and chromatin decondensation leading to photoreceptor dysfunction in Spinocerebellar ataxia type 7. PLoS Biol 4:e67
58. La Spada AR, Fu YH, Sopher BL, Libby RT, Wang X, Li LY, Einum DD, Huang J, Possin DE, Smith AC, Martinez RA, Koszdin KL, Treuting PM, Ware CB, Hurley JB, Ptacek LJ, Chen S (2001) Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. Neuron 31:913–927
59. McMahon SJ, Pray-Grant MG, Schieltz D, Yates JR 3rd, Grant PA (2005) Polyglutamine-expanded spinocerebellar ataxia-7 protein disrupts normal SAGA and SLIK histone acetyltransferase activity. Proc Natl Acad Sci USA 102:8478–8482
60. Kizilyaprak C, Spehner D, Devys D, Schultz P (2011) The linker histone H1C contributes to the SCA7 nuclear phenotype. Nucleus 2:444–454
61. Chen YC, Gatchel JR, Lewis RW, Mao CA, Grant PA, Zoghbi HY, Dent SY (2012) Gcn5 loss-of-function accelerates cerebellar and retinal degeneration in a SCA7 mouse model. Hum Mol Genet 21:394–405
62. McCullough SD, Xu X, Dent SY, Bekiranov S, Roeder RG, Grant PA (2012) Reelin is a target of polyglutamine expanded ataxin-7 in human spinocerebellar ataxia type 7 (SCA7) astrocytes. Proc Natl Acad Sci USA 109:21319–21324

63. Yang H, Liu S, He WT, Zhao J, Jiang LL, Hu HY (2015) Aggregation of polyglutamine-expanded ataxin 7 protein specifically sequesters ubiquitin-specific protease 22 and deteriorates its deubiquitinating function in the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex. J Biol Chem 290:21996–22004
64. Lan X, Koutelou E, Schibler AC, Chen YC, Grant PA, Dent SY (2015) Poly(Q) expansions in ATXN7 affect solubility but not activity of the SAGA deubiquitinating module. Mol Cell Biol 35:1777–1787
65. Tan JY, Vance KW, Varela MA, Sirey T, Watson LM, Curtis HJ, Marinello M, Alves S, Steinkraus BR, Cooper S, Nesterova T, Brockdorff N, Fulga TA, Brice A, Sittler A, Oliver PL, Wood MJ, Ponting CP, Marques AC (2014) Cross-talking noncoding RNAs contribute to cell-specific neurodegeneration in SCA7. Nat Struct Mol Biol 21:955–961
66. Strom AL, Forsgren L, Holmberg M (2005) A role for both wild-type and expanded ataxin-7 in transcriptional regulation. Neurobiol Dis 20:646–655
67. Ajayi A, Yu X, Lindberg S, Langel U, Strom AL (2012) Expanded ataxin-7 cause toxicity by inducing ROS production from NADPH oxidase complexes in a stable inducible spinocerebellar ataxia type 7 (SCA7) model. BMC Neurosci 13:86
68. Yefimova MG, Messaddeq N, Karam A, Jacquard C, Weber C, Jonet L, Wolfrum U, Jeanny JC, Trottier Y (2010) Polyglutamine toxicity induces rod photoreceptor division, morphological transformation or death in Spinocerebellar ataxia 7 mouse retina. Neurobiol Dis 40:311–324
69. Forsgren L, Libelius R, Holmberg M, von Dobeln U, Wibom R, Heijbel J, Sandgren O, Holmgren G (1996) Muscle morphology and mitochondrial investigations of a family with autosomal dominant cerebellar ataxia and retinal degeneration mapped to chromosome 3p12–p21.1. J Neurol Sci 144:91–98
70. Chen S, Peng GH, Wang X, Smith AC, Grote SK, Sopher BL, La Spada AR (2004) Interference of Crx-dependent transcription by ataxin-7 involves interaction between the glutamine regions and requires the ataxin-7 carboxy-terminal region for nuclear localization. Hum Mol Genet 13:53–67
71. Marc RE, Jones BW (2003) Retinal remodeling in inherited photoreceptor degenerations. Mol Neurobiol 28:139–147
72. Merienne K, Helmlinger D, Perkin GR, Devys D, Trottier Y (2003) Polyglutamine expansion induces a protein-damaging stress connecting heat shock protein 70 to the JNK pathway. J Biol Chem 278:16957–16967
73. Merienne K, Friedman J, Akimoto M, Abou-Sleymane G, Weber C, Swaroop A, Trottier Y (2007) Preventing polyglutamine-induced activation of c-Jun delays neuronal dysfunction in a mouse model of SCA7 retinopathy. Neurobiology of disease 25:571–581
74. Custer SK, Garden GA, Gill N, Rueb U, Libby RT, Schultz C, Guyenet SJ, Deller T, Westrum LE, Sopher BL, La Spada AR (2006) Bergmann glia expression of polyglutamine-expanded ataxin-7 produces neurodegeneration by impairing glutamate transport. Nat Neurosci 9:1302–1311
75. Noma S, Ohya-Shimada W, Kanai M, Ueda K, Nakamura T, Funakoshi H (2012) Overexpression of HGF attenuates the degeneration of Purkinje cells and Bergmann glia in a knockin mouse model of spinocerebellar ataxia type 7. Neurosci Res 73:115–121
76. Furrer SA, Mohanachandran MS, Waldherr SM, Chang C, Damian VA, Sopher BL, Garden GA, La Spada AR (2011) Spinocerebellar ataxia type 7 cerebellar disease requires the coordinated action of mutant ataxin-7 in neurons and glia, and displays non-cell-autonomous Bergmann glia degeneration. J Neurosci Official J Soc Neurosci 31:16269–16278
77. Fern RF, Matute C, Stys PK (2014) White matter injury: ischemic and nonischemic. Glia 62:1780–1789
78. Lee SG, Su ZZ, Emdad L, Gupta P, Sarkar D, Borjabad A, Volsky DJ, Fisher PB (2008) Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. J Biol Chem 283:13116–13123

79. Maltecca F, Baseggio E, Consolato F, Mazza D, Podini P, Young SM Jr, Drago I, Bahr BA, Puliti A, Codazzi F, Quattrini A, Casari G (2015) Purkinje neuron Ca^{2+} influx reduction rescues ataxia in SCA28 model. J Clin Invest 125:263–274
80. Chew LJ, DeBoy CA (2015) Pharmacological approaches to intervention in hypomyelinating and demyelinating white matter pathology. Neuropharmacology
81. Murakami Y, Notomi S, Hisatomi T, Nakazawa T, Ishibashi T, Miller JW, Vavvas DG (2013) Photoreceptor cell death and rescue in retinal detachment and degenerations. Prog Retin Eye Res 37:114–140
82. Ieraci A, Forni PE, Ponzetto C (2002) Viable hypomorphic signaling mutant of the Met receptor reveals a role for hepatocyte growth factor in postnatal cerebellar development. Proc Natl Acad Sci USA 99:15200–15205
83. Sun W, Funakoshi H, Nakamura T (2002) Overexpression of HGF retards disease progression and prolongs life span in a transgenic mouse model of ALS. J Neurosci Official J Soc Neurosci 22:6537–6548
84. Furrer SA, Waldherr SM, Mohanachandran MS, Baughn TD, Nguyen KT, Sopher BL, Damian VA, Garden GA, La Spada AR (2013) Reduction of mutant ataxin-7 expression restores motor function and prevents cerebellar synaptic reorganization in a conditional mouse model of SCA7. Hum Mol Genet 22:890–903
85. Ramachandran PS, Boudreau RL, Schaefer KA, La Spada AR, Davidson BL (2014) Nonallele specific silencing of ataxin-7 improves disease phenotypes in a mouse model of SCA7. Mol Ther 22:1635–1642
86. Greenberg J, Solomon GA, Vorster AA, Heckmann J, Bryer A (2006) Origin of the SCA7 gene mutation in South Africa: implications for molecular diagnostics. Clin Genet 70: 415–417
87. Scholefield J, Watson L, Smith D, Greenberg J, Wood MJ (2014) Allele-specific silencing of mutant Ataxin-7 in SCA7 patient-derived fibroblasts. Eur J Hum Genet 22:1369–1375

Chapter 10
Spinocerebellar Ataxia Type 17 (SCA17)

Yasuko Toyoshima and Hitoshi Takahashi

Abstract In 1999, a polyglutamine expansion was identified in the transcription factor TATA-binding protein (TBP) in a patient with ataxia with negative family history. Subsequently, CAG/CAA repeat expansions in the TBP gene were identified in families with spinocerebellar ataxia (SCA), establishing this repeat expansion as the underlying mutation in SCA type 17 (SCA17). There are several characteristic differences between SCA17 and other polyglutamine diseases. First, SCA17 shows a complex and variable clinical phenotype, in some cases overlapping that of Huntington's disease. Second, compared to the other SCA subtypes caused by expanded trinucleotide repeats, anticipation in SCA17 kindreds is rare because of the characteristic structure of the TBP gene. And thirdly, SCA17 patients often have diagnostic problems that may arise from non-penetrance. Because the gap between normal and abnormal repeat numbers is very narrow, it is difficult to determine a cutoff value for pathologic CAG repeat number in SCA17. Herein, we review the clinical, genetic and pathologic features of SCA17.

Keywords Spinocerebellar ataxia · Huntington's disease-like · Chorea
Dystonia · Dementia

10.1 Clinical Features

SCA17 is an autosomal dominant cerebellar ataxia caused by abnormal expansion of a CAG/CAA repeat encoding a polyglutamine (polyQ) tract in the TATA-box binding protein (TBP) gene on chromosome 6q [1–4]. Though the genetic abnormalities are mostly observed as a hereditary trait, de novo mutations have also been reported [2, 5, 6].

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The clinical symptoms of SCA17 are mainly ataxia and/or dementia, as is the case for other forms of autosomal dominant SCA [7]. However, SCA17 is a more complex disease with extensive phenotypic variability, and the age at onset spans several decades. Clinical heterogeneity can be observed even within the same family [8]. In the present literature review of SCA17 patients (Table 10.1), the age at onset ranged from 3 to 60 years, and about half of the patients developed ataxia as the initial symptom. The age at onset appeared to correlate weakly with the number of repeats (Fig. 10.1). During the disease course, most of the patients (>90%) developed ataxia, which was manifested as gait instability, and slurred speech. Cognitive dysfunction and memory disturbance have also been recognized as an initial symptom [9]; dementia is the second most common symptom (73%) during the disease course. In childhood, mental deterioration may occur instead of dementia. Psychiatric symptoms, such as aggression [10], paranoia [1], euphoria [11] and depression [12, 13] are observed frequently. Behavior or personality changes as initial symptoms may indicate the presence of psychiatric disorders.

Involuntary movement is one of the characteristic features of SCA17 [14, 15]. As chorea is a well-known symptom of SCA 17, the clinical phenotype sometimes overlaps that of Huntington's disease (HD), being characterized by the triad of movement disorder, psychiatric manifestations and cognitive impairment [16]. In many cases of clinically suspected HD, patients lack the CAG repeat expansion that causes HD. Such individuals are said to have HD phenocopy syndromes or HD-like disorders [17–23]. SCA17 has therefore also been termed Huntington's disease-like 4 (HDL4; OMIM #607136) [24]. Wild et al. [21] identified gene abnormalities in 285 HD phenocopy patients in the United Kingdom. Among the patients, five (1.8%) were found to have expansions in *TBP* causing SCA 17. One patient (0.4%) had a 6-octapeptide insertion in the prion protein gene (*PRNP*) [25], and one had HDL2 caused by a pathogenic expansion in the janctofillin gene (*JPH3*) [26]. In addition, one patient was diagnosed later as having Friedreich's ataxia with homozygous expansion in the flataxin gene (*FXN*) [27]. Moss et al. [28] recently reported that ten (1.95%) of 514 HD phenocopy patients had an expanded hexanucleotide repeat in the C9orf72 gene. If patients with HD-like disease have no mutations in huntingtin, the TBP and C9orf72 genes should be examined.

We have previously examined the relationship between repeat number and clinical symptoms (Toyoshima et al. 1993), and found that more than 75% of patients with a CAG/CAA repeat size of 43–50 had intellectual deterioration; in some individuals, intellectual problems and involuntary movements were the only signs. Psychiatric problems or dementia, parkinsonism and chorea, a clinical constellation resembling Huntington disease, are observed more frequently in individuals with CAG/CAA repeats in this range than in those with larger repeats. All individuals showing a CAG/CAA repeat size of 50–60 have ataxia and 75% have reduced intellectual function. Pyramidal signs (e.g., increased deep tendon reflexes) and dystonia are more common in these individuals than in those with smaller repeats. These features were also confirmed in the present literature review (Table 10.1). Two children with over 60 repeats have been reported. One, a familial example, with a CAG/CAA expansion of 66 repeats developed gait disturbance at

Table 10.1 Summary of the clinical features in the SCA17 patients

sex	age at onset	initial symptom	ataxia	dementia	involuntary movement	pyramidal signs	extra pyramidal signs	psychiatric symptoms	CAG/CAA expansion	references
F	60	chorea	–	nc	chorea	–	nc	depression	41	Park et al. [44]
F	35	hand discoordination	nc	nc	chorea, trunk titubation	+	+	depression	41	Herrera et al. [12]
M	59	slurred speech	+	nc	poly-mini-myoclonus	–	–	–	41	Doherty et al. [32]
M	50	ataxia	+	+	nc	+	nc	nc	41	Nanda et al. [42]
M	42	reduced speed working	+	++	–	+	+	nc	43	Nielsen et al. [9]
F	29	gait instability,behavioral change	++	+	chorea	+	–	nc	44	Stevanin et al. [14]
M	48	gait instability	+	+++	dyskinesia	nc	nc	nc	44	Mariotti et al. [13]
F	40	gait instability	+	nc	nc	nc	nc	introvert	44	
F	36	gait instability	+	+	nc	nc	nc	nc	44	
M	41	behavioral change	+	+	dyskinesia	+	nc	nc	45	
F	38	depression	nc	+++	nc	+++	nc	depression	45	
F	40	gait instability	+	+	dyskinesia	nc	nc	nc	45	
M	34	gait instability	+	–	dyskinesia, tremor	+	nc	nc	45	
M	55	chorea	+	nc	chorea, dyskinesia	nc	nc	nc	45	
F	52	gait instability	+	+	dyskinesia	nc	nc	nc	45	
M	55	gait instability	+	+	nc	nc	nc	nc	45	
M	nc	ataxia, urinary dist.	+++	+	–	+	++	euphoria	45	Lin et al. [11]
F	30	speech dist., depression	+	nc	nc	nc	++	depression	45	Rolfs et al. [31]
M	43	gait ataxia	+	nc	nc	nc	nc	depression	45	
F	30	depression	+	+	dystonia, chorea	+	nc	depression, aggression, paranoia	45	
M	23	speech dist, gait ataxia	+	nc	nc	+	nc	–	45	
M	19	behavioral change	+	+	dyskinesia	nc	nc	nc	46	Mariotti et al. [13]
F	44	gait instability, fall, IVM	+	++	chorea, myoclonus	–	+	depression, aggression	46	Schneider et al. [20]
M	37	gait instability, fall, IVM	+	++	chorea, myoclonus	nc	nc	depression, aggression	46	
F	45	gait instability, fall, IVM	+	++	chorea, myoclonus	nc	nc	depression, aggression	46	
F	23	behavioral change, chorea	+	+	chorea	+	+	nc	46	Stevanin et al. [14]
M	37	paranoia psychosis	+	+	choreoathetosis	+	–	paranoia, psychosis	46	Fujigasaki et al. [1]
F	55	ataxia, dementia	+	+	–	+	+	nc	46	
nc	40	nc	+	+	chorea	+	+	nc	47	Nakamura et al. [3]
nc	28	nc	–	+	–	+	+	nc	47	
nc	39	nc	+	+	dystonia	+	+	nc	48	
nc	48	nc	+	+	chorea	+	+	nc	48	
F	38	depression	–	nc	nc	nc	nc	hallcination, suicide attempts	49	Rolfs et al. [31]
F	16	cognitive decline, gait ataxia	+	+++	nc	nc	nc	nc	49	
F	48	chorea	+	nc	chorea	nc	+	paranoia	50	Mariotti et al. [13]
M	55	behavioral change	+	+	dystonia, dyskinesia	nc	nc	nc	50	
M	13	mental deterioration	+	+	–	+	+	nc	51	Zühlke et al. [4]
M	35	ataxia	+	+	nc	nc	nc	nc	52	Belluzzo et al. [30]
F	35	depression	+	+	dyskinesia	nc	nc	depression	52	Mariotti et al. [13]
F	25	paranoia	nc	+++	nc	+++	nc	paranoia	53	
F	35	ataxia	+	+++	dystonia	nc	+	++	53	Maltecca et al. [29]
M	34	ataxia	+	++	dystonia	nc	nc	++	53	
M	23	ataxia	+	++	dystonia	+	+	++	53	
F	34	ataxia, dystonic posture, personality change	+	+	dystonia	++	++	mood change	53	Zühlke et al. [4]
F	34	personality change	+	+	nc	+	+	euphoria	53	Fujigasaki et al. [1]
F	25	gait ataxia	+	++	dystonia, chorea	+	+	nc	54	Koutsis et al. [8]
F	22	gait ataxia	+	+	nc	–	+	depression	54	
F	25	speech dist., memory dist.	+++	+	dystonia	++	++	nc	54*	Bech et al. [5]
M	36	ataxia, psychiatric sign	+	+++	dystonia, chorea	+	nc	aggression, paranoia	54	Rolfs et al. [31]
M	18	ataxia, dementia	+++	++	nc	+	nc	nc	54	
F	23	hallucination	++	+	dystonia, chorea	+	nc	mania	54	
F	18	speech dist.	+	nc	nc	nc	nc	–	54	
M	18	ataxia	+	nc	dystonia	nc	nc	–	54	
nc	20	cognitive dysfunction	+	++	nc	nc	nc	nc	55*	Wu et al. [74]
F	20	dystonic mov. of fingers	+	–	dystonia torcicollis	–	–	nc	55	Zühlke et al. [4]
nc	19	nc	+	+	dystonia	+	+	nc	55	Nakamura et al. [3]
nc	25	nc	+	+	dystonia	+	+	nc	55	
F	6	mental deterioration, gait dist.	+	++	–	+	–	nc	63*	Koide et al. [2]
F	3	ataxia	+	+++	dystonia	+	+	++	66	Maltecca et al. [29]

-, unaffected. +, mild. ++, moderate. +++, severe. nc = not commented, IVM = involuntary movement

Shadowed columns; gray = over 50 CAG/CAA repeats, patients having chorea (red), dystonia (green), and the both (blue). *de novo mutation. References column filled with the first author's name

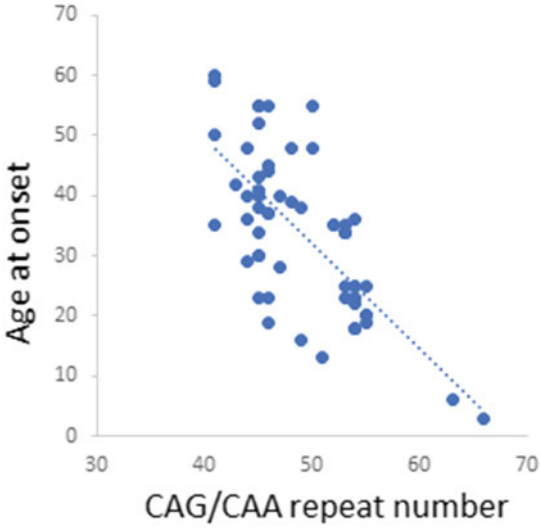

Fig. 10.1 Correlation between age at onset (years) and CAA/CAG repeat number on the expanded allele

the age of 3 years followed by spasticity, dementia and psychiatric symptoms [29]. The other, with a de novo CAG/CAA expansion of 63 repeats, developed ataxia and intellectual deterioration at the age of 6 years, followed later by spasticity [2].

Less common symptoms reported are epilepsy [1–3, 13, 29–31] (20%), autonomic symptoms [4, 11, 31, 32] (9%), apraxia [31] (7%) and peripheral nerve symptoms [12, 13, 29] (3%). Lin et al. [11] reported a patient who developed ophthalmoplegia with parkinsonism, and Rolfs et al. [31] reported a patient showing hypogonadism.

Neuroradiological examination has been reported to demonstrate several characteristic features. In early reports, some degree of cerebellar and cerebral atrophy was shown on MRI [1, 3]. Later, putaminal rim hyperintensity on T2-weighted images was also reported [33]. Striatal hyperintensity on MRI has been reported in a range of disease conditions, including SCA2 [34], dentatorubral-pallidoluysian atrophy (DRPLA) [35], neuroacanthocytosis [36], mitochondrial cytopathy [37] and multiple system atrophy (MSA) [38, 39]. Functional imaging using radioisotopes is helpful for understanding the pathophysiological conditions in the basal ganglia. Günther et al. [40] measured striatal pre-synaptic dopamine transporter (DAT) availability and striatal dopamine D2 receptor (D2R) expression using [^{123}I]FP-CIT and [^{123}I]IBZM, respectively, applying a brain-dedicated single-photon emission computed tomography (SPECT) system; [^{18}F] Fluordeoxyglucose positron emission tomography scanning (PET) was also used for measurement of glucose metabolism. They found that the striatum had a reduction in the availability of presynaptic dopamine transporters, although postsynaptic dopamine D2 receptor binding capacity was reduced only slightly, whereas marked reduction of glucose metabolism was evident in the basal ganglia. Lin et al. [11] reported a patient with parkinsonism partially responsive to L-dopa administration, in whom presynaptic degeneration of the nigrostriatal dopaminergic system was manifested by a marked decrease of dopa uptake in the striatum, as demonstrated by [^{18}F]-6-fluorodopa PET.

10.2 Genetic Cause and Penetrance of SCA17

SCA17 is caused by abnormal expansion of the TBP gene on chromosome 6q. TBP is a transcription factor with a polyglutamine tract encoded by the CAG/CAA sequence [1–3]. The normal repeat range reported is from 25 up to 42–45 units, varying among studies. The highest number of repeats reported to date is 66 [29]. Expansions of between 41 and 49 repeats may constitute an intermediate range with incomplete penetrance. In our previous review [41], the pathologic number of repeats was 43 or more. However, there have been reports of patients with 41 repeats, presenting with progressive cerebellar ataxia and/or involuntary movements [12, 32, 42], late-onset chorea and psychiatric symptoms [43] and parkinsonism with chorea [44]. On the other hand, healthy individuals with 45 [45] and 49 repeats [46] have also been recognized. Because the gap between normal and abnormal numbers of repeats is very narrow, it is difficult to determine the cutoff value for the pathologic number of CAG repeats in SCA17. Shin et al. [47] reported very interesting results from large genetic study conducted in Korea. They examined 2099 patients (classified by dominant clinical phenotype: parkinsonism, n = 1706; ataxia, n = 345; chorea, n = 37; and dystonia, n = 11) and 522 normal controls, and reported that 64 patients had 42 repeats or less (3%) in the TBP gene. Forty-five repeats were the greatest number in normal populations. They recommended that 41 through 45 repeats should be considered as the intermediate range, requiring cautious interpretation.

Compared to the other SCA subtypes caused by expanded trinucleotide repeats, anticipation [48] is rare in SCA17 kindreds because interruption of CAA within the CAG repeat configuration of the TBP gene stabilizes the microsatellite [49, 50]. The allele basic structure is (CAG)3 (CAA)3 (CAG)n CAA CAG CAA (CAG)n CAA CAG. However, when the basic structure is broken, anticipation may be observed [50]. Maltecca et al. [29] reported a family showing marked anticipation. PCR analysis of the CAG repeat region within the TBP gene in the third generation showed a 300-base-pair (bp) band (53 CAG/CAA repeats), whereas the patient in the fourth generation showed a band of ~330 bp (66 repeats). The allele structure of the father was (CAG)3 (CAA)4 (CAG)44 CAA CAG, and that of the daughter was (CAG)3 (CAA)4 (CAG)57 CAA CAG. The basic allele structure was not conserved in patients whose alleles had a simplified pattern with loss of CAA interruptions, possibly leading to a reduction of repeat stability.

We previously reported a patient having 48 CAA/CAG repeats as a homozygous state [15]. The clinical features of that patient essentially did not differ from those of heterozygotes, although homozygosity might have exerted some influence on the severity and progression of dementia. Zühlke et al. [51] also reported a patient who had 47 homozygous glutamine residues caused by apparent partial isodisomy 6. Compared with the heterozygote, the patient had no apparent differences in clinical features.

Almost all SCA17 patients have a family history and the disease is inherited as an autosomal dominant trait. However, a few cases develop de novo mutations in

the TBP gene. One example is the child with 63 repeats mentioned above [2]. In addition, [5] reported a 33-year-old woman presenting with a HD-like phenotype with a de novo 54 CAG/CAA repeat expansion, and Wu et al. (2004) described a de novo 55 CAG repeat expansion in a patient with a Pakinson's disease phenotype. The presence of such cases indicates that even in the absence of a positive family history, genetic testing for SCA17 should be considered in patients with a HD-like, or PD-like, phenocopy.

10.3 Pathological Findings

Several reports have described the pathological findings in SCA17 patients [1, 52]. We previously reported a homozygous patient focusing on the histopathological findings; the patient did not show earlier onset of the disease than heterozygotes reported with similar CAA/CAG-repeat sizes, and the pathological features appeared to be very similar, if not identical, to those described for heterozygotes [15].

The patient died at the age of 49 years, about 6 years after disease onset. The main symptoms were dementia and choreic movement. At autopsy, the brain showed mild atrophy in the caudate nucleus and putamen (Fig. 10.2). Histologically, mild neuronal loss and gliosis were observed in the cerebral cortex, especially the deep layers. Neuronal loss was moderate in the neostriatum, affecting both small and large neurons, and in the Purkinje cell layer with Bergmann gliosis (Fig. 10.3). Mild neuronal loss was also evident in the cornu ammonis region 1 (CA1) of Ammon's horn, subiculum, parahippocampal gyrus, substantia nigra, brainstem reticular formation, and inferior olivary nucleus. It is known that the formation of neuronal intranuclear inclusions (NIIs) is a common hallmark of the CAG repeat diseases [53]. In the above SCA17 patient, immunohistochemistry showed that expanded polyQ stretches had accumulated in the neuronal nuclei in a diffuse pattern [54, 55] (Fig. 10.4a), and no labeling was detected in their cytoplasm or in the neuropil. NII formation was rare (<1%) in affected neurons and was restricted to brain regions

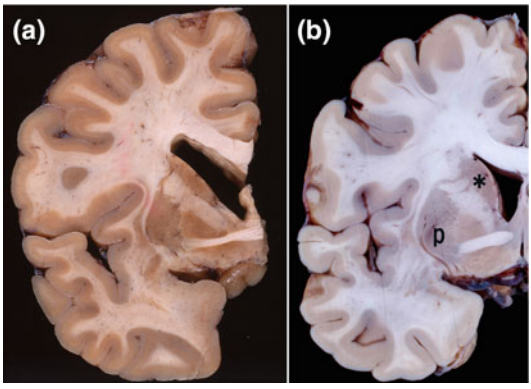

Fig. 10.2 Coronal section of the patient (**a**) showing mild atrophy of the caudate nucleus (*) and putamen (p). (**b**) Age matched control

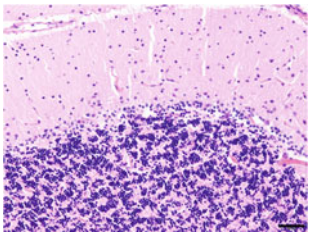

Fig. 10.3 There is moderate loss of Purkinje cells with Bergmann gliosis in the cerebellum. The granule cells are well preserved. Hematoxylin and eosin. Scale bar = 50 μm

such as the cerebral cortex (Fig. 10.4b), putamen, and midbrain reticular formation. The intranuclear diffuse accumulation of polyQ involved many neurons in a wide range of CNS regions far beyond the lesion distribution assessed by neuronal loss. Regions in which more than 40% of neurons were 1C2- immunoreactive included the sixth layer of the cerebral cortex (Fig. 10.5a), neostriatum, hippocampal formation (CA1 and subiculum) (Fig. 10.5b) and Purkinje cell layer. Of note was the high frequency (>60%) of nuclear 1C2 immunoreactivity in the large neurons of the neostriatum. Brain regions showing a frequency of 20–40% included the cerebral cortical layers III and VI, subthalamic nucleus, and inferior olive. In the white matter, a few glial nuclei also were immunolabeled for 1C2. No positive staining was observed in the visceral organs.

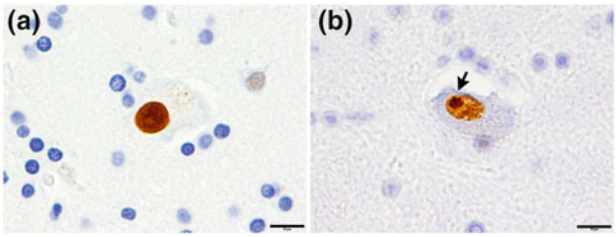

Fig. 10.4 The neuronal nuclei are often stained diffusely by 1C2 (**a**). There are a few neurons showing intra nuclear inclusion (**b**, arrow). **a** putamen. **b** brainstem reticular formation. Immunohistochemistry with a monoclonal antibody (1C2). Scale bar = 10 μm

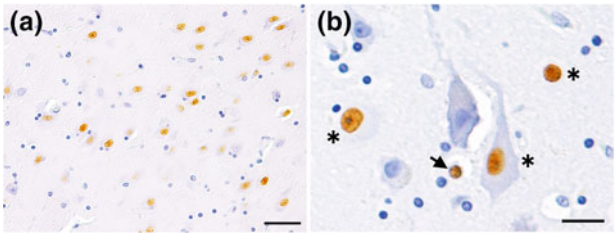

Fig. 10.5 There are many 1C2-positive nuclei in the subiculum of the hippocampal formation (**a**). 1C2-positive neuronal nuclei in the neurons (*) and an oligodendrocyte (arrow) (**b**, frontal cortex). Immunohistochemistry with 1C2. Scale bar = **a** 50 μm, **b** 20 μm

Intranuclear accumulation of mutant proteins has been recognized in many polyQ-disease brains of humans [56, 57] and transgenic mice [54]. The wide neuronal intranuclear distribution of the mutant proteins in the CNS far beyond the lesions assessed by neuronal loss must be important when considering the clinical and pathological correlations in polyQ diseases, including SCA17 [54, 56, 57].

10.4 Pathophysiological Reviews

The molecular mechanisms responsible for the pathogenesis of the polyQ diseases have not yet been completely explained [58, 59]. PolyQ expansion renders the protein more prone to aggregation and formation of inclusion bodies that are a pathological hallmark of polyglutamine diseases [60, 61]. Early discussions focused on whether the tendency of mutant polyQ proteins to aggregate was responsible for the disease-associated neurodegeneration (gain of function). Yet, several studies have indicated that disease severity can be disassociated from the presence of inclusions [62–64]. Moreover, there are data indicating that inclusions may be protective, perhaps as a result of sequestration of the mutant protein [62, 65, 66].

Several disease models, including cells [67, 68], *Drosophila* [59, 69, 70] and rodents [71–74], have been generated to clarify the pathomechanisms of SCA17. Overexpression of full-length-mutant TBP and truncated-mutant TBP lacking the DNA-binding domains (DBDs) was found to cause formation of inclusions, suggesting that insoluble aggregates are causative factors and that the neurotoxicity of mutant TBP is independent of DNA binding [69]. Thus, the pathogenesis of SCA17 seems similar to that of other polyQ diseases [75]. On the other hand, whether or not expanded polyQ tracts affect the function of TBP has yet to be comprehensively addressed. TBP is a general transcription factor [76, 77] essential for formation of the transcription preinitiation complex and transcription of RNA polymerases I, II and III (Pol I, II and III). Aberrant TBP activity is expected to profoundly affect normal cellular function. Inactivation of TBP in mice causes downregulation of Pol I and Pol III transcription, growth arrest and cell death [78]. Friedmann et al. [79] have reported that polyQ expansion reduces in vitro binding of TBP to DNA. Furthermore, they observed that polyQ-expanded TBP fragments, which were incapable of binding DNA, formed nuclear inclusions and caused a severe neurological phenotype in transgenic mice. Huang et al. [80] reported muscle dysfunction in a knock-in mouse model that had long polyQ repeats. They considered that decreased interaction between mutant TBP and MyoD, a muscle-specific transcription factor [81, 82], might affect the association between MyoD and the DNA promoter, thus reducing its transcriptional activity. Hsu et al. [69] recently reported that deactivation of TBP may contribute to SCA17 pathogenesis. They generated novel *Drosophila* models for SCA17 that overexpressed polyQ-expanded TBP, and demonstrated neurotoxic aggregates, the mutant TBP sequestering wild-type TBP in the neuroblasts of the flies. Moreover, they generated *Drosophila* mutants with loss of TDP (dTbp) to examine whether the neurodegeneration was the same as that

of the SCA-17 model flies. They confirmed that loss of TDP function caused age-associated neurodegeneration in *Drosophila*. Interestingly, they reported that dTbp expression exacerbated retinal degeneration induced in polyQ-expanded SCA3 and Huntington's disease fly models. These findings suggest that dysfunction of TBP may play a universal role in polyQ-induced neurodegeneration. Therefore, it is very significant to study the pathophysiology of SCA17 to clarify the causes of other polyglutamine diseases.

References

1. Fujigasaki H, De Martin JJ, Deyn PP, Camuzat A, Deffond D, Stevanin G, Van Dermaut B, Broeckhoven C, Durr A, Brice A (2001) CAG repeat expansion in the TATA box-binding protein gene causes autosomal dominant cerebellar ataxia. Brain 124:1939–1947
2. Koide R, Kobayashi S, Shimohata T, Ikeuchi T, Maruyama M, Saito M, Yamada M, Takahashi H, Tsuji S (1999) A neurological disease caused by an expanded CAG trinucleotide repeat in the TATA-binding protein gene: a new polyglutamine disease? Hum Mol Genet 8:2047–2053
3. Nakamura K, Jeong SY, Uchihara T, Anno M, Nagashima K, Nagashima T, Ikeda S, Tsuji S, Kanazawa I (2001) SCA17, a novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. Hum Mol Genet 10:1441–1448
4. Zuhlke C, Hellenbroich Y, Dalski A, Kononowa N, Hagenah J, Vieregge P, Riess O, Klein C, Schwinger E (2001) Different types of repeat expansion in the TATA-binding protein gene are associated with a new form of inherited ataxia. Eur J Hum Genet 9:160–164
5. Bech S, Petersen T, Norremolle A, Gjedde A, Ehlers L, Eiberg H, Hjermind LE, Hasholt L, Lundorf E, Nielsen JE (2010) Huntington's disease-like and ataxia syndromes: identification of a family with a de novo SCA17/TBP mutation. Parkinsonism Relat Disord 16:12–15
6. Shatunov A, Fridman EA, Pagan FI, Leib J, Singleton A, Hallett M, Goldfarb LG (2004) Small de novo duplication in the repeat region of the TATA-box-binding protein gene manifest with a phenotype similar to variant Creutzfeldt-Jakob disease. Clin Genet 66:496–501
7. Schols L, Bauer P, Schmidt T, Schulte T, Riess O (2004) Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 3:291–304
8. Koutsis G, Panas M, Paraskevas GP, Bougea AM, Kladi A, Karadima G, Kapaki E (2014) From mild ataxia to huntington disease phenocopy: the multiple faces of spinocerebellar ataxia 17. Case Rep Neurol Med 2014:643289
9. Nielsen TT, Mardosiene S, Lokkegaard A, Stokholm J, Ehrenfels S, Bech S, Friberg L, Nielsen JK, Nielsen JE (2012) Severe and rapidly progressing cognitive phenotype in a SCA17-family with only marginally expanded CAG/CAA repeats in the TATA-box binding protein gene: a case report. BMC Neurol 12:73
10. van de Schneider SA, Warrenburg BP, Hughes TD, Davis M, Sweeney M, Wood N, Quinn NP, Bhatia KP (2006) Phenotypic homogeneity of the Huntington disease-like presentation in a SCA17 family. Neurology 67:1701–1703
11. Lin IS, Wu RM, Lee-Chen GJ, Shan DE, Gwinn-Hardy K (2007) The SCA17 phenotype can include features of MSA-C, PSP and cognitive impairment. Parkinsonism Relat Disord 13:246–249
12. Herrema H, Mikkelsen T, Robin A, LeWitt P, Sidiropoulos C (2014) SCA 17 phenotype with intermediate triplet repeat number. J Neurol Sci 345:269–270

13. Mariotti C, Alpini D, Fancellu R, Soliveri P, Grisoli M, Ravaglia S, Lovati C, Fetoni V, Giaccone G, Castucci A, Taroni F, Gellera C Di, Donato S (2007) Spinocerebellar ataxia type 17 (SCA17): oculomotor phenotype and clinical characterization of 15 Italian patients. J Neurol 254:1538–1546
14. Stevanin G, Fujigasaki H, Lebre AS, Camuzat A, Jeannequin C, Dode C, Takahashi J, San C, Bellance R, Brice A, Durr A (2003) Huntington's disease-like phenotype due to trinucleotide repeat expansions in the TBP and JPH3 genes. Brain 126:1599–1603
15. Toyoshima Y, Yamada M, Onodera O, Shimohata M, Inenaga C, Fujita N, Morita M, Tsuji S, Takahashi H (2004) SCA17 homozygote showing Huntington's disease-like phenotype. Ann Neurol 55:281–286
16. Walker FO (2007) Huntington's disease. Lancet 369:218–228
17. Bauer P, Laccone F, Rolfs A, Wullner U, Bosch S, Peters H, Liebscher S, Scheible M, Epplen JT, Weber BH, Holinski-Feder E, Weirich-Schwaiger H, Morris-Rosendahl DJ, Andrich J, Riess O (2004) Trinucleotide repeat expansion in SCA17/TBP in white patients with Huntington's disease-like phenotype. J Med Genet 41:230–232
18. Kambouris M, Bohlega S, Al-Tahan A, Meyer BF (2000) Localization of the gene for a novel autosomal recessive neurodegenerative Huntington-like disorder to 4p15.3. Am J Hum Genet 66:445–452
19. Margolis RL, O'Hearn E, Rosenblatt A, Willour V, Holmes SE, Franz ML, Callahan C, Hwang HS, Troncoso JC, Ross CA (2001) A disorder similar to Huntington's disease is associated with a novel CAG repeat expansion. Ann Neurol 50:373–380
20. Schneider SA, Walker RH, Bhatia KP (2007) The Huntington's disease-like syndromes: what to consider in patients with a negative Huntington's disease gene test. Nat Clin Pract Neurol 3:517–525
21. Wild EJ, Mudanohwo EE, Sweeney MG, Schneider SA, Beck J, Bhatia KP, Rossor MN, Davis MB, Tabrizi SJ (2008) Huntington's disease phenocopies are clinically and genetically heterogeneous. Mov Disord 23:716–720
22. Wild EJ, Tabrizi SJ (2007) Huntington's disease phenocopy syndromes. Curr Opin Neurol 20:681–687
23. Xiang F, Almqvist EW, Huq M, Lundin A, Hayden MR, Edstrom L, Anvret M, Zhang Z (1998) A Huntington disease-like neurodegenerative disorder maps to chromosome 20p. Am J Hum Genet 63:1431–1438
24. Stevanin G, Brice A (2008) Spinocerebellar ataxia 17 (SCA17) and Huntington's disease-like 4 (HDL4). Cerebellum 7:170–178
25. Moore RC, Xiang F, Monaghan J, Han D, Zhang Z, Edstrom L, Anvret M, Prusiner SB (2001) Huntington disease phenocopy is a familial prion disease. Am J Hum Genet 69:1385–1388
26. Holmes SE, O'Hearn E, Rosenblatt A, Callahan C, Hwang HS, Ingersoll-Ashworth RG, Fleisher A, Stevanin G, Brice A, Potter NT, Ross CA, Margolis RL (2001) A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. Nat Genet 29:377–378
27. Hanna MG, Davis MB, Sweeney MG, Noursadeghi M, Ellis CJ, Elliot P, Wood NW, Marsden CD (1998) Generalized chorea in two patients harboring the Friedreich's ataxia gene trinucleotide repeat expansion. Mov Disord 13:339–340
28. Hensman Moss DJ, Poulter M, Beck J, Hehir J, Polke JM, Campbell T, Adamson G, Mudanohwo E, McColgan P, Haworth A, Wild EJ, Sweeney MG, Houlden H, Mead S, Tabrizi SJ (2014) C9orf72 expansions are the most common genetic cause of Huntington disease phenocopies. Neurology 82:292–299
29. Maltecca F, Filla A, Castaldo I, Coppola G, Fragassi NA, Carella M, Bruni A, Cocozza S, Casari G, De Servadio A, Michele G (2003) Intergenerational instability and marked anticipation in SCA-17. Neurology 61:1441–1443
30. Belluzzo M, Musho-Ilbeh S, Monti F, Pizzolato G (2012) A case of nocturnal frontal lobe epilepsy in a patient with spinocerebellar ataxia type 17. Seizure 21:805–806

31. Rolfs A, Koeppen AH, Bauer I, Bauer P, Buhlmann S, Topka H, Schols L, Riess O (2003) Clinical features and neuropathology of autosomal dominant spinocerebellar ataxia (SCA17). Ann Neurol 54:367–375
32. Doherty KM, Warner TT, Lees AJ (2014) Late onset ataxia: MSA-C or SCA 17? A gene penetrance dilemma. Mov Disord 29:36–38
33. Loy CT, Sweeney MG, Davis MB, Wills AJ, Sawle GV, Lees AJ, Tabrizi SJ (2005) Spinocerebellar ataxia type 17: extension of phenotype with putaminal rim hyperintensity on magnetic resonance imaging. Mov Disord 20:1521–1523
34. Payami H, Nutt J, Gancher S, Bird T, McNeal MG, Seltzer WK, Hussey J, Lockhart P, Gwinn-Hardy K, Singleton AA, Singleton AB, Hardy J, Farrer M (2003) SCA2 may present as levodopa-responsive parkinsonism. Mov Disord 18:425–429
35. Imamura A, Sugai K, Watanabe S, Hamada F, Kurashige T, Takashima S (1994) High intensity in the globus pallidus on proton and T2-weighted MRI in a case of dentato-ruburo-pallido-luysian atrophy of myoclonus epilepsy type. Acta Paediatr Jpn 36:527–530
36. Tanaka M, Hirai S, Kondo S, Sun X, Nakagawa T, Tanaka S, Hayashi K, Okamoto K (1998) Cerebral hypoperfusion and hypometabolism with altered striatal signal intensity in chorea-acanthocytosis: a combined PET and MRI study. Mov Disord 13:100–107
37. Wray SH, Provenzale JM, Johns DR, Thulborn KR (1995) MR of the brain in mitochondrial myopathy. AJNR Am J Neuroradiol 16:1167–1173
38. Feng JY, Huang B, Yang WQ, Zhang YH, Wang LM, Wang LJ, Zhong XL (2015) The putaminal abnormalities on 3.0T magnetic resonance imaging: can they separate parkinsonism-predominant multiple system atrophy from Parkinson's disease? Acta Radiol 56:322–328
39. Schrag A, Kingsley D, Phatouros C, Mathias CJ, Lees AJ, Daniel SE, Quinn NP (1998) Clinical usefulness of magnetic resonance imaging in multiple system atrophy. J Neurol Neurosurg Psychiatry 65:65–71
40. Gunther P, Storch A, Schwarz J, Sabri O, Steinbach P, Wagner A, Hesse S (2004) Basal ganglia involvement of a patient with SCA 17—a new form of autosomal dominant spinocerebellar ataxia. J Neurol 251:896–897
41. Toyoshima Y, Onodera O, Yamada M, Tsuji S, Takahashi H (2005) Spinocerebellar ataxia type 17. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH, Stephens K (eds) GeneReviews(R). Seattle (WA)
42. Nanda A, Jackson SA, Schwankhaus JD, Metzer WS (2007) Case of spinocerebellar ataxia type 17 (SCA17) associated with only 41 repeats of the TATA-binding protein (TBP) gene. Mov Disord 22:436
43. Alibardi A, Squitieri F, Fattapposta F, Missori P, Pierelli F, Trompetto C, Curra A (2014) Psychiatric onset and late chorea in a patient with 41 CAG repeats in the TATA-binding protein gene. Parkinsonism Relat Disord 20:678–679
44. Park H, Jeon BS, Shin JH, Park SH (2016) A patient with 41 CAG repeats in SCA17 presenting with parkinsonism and chorea. Parkinsonism Relat Disord 22:106–107
45. Oda M, Maruyama H, Komure O, Morino H, Terasawa H, Izumi Y, Imamura T, Yasuda M, Ichikawa K, Ogawa M, Matsumoto M, Kawakami H (2004) Possible reduced penetrance of expansion of 44 to 47 CAG/CAA repeats in the TATA-binding protein gene in spinocerebellar ataxia type 17. Arch Neurol 61:209–212
46. Zuhlke C, Dalski A, Schwinger E, Finckh U (2005) Spinocerebellar ataxia type 17: report of a family with reduced penetrance of an unstable Gln49 TBP allele, haplotype analysis supporting a founder effect for unstable alleles and comparative analysis of SCA17 genotypes. BMC Med Genet 6:27
47. Shin JH, Park H, Ehm GH, Lee WW, Yun JY, Kim YE, Lee JY, Kim HJ, Kim JM, Jeon BS, Park SS (2015) The pathogenic role of low range repeats in SCA17. PLoS ONE 10:e0135275

48. Duyao M, Ambrose C, Myers R, Novelletto A, Persichetti F, Frontali M, Folstein S, Ross C, Franz M, Abbott M et al (1993) Trinucleotide repeat length instability and age of onset in Huntington's disease. Nat Genet 4:387–392
49. Dorsman JC, Bremmer-Bout M, van Pepers B, Den Ommen GJ, Dunnen JT (2002) Interruption of perfect CAG repeats by CAA triplets improves the stability of glutamine-encoding repeat sequences. Biotechniques 33:976–978
50. Gao R, Matsuura T, Coolbaugh M, Zuhlke C, Nakamura K, Rasmussen A, Siciliano MJ, Ashizawa T, Lin X (2008) Instability of expanded CAG/CAA repeats in spinocerebellar ataxia type 17. Eur J Hum Genet 16:215–222
51. Zuhlke CH, Spranger M, Spranger S, Voigt R, Lanz M, Gehlken U, Hinrichs F, Schwinger E (2003) SCA17 caused by homozygous repeat expansion in TBP due to partial isodisomy 6. Eur J Hum Genet 11:629–632
52. Bruni AC, Takahashi-Fujigasaki J, Maltecca F, Foncin JF, Servadio A, Casari G, D'Adamo P, Maletta R, Curcio SA De, Michele G, Filla A El, Hachimi KH, Duyckaerts C (2004) Behavioral disorder, dementia, ataxia, and rigidity in a large family with TATA box-binding protein mutation. Arch Neurol 61:1314–1320
53. Yamada M, Tsuji S, Takahashi H (2000) Pathology of CAG repeat diseases. Neuropathology 20:319–325
54. Sato T, Miura M, Yamada M, Yoshida T, Wood JD, Yazawa I, Masuda M, Suzuki T, Shin RM, Yau HJ, Liu FC, Shimohata T, Onodera O, Ross CA, Katsuki M, Takahashi H, Kano M, Aosaki T, Tsuji S (2009) Severe neurological phenotypes of Q129 DRPLA transgenic mice serendipitously created by en masse expansion of CAG repeats in Q76 DRPLA mice. Hum Mol Genet 18:723–736
55. Yamada M, Sato T, Tsuji S, Takahashi H (2008) CAG repeat disorder models and human neuropathology: similarities and differences. Acta Neuropathol 115:71–86
56. Yamada M, Hayashi S, Tsuji S, Takahashi H (2001) Involvement of the cerebral cortex and autonomic ganglia in Machado-Joseph disease. Acta Neuropathol 101:140–144
57. Yamada M, Wood JD, Shimohata T, Hayashi S, Tsuji S, Ross CA, Takahashi H (2001) Widespread occurrence of intranuclear atrophin-1 accumulation in the central nervous system neurons of patients with dentatorubral-pallidoluysian atrophy. Ann Neurol 49:14–23
58. Lupton CJ, Steer DL, Wintrode PL, Bottomley SP, Hughes VA, Ellisdon AM (2015) Enhanced molecular mobility of ordinarily structured regions drives polyglutamine disease. J Biol Chem 290:24190–24200
59. Xu Z, Tito AJ, Rui YN, Zhang S (2015) Studying polyglutamine diseases in Drosophila. Exp Neurol 274:25–41
60. Ross CA, Margolis RL, Becher MW, Wood JD, Engelender S, Cooper JK, Sharp AH (1998) Pathogenesis of neurodegenerative diseases associated with expanded glutamine repeats: new answers, new questions. Prog Brain Res 117:397–419
61. Trottier Y, Lutz Y, Stevanin G, Imbert G, Devys D, Cancel G, Saudou F, Weber C, David G, Tora L et al (1995) Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. Nature 378:403–406
62. Cummings CJ, Reinstein E, Sun Y, Antalffy B, Jiang Y, Ciechanover A, Orr HT, Beaudet AL, Zoghbi HY (1999) Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. Neuron 24:879–892
63. Klement IA, Skinner PJ, Kaytor MD, Yi H, Hersch SM, Clark HB, Zoghbi HY, Orr HT (1998) Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 95:41–53
64. Saudou F, Finkbeiner S, Devys D, Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95:55–66
65. Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature 431:805–810

66. Bowman AB, Lam YC, Jafar-Nejad P, Chen HK, Richman R, Samaco RC, Fryer JD, Kahle JJ, Orr HT, Zoghbi HY (2007) Duplication of Atxn1l suppresses SCA1 neuropathology by decreasing incorporation of polyglutamine-expanded ataxin-1 into native complexes. Nat Genet 39:373–379
67. Reid SJ, van Rees MI, Roon-Mom WM, Jones AL, MacDonald ME, Sutherland G, During MJ, Faull RL, Owen MJ, Dragunow M, Snell RG (2003) Molecular investigation of TBP allele length: a SCA17 cellular model and population study. Neurobiol Dis 13:37–45
68. Schaffar G, Breuer P, Boteva R, Behrends C, Tzvetkov N, Strippel N, Sakahira H, Siegers K, Hayer-Hartl M, Hartl FU (2004) Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. Mol Cell 15:95–105
69. Hsu TC, Wang CK, Yang CY, Lee LC, Hsieh-Li HM, Ro LS, Chen CM, Lee-Chen GJ, Su MT (2014) Deactivation of TBP contributes to SCA17 pathogenesis. Hum Mol Genet 23:6878–6893
70. Ren J, Jegga AG, Zhang M, Deng J, Liu J, Gordon CB, Aronow BJ, Lu LJ, Zhang B, Ma J (2011) A Drosophila model of the neurodegenerative disease SCA17 reveals a role of RBP-J/Su(H) in modulating the pathological outcome. Hum Mol Genet 20:3424–3436
71. Friedman MJ, Shah AG, Fang ZH, Ward EG, Warren ST, Li S, Li XJ (2007) Polyglutamine domain modulates the TBP-TFIIB interaction: implications for its normal function and neurodegeneration. Nat Neurosci 10:1519–1528
72. Huang S, Ling JJ, Yang S, Li XJ, Li S (2011) Neuronal expression of TATA box-binding protein containing expanded polyglutamine in knock-in mice reduces chaperone protein response by impairing the function of nuclear factor-Y transcription factor. Brain 134:1943–1958
73. Kelp A, Koeppen AH, Petrasch-Parwez E, Calaminus C, Bauer C, Portal E, Yu-Taeger L, Pichler B, Bauer P, Riess O, Nguyen HP (2013) A novel transgenic rat model for spinocerebellar ataxia type 17 recapitulates neuropathological changes and supplies in vivo imaging biomarkers. J Neurosci 33:9068–9081
74. Lee GC, Lin CH, Tao YC, Yang JM, Hsu KC, Huang YJ, Huang SH, Kung PJ, Chen WL, Wang CM, Wu YR, Chen CM, Lin JY, Hsieh-Li HM, Lee-Chen GJ (2015) The potential of lactulose and melibiose, two novel trehalase-indigestible and autophagy-inducing disaccharides, for polyQ-mediated neurodegenerative disease treatment. Neurotoxicology 48:120–130
75. Koshy BT, Zoghbi HY (1997) The CAG/polyglutamine tract diseases: gene products and molecular pathogenesis. Brain Pathol 7:927–942
76. Burley SK (1996) The TATA box binding protein. Curr Opin Struct Biol 6:69–75
77. Sharp PA (1992) TATA-binding protein is a classless factor. Cell 68:819–821
78. Martianov I, Viville S, Davidson I (2002) RNA polymerase II transcription in murine cells lacking the TATA binding protein. Science 298:1036–1039
79. Friedman MJ, Wang CE, Li XJ, Li S (2008) Polyglutamine expansion reduces the association of TATA-binding protein with DNA and induces DNA binding-independent neurotoxicity. J Biol Chem 283:8283–8290
80. Huang S, Yang S, Guo J, Yan S, Gaertig MA, Li S, Li XJ (2015) Large polyglutamine repeats cause muscle degeneration in SCA17 mice. Cell Rep 13:196–208
81. Di Marco S, Mazroui R, Dallaire P, Chittur S, Tenenbaum SA, Radzioch D, Marette A, Gallouzi IE (2005) NF-kappa B-mediated MyoD decay during muscle wasting requires nitric oxide synthase mRNA stabilization, HuR protein, and nitric oxide release. Mol Cell Biol 25:6533–6545
82. Guttridge DC, Mayo MW, Madrid LV, Wang CY, Baldwin AS Jr (2000) NF-kappaB-induced loss of MyoD messenger RNA: possible role in muscle decay and cachexia. Science 289:2363–2366

Chapter 11
The Neuropathology of Spinocerebellar Ataxia Type 3/Machado-Joseph Disease

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Abstract Spinocerebellar ataxia type 3 (SCA-3)/Machado-Joseph disease (MJD), the most common autosomal dominant ataxia, affects many regions of the brain and spinal cord. Similar to SCA-1, SCA-2, SCA-6, SCA-7, and SCA-17, the mutation consists of a pathogenic translated cytosine-adenine-guanine (CAG) trinucleotide repeat expansion. Almost invariably, the substantia nigra and the dentate nucleus of the cerebellum bear the brunt of the disease, and these lesions account for the Parkinsonian and ataxic phenotypes. Lesions of motor nuclei in the brain stem cause the complex disturbance of ocular motility and weakness of the tongue. Atrophy of the basis pontis is common, and polyglutamine-positive neuronal intranuclear inclusion bodies are most readily found in the pontine gray. Abnormalities of basal ganglia, thalamus, spinal cord, dorsal root ganglia, and sensory peripheral nerves are more variable. This report of the main neuropathological lesions is based on the study of 12 genetically confirmed autopsy cases of SCA-3/MJD. In the cerebellum, all layers of the cortex remain normal, but the dentate nucleus exhibits neuronal loss and a peculiar proliferation of synaptic terminals termed grumose regeneration. The clusters surrounding residual neuronal cell bodies and dendrites are interpreted as a response to loss of γ-aminobutyric acid (GABA)-A-receptors and lack of gephyrin, a protein that accomplishes the proper positioning of GABA-A- and glycine receptors. At the spinal level, dorsal root ganglia reveal proliferation of satellite cells, active neuronal destruction, and residual nodules. The spinal cord shows total or subtotal loss of neurons in the dorsal nuclei, anterior horn cell atrophy, and variable long tract degeneration. While misfolding of ataxin-3 due to overly long polyglutamine stretches is a critical contributor to the pathogenesis of SCA-3/MJD, the great neuropathological complexity of the disorder remains largely unexplained.

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Keywords Spinocerebellar ataxia type 3 · Machado-Joseph disease
Dentate nucleus · Substantia nigra · Inclusion bodies

11.1 History

The first neuropathological description of spinocerebellar ataxia type 3 (SCA-3)/Machado-Joseph disease (MJD) was titled "nigro-spino-dentatal degeneration with nuclear ophthalmoplegia" [1] and coincided with the recognition that MJD is a disease entity [2]. Several subsequent reports [3, 4] antedated the discovery that MJD and SCA-3 are the same autosomal dominant disorder, sharing a pathogenic polyglutamine expansion [5]. The early descriptions recognized the main targets of SCA-3/MJD, namely, dentate nucleus and substantia nigra. Sparing of cerebellar cortex and inferior olivary nuclei was confirmed in many papers that followed. This constellation of lesions differs greatly from SCA types that affect the cerebellar cortex and cause retrograde degeneration of the inferior olivary nuclei. The involvement of regions of the central nervous system other than dentate nucleus and substantia nigra is more variable: striatum and globus pallidus, thalamus, basis pontis, oculomotor nuclei, paramedian reticular formation, hypoglossal nuclei, anterior horn cells and dorsal nuclei of the spinal cord, and dorsal columns and spinocerebellar tracts (review by [6]). It is peculiar that the dentate nuclei are not always atrophic [7]. The dominant cerebellar ataxia described by Becker et al. [8] was retrospectively identified as SCA-3/MJD, but neuropathological examination of two siblings, brother and sister, from the same pedigree also revealed intact dentate nuclei. The peripheral nervous system [2, 9] and dorsal root ganglia (DRG) may also be affected [3].

11.2 Gross and Microscopic Neuropathology

This report is based on 12 genetically confirmed autopsy cases of SCA-3/MJD. All showed the characteristic combination of lesions in dentate nucleus and substantia nigra. Four of the 12 autopsies included lumbar DRG among which only one was entirely normal. In one case, a sural nerve was available. It revealed no abnormalities.

The size of the cerebellum may be reduced (Fig. 11.1a) because of shrinkage of the dentate nucleus and atrophy of its efferent fibers in the superior cerebellar peduncle (Fig. 11.1b). For the same reasons, the size of the fourth ventricle may be larger than normal. The mesial section of the brain shows attenuation of the basis pontis (Fig. 11.1a, arrow), but pontine atrophy rarely reaches the severe degree of "olivopontocerebellar atrophy" (OPCA) that characterizes SCA-2, SCA-7, and multiple system atrophy. Slices of the cerebellum reveal the collapse of the dentate nucleus and thinning of its efferent fiber tract (Fig. 11.1b, arrow). Serial transverse

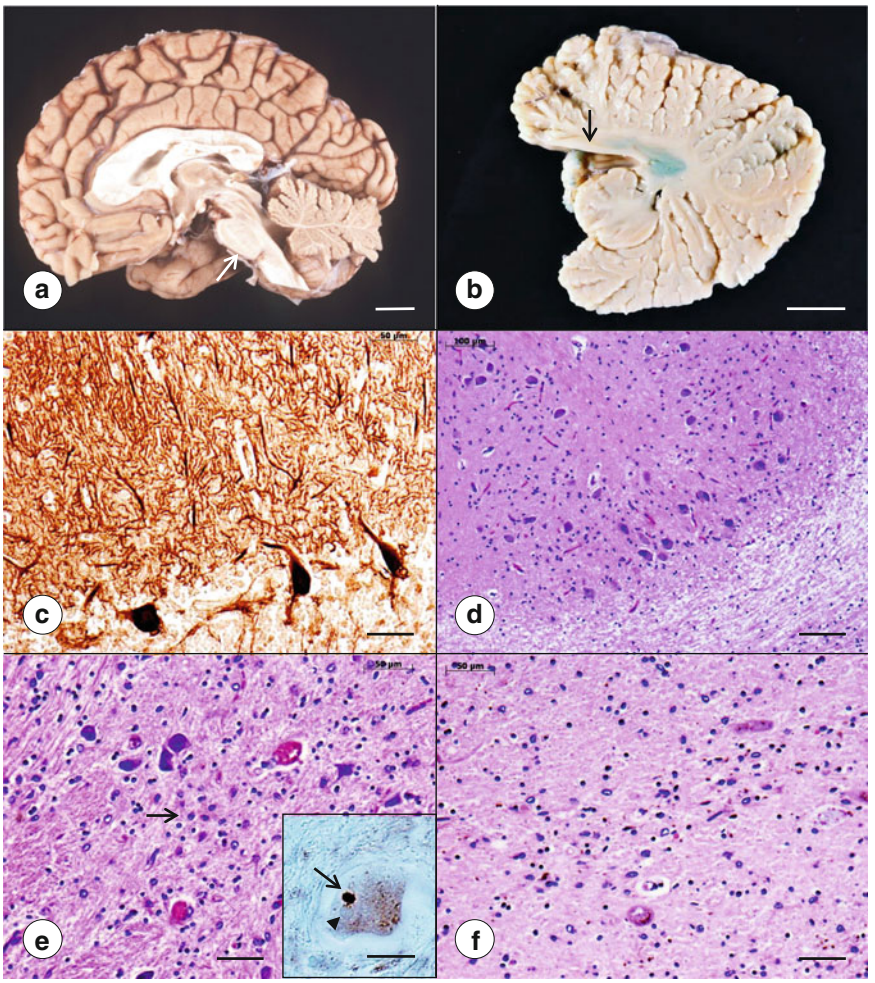

Fig. 11.1 Gross and microscopic findings in SCA-3/MJD, **a** A midline division of the brain shows modest dilatation of the fourth ventricle and attenuation of the lower pontine base (arrow). The cerebellum is of normal size. **b** A parasagittal slice of the cerebellum shows a diminutive dentate nucleus that is highlighted by a macro-iron stain. Cerebellar hilum and superior cerebellar peduncle are atrophic (arrow). **c** Cerebellar cortex. An immunohistochemical stain with anti-calbindin D-28K shows intense reaction product in the perikarya of Purkinje cells and their dendritic trees. **d** Inferior olivary nucleus. A hematoxylin-and-eosin stain shows a normal population of neurons. **e** Basis pontis. A hematoxylin-and-eosin stain reveals loss of neurons and replacement gliosis (arrow); the inset illustrates a polyglutamine-reactive intranuclear neuronal inclusion body (arrow) that lies adjacent to the nucleolus (arrowhead) (monoclonal antibody 1C2 to polyglutamine). **f** Substantia nigra. The hematoxylin-and-eosin stain shows subtotal depletion of pigmented neurons and gliosis. Bars: **a** 2 cm; **b** 1 cm; **c** 50 μm; **d** 100 μm; **e–f** 50 μm; inset in (**e**) 10 μm

slices of the brain stem disclose loss of pigment in the substantia nigra and pontine atrophy in some but not all cases. The meandering bands of the inferior olivary nuclei remain distinctly visible.

Microscopy reveals integrity of Purkinje cells and their arborizations (Fig. 11.1c) and, as expected, intact inferior olivary nuclei (Fig. 11.1d). The lesion of the substantia nigra differs from idiopathic Parkinson's disease in that it tends to be focal rather than diffuse across the compact and lateral portions. Total neuronal depletion and glial scarring, as shown in Fig. 11.1f, may alternate with relatively intact adjacent portions. One case among those reported here revealed only a lack of melanin but no numerical loss of nerve cells. Lewy bodies are absent. Loss of neurons in the basis pontis (Fig. 11.1e) is not as severe as in typical hereditary or sporadic OPCA, but glial scarring may be quite prominent (Fig. 11.1e, arrow). While pontine atrophy may be entirely absent, the gray matter is an excellent location to search for neuronal intranuclear polyglutamine-containing inclusion bodies (Fig. 11.1e, inset, arrow). Disturbances of ocular motility are well known from among the clinical features of SCA-3/MJD, and lesions of oculomotor nuclei and the paramedian reticular formation are well established [6]. At the level of the medulla oblongata, neuronal loss in the hypoglossal nuclei is also common, explaining bulbar palsy in SCA-3/MJD. Reduced thickness of the spinal cord is often very evident (Fig. 11.2a), and a stain for myelin shows fiber loss in the dorsal columns (Fig. 11.2a), the spinocerebellar tracts, or a combination of these pathways. Figure 11.2b shows complete atrophy of the neurons in the dorsal nuclei. Anterior horn cell atrophy may occur at all levels of the spinal cord (Fig. 11.2c), and commensurate group lesions in skeletal muscle are part of the neuropathological phenotype [2].

Dorsal root ganglia in SCA-3/MJD often show hypercellularity, active destruction of neurons (Fig. 11.2d, arrow), and residual nodules (Fig. 11.2d, arrowheads). Stains for class-III-β-tubulin suggest that DRG neurons (Fig. 11.2e) are dysfunctional while still stainable by hematoxylin and eosin (Fig. 11.2d). Immunostains with anti-S100 show the formation of multiple layers of satellite cells around neurons and immunoreactivity of residual nodules (Fig. 11.2f).

Figure 11.3 presents the critical histopathological findings in the dentate nucleus of SCA-3/MJD: loss of large neurons (Fig. 11.3a), preservation of small neurons (Fig. 11.3b), grumose regeneration (Figs. 11.3a, c, d); loss of neurons expressing the glycine (Fig. 11.3e) and γ-aminobutyric acid (GABA) A receptors (Fig. 11.3f), and of gephyrin, the protein that anchors glycine and GABA A receptors to the neuronal plasma membrane (Fig. 11.3g). A comparison of the stains for glutamic acid decarboxylase (GAD; Fig. 11.3c) and synaptophysin (Fig. 11.3d) establishes that grumose regeneration is due to proliferation of GABA-ergic terminals arising from Purkinje cells. Small nerve cells stained for class-III-β-tubulin (Fig. 11.3b) are also reactive with anti-GAD, implying their identity as GABA-ergic neurons.

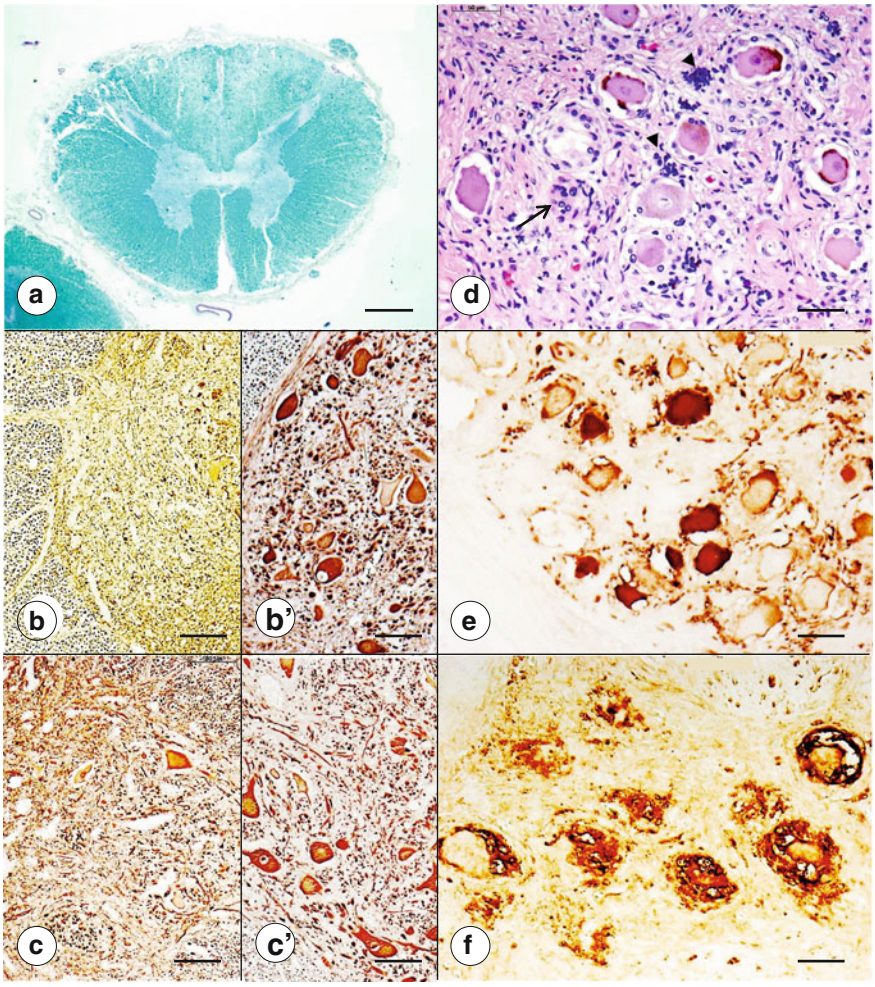

Fig. 11.2 Microscopy of spinal cord and DRG in SCA-3/MJD, **a**–**c** Spinal cord; **d**–**f** DRG. **b′** and **c′** Show microphotographs from normal controls to match **b** and **c**, respectively. **a** Thoracic spinal cord, stained with Luxol Fast Blue for myelin. The transectional area measures 27.1 mm^2 (mean normal: 40.3 mm^2). The fasciculus gracilis shows loss of myelinated fibers. **b** Nucleus dorsalis, immunostained for class-III-β-tubulin. The nucleus shows total depletion of the characteristically large nerve cells (**b′**, normal control). **c** Lumbar anterior horn, immunostained for class-III-β-tubulin. The anterior horn shows a paucity of motor neurons in comparison with a control section **c′**. **d** DRG, hematoxylin and eosin. The neural parenchyma is hypercellular and displays residual nodules. The arrow points to active invasion of a nerve cell. **e** DRG, immunostain for class-III-β-tubulin. The section shows loss of reaction product from small and intermediate-size neurons. In some nerve cells, the immunoreaction is restricted to the cytosol just beneath the neuronal plasma membrane. **f** DRG, S100 immunostain. Several nerve cells show multiple layers of satellite cells, and S100-reactive residual nodules represent destruction of neurons. Bars: **a** 1 mm; **b**–**c** 100 μm; **d**–**f** 50 μm

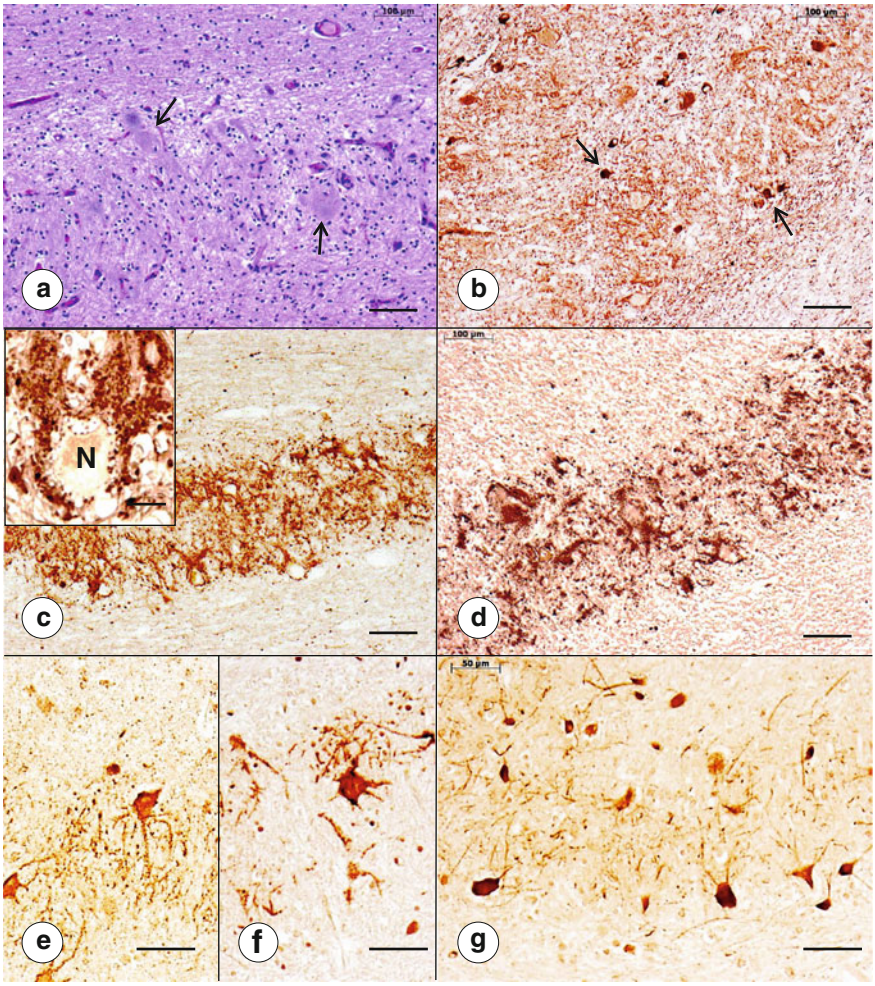

Fig. 11.3 Microscopy of the dentate nucleus in SCA-3/MJD, **a** A hematoxylin-and-eosin stain shows loss of neurons and numerous clusters of grumose regeneration (arrows); **b** An immunohistochemical stain for class-III-β-tubulin reveals total loss of large glutamatergic neurons and preservation of small neurons (arrows). **c** An immunohistochemical stain for GAD shows lack of negative images of large glutamatergic neurons and grumose regeneration. The inset displays studding of the plasma membrane with multiple layers of GAD-reactive terminals (N, neuronal perikaryon). **d** Immunohistochemical reaction product of synaptophysin resembles the visualization of GAD **c** Including grumose regeneration. **e** Immunohistochemistry with anti-GABA-A-receptor γ2 subunit shows only a few small and intermediate neurons. **f** Immunohistochemical visualization of the glycine receptor α1/2 subunits resembles the stain for GABA-A-receptor γ2 subunit (**e**). **g** Immunohistochemical gephyrin reaction product labels neurons that are of small or intermediate size. Bars: **a**–**d** 100 μm; inset in **c** 10 μm; **e**–**g** 50 μm

11.3 Functional Consequences and Pathogenesis

The cytosine-adenine-guanine (CAG) trinucleotide repeat expansion of the *ATXN3* gene (chromosome 14.q32.12; OMIM 109150) in SCA-3/MJD is translated to an abnormally long polyglutamine stretch in the gene product, ataxin 3. One of the first and most intriguing observations was that presence and abundance of polyglutamine-containing neuronal inclusion bodies did not correlate with the degree of tissue destruction. It is widely held that expanded and misfolded ataxin 3, or its proteolytic fragments, are toxic to the neuropil. One explanation of the disparity of inclusion bodies and tissue damage is that intranuclear inclusions are an epiphenomenon while expanded ataxin 3 damages organelles in the neuronal cytoplasm. Several animal models have yielded insight into the disease mechanism of SCA-3/MJD but have not provided advanced information on the complex neuropathological phenotype in humans (review in [10]). Parkinsonism may be attributed to the lesions of substantia nigra while ataxia is the expected result of damage to the dentate nucleus and atrophy of the dorsal nuclei in Clarke columns. The clinical phenotype, however, is much more variable and reflects the broad spectrum of structural lesions in the central and peripheral nervous systems (reviews in [6, 11–14]).

SCA-3/MJD and Friedreich ataxia are very different heritable disorders though they exhibit a remarkable resemblance in their lesions of the dentate nuclei, spinal cord, DRG, and sensory peripheral nerves. Similar to Friedreich ataxia, SCA-3/MJD causes loss of large neurons in the dentate nucleus while small neurons persist (Fig. 11.3b). The author and his collaborators [15] have proposed that the grape-like clusters in the dentate nucleus of Friedreich ataxia, SCA-3/MJD (Figs. 11.3a, c, d), and progressive supranuclear palsy be named grumose *regeneration*, rather than *degeneration,* because the immunohistochemical evidence strongly supports proliferation from GABA-ergic synaptic terminals. This luxuriant growth is not present in all cases and may only indicate relatively short disease duration. Koeppen et al. [15] suggested that grumose regeneration is a response to the loss of GABA-A- and glycine-receptors on large nerve cells of the dentate nucleus. Disappearance of these receptors, in turn, may be the result of gephyrin deficiency in the postsynaptic membrane. This protein, named after the Greek term for bridge [16] is essential for the proper positioning of GABA-A- and glycine-receptors. Persistence of all three proteins within the plasma membranes and cytoplasm of small and intermediate-size neurons of the dentate nucleus (Fig. 11.3e–g) implies that SCA-3/MJD selectively damages large glutamatergic nerve cells.

Destruction of the dentate nucleus by strokes, trauma, tumors, or multiple sclerosis causes degeneration of the dentato-olivary tract and a characteristic clinical phenomenon, palatal myoclonus. Though the dentate nucleus shows advanced degeneration, neither SCA-3/MJD nor Friedreich ataxia causes palatal myoclonus. The reason is the survival of small GABA-ergic neurons that are the source of the dentato-olivary fibers. Koeppen et al. [17] suggested that GABA-ergic input to the

inferior olivary nuclei has trophic properties and prevents the development of olivary hypertrophy, which is the anatomical substrate of palatal myoclonus. The integrity of the inferior olivary nuclei also accounts for intact climbing fiber connections to Purkinje cells in SCA-3/MJD. Therefore, the main ataxia-causing deficit resides in the dentate nucleus and failure of glutamatergic output to the thalamus.

In comparison with the dentate nucleus, much less is known about the destruction of the substantia nigra, neuronal loss in basal ganglia, motor and non-motor nuclei of the brain stem and spinal cord, DRG, and peripheral nerves.

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References

1. Woods BT, Schaumburg HH (1972) Nigro-spino-dentatal degeneration with nuclear ophthalmoplegia. A unique and partially treatable clinico-pathological entity. J Neurol Sci 17:149–166
2. Nakano KK, Dawson DM, Spence A (1972) Machado disease: a hereditary ataxia in Portuguese emigrants to Massachusetts. Neurology 22:49–55
3. Coutinho P, Guimarães A, Scaravilli F (1982) The pathology of Machado-Joseph disease. Acta Neuropathol 58:48–54
4. Rosenberg RN, Nyhan WL, Bay C, Shore P (1976) Autosomal dominant striatonigral degeneration. A clinical, pathologic, and biochemical study of a new genetic disorder. Neurology 26:703–714
5. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I, Kimura J, Narumiya S, Kakizuka A (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8:221–228
6. Riess O, Rüb U, Pastore A, Bauer P, Schöls L (2008) SCA3: neurological features, pathogenesis and animal models. Cerebellum 7:125–137
7. Romanul FCA, Fowler HL, Radvany J, Feldman RG, Feingold M (1977) Azorean disease of the nervous system. New Engl J Med 296:1505–1508
8. Becker PE, Sabuncu N, Hopf HC (1971) Dominant erblicher Typ von “cerebellarer Ataxie”. Z Neurol 199:116–139
9. Coutinho P, Guimarães A, Pires MM, Scaravilli F (1986) The peripheral neuropathy in Machado-Joseph disease. Acta Neuropathol 71:119–124
10. Colomer Gould VF (2012) Mouse models of spinocerebellar ataxia type 3 (Machado-Joseph disease). Neurotherapeutics 9:285–296
11. Bettencourt C, Lima M (2011) Machado-Joseph disease: from first descriptions to new perspectives. Orphanet J Rare Dis 6:35
12. Matilla-Dueñas A (2012) Machado-Joseph disease and other rare spinocerebellar ataxias. Adv Exp Med Biol 724:172–188
13. Paulson HL (2007) Dominantly inherited ataxias: lessons learned from Machado-Joseph disease/spinocerebellar ataxia type 3. Semin Neurol 27:133–142

14. Rüb U, Brunt ER, Deller T (2008) New insights into the pathoanatomy of spinocerebellar ataxia type 3 (Machado-Joseph disease). Curr Opin Neurol 21:111–116
15. Koeppen AH, Ramirez RL, Becker AB, Feustel PJ, Mazurkiewicz JE (2015) Friedreich ataxia: failure of GABA-ergic and glycinergic synaptic transmission in the dentate nucleus. J Neuropathol Exp Neurol 74:166–176
16. Prior P, Schmitt B, Grenningloh G, Pribilla I, Multhaup G, Beyreuther K, Maulet Y, Werner P, Langosh D, Kirsch J, Betz H (1992) Primary structure and alternative splice variants of gephyrin, a putative glycine-receptor-tubulin linker protein. Neuron 8:1161–1170
17. Koeppen AH, Davis AN, Morral JA (2011) The cerebellar component of Friedreich's ataxia. Acta Neuropathol 122:323–330

Chapter 12
Origins and Spread of Machado-Joseph Disease Ancestral Mutations Events

Sandra Martins and Jorge Sequeiros

Abstract Machado-Joseph disease (MJD) is the most common autosomal dominant spinocerebellar ataxia reported worldwide, but it shows marked geographic differences in prevalence. The study of ancestral origins and spreading routes of MJD mutational events has contributed to explain such differences. During human evolution, at least two independent de novo MJD expansions occurred in distinct haplotype backgrounds: TTACAC and GTGGCA (named Joseph and Machado lineages). The most ancient Joseph lineage, probably of Asian origin, has been introduced recently in Europe, where founder effects are responsible for the high MJD prevalence, as occurs in the Portuguese/Azorean island of Flores and Northeastern mainland. The Machado lineage is geographically more restricted, with most known families in Portugal (island of São Miguel and along the Tagus valley). The hypothesis of other mutational origins has been raised, namely to explain the disease among Australian aborigines; however, a comprehensive haplotype study suggested the introduction of the Joseph lineage in that community via Asia. Also, additional SNP-based haplotypes (TTAGAC, TTGGAC and GTGCCA) were observed in other MJD families, but phylogenetic analysis with more polymorphic flanking markers did not point to independent mutational events, reinforcing the hypothesis of a very low mutation rate underlying this repeat expansion *locus*.

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12.1 The Original Descriptions of Machado-Joseph Disease

A novel form of autosomal dominant cerebellar ataxia, with a "peripheral neuropathy" and onset after age 40 years, was described by Nakano, Dawson and Spence, in 1972, among Portuguese immigrants in the United States [1]. The *new* disease was named after the affected family, as *Machado disease*. The kindred was traced back to Guilherme (William) Machado, a native of the island of São Miguel, in the archipelago of the Azores (Portugal), who had migrated to southern Massachusetts in the late XIX to early XX centuries.

The same year, another family (descending from Joe Thomas), also of Azorean ancestry but from the island of Flores, was reported as having a cerebellar ataxia, also with dominant inheritance; however, an earlier age-at-onset (17 through 46 years) and the presence of a pyramidal syndrome and progressive external ophthalmoplegia (PEO) led Woods & Schaumburg to propose this was a *new* entity, distinct from Machado disease, and named it as *nigro-spino-dentatal degeneration with nuclear ophthalmoplegia* [2].

Four years later, Rosenberg et al. reported still another large family of Azorean extraction with an *autosomal dominant striatonigral degeneration* [3]. One patient resembled Machado disease, but others shared some clinical features with the Thomas family: onset was at age 17 to 42 years, cerebellar ataxia was absent (or minor), extrapyramidal signs were prominent and neuropathological findings also seemed to differ. The family descended from Antone Joseph, born in the island of Flores in 1815 (as António Jacinto Bastiana, as much later was found) and who reached the San Francisco bay area aboard a whaling ship, in 1844. He died in Pleasanton (in 1870), leaving many affected descendants mostly in northern California. Their disorder eventually came to be named as *Joseph disease* [4].

In 1976, Paula Coutinho and Corino Andrade investigated a familial neurological disease with high prevalence in the Azorean islands, after the national health authority called for their attention and help [5]; they suggested that all the 15 families found (mostly in Flores and São Miguel), as well as the previously described Machado, Thomas and Joseph families, suffered from the same disease, despite some differences in its expression, as the various clinical presentations could be found within a single family, and even among sibs [6]. They called it *autosomal dominant system degeneration* in Portuguese families of the Azores islands.

A similar conclusion was independently reached by other authors, including after the observation of the three presentations previously reported in still another Portuguese-Azorean family in Massachusetts [7]; the name *Azorean disease* was

now suggested. But the disease was to be found in mainland Portugal (in 1978) [8] and in an American black family from North Carolina (in 1980) [9], as well as in families of Japanese [10–12], Indian [13], Chinese [14] and other origins. Additional descriptions showed its presence in four aboriginal families from Arnhem Land, in northern Australia [15], and in a Yemenite Jewish kindred from the Ta'izz village [16].

The name *Machado-Joseph disease* (MJD) was proposed by Coutinho and Sequeiros and adopted, in 1980, at the first international meeting on this disease, where all the clinical and research groups involved so far were represented; this was meant to unify the various denominations given, as well as to honour the first family described and the largest and better studied one (also of relevance was the fact that the Thomas descendants were reluctant to provide their surname to the disease).

The epidemiology of MJD, based on families described until 1993 (before the gene was known), was reviewed by Sequeiros and Coutinho [17]. In 1995, a British family was molecularly diagnosed with MJD, among nine others of different origins living in the United Kingdom. That was the historical *Drew family of Walworth*, described 100 years earlier, with a *heredo-familial disease resembling disseminated sclerosis* [18, 19]. This way, the clinical diagnosis of MJD, as suggested by Paula Coutinho, was confirmed by the group lead by Anita Harding [20].

12.2 The Current Distribution of MJD in the World

Epidemiological data on the spinocerebellar ataxias (SCAs) are essential to study the natural history of underlying mutations, but also important for genetic testing in every population, helping the clinical diagnosis since phenotypes of different SCAs often overlap. Nationwide surveys are scarce to estimate prevalence of MJD (also known as SCA3) and other SCAs in most populations; thus, diagnostic testing is often informed by the relative frequency of SCA subtypes, even though the number of dominant ataxias of unknown aetiology may exceed half of the cases in many series. A marked geographical and ethnic variability in SCA frequency exists (mainly due to demographic and population effects, such as migration, founder effects or genetic drift), but MJD proved to be the most frequent dominant ataxia worldwide. Currently, MJD is particularly common in China (62.1%) [21], Brazil (59.6%) [22], Portugal (57.8%) [23], Thailand (46.5%) [24], Germany (42%) [25], Singapore (41%) [26], Taiwan (32%) [27], France (32%) [28], Japan (28%) [29], The Netherlands (28%) [30], Venezuela (25%) [31], the USA (21%) [32], and Spain (15%, the same frequency as SCA2) [33]. On the other hand, MJD seems absent in Greece [34], Czech Republic [35], Cyprus [36], Sri Lanka [37], Poland [38], Finland [39], Serbia [40], and rare in Turkey (0.6%) [41] and South Africa (1.1%) [42].

12.3 Identification of Mutations with Independent Origin

The knowledge on how many mutational events occurred (independently of the demographic and evolutionary forces that conditioned their success and consequent present-day frequency) is of utmost importance to determine the local mutation rate and better calibrate the respective molecular clock. The first study to identify MJD mutational origins focused on the analysis of 3 single-nucleotide polymorphisms (SNPs) flanking the causative CAG repeat at *ATXN3* (the gene responsible for MJD) [43]. SNPs are bi-allelic markers, characterized by a very low mutation rate, estimated at around 2×10^{-8} per *locus*, per generation, in humans [44]. Given the low probability of having two independent base changes occurring at a single position, SNPs often result from unique events over the evolution of a species. To date, CAG expanded/pathogenic alleles have been observed exclusively in humans, suggesting that large expansions responsible for MJD and other polyglutamine disorders occurred after the human-chimp split [45–47]. Therefore, at the moment a de novo expansion occurs from a pool of genetically diverse normal alleles, a single haplotype background is in total linkage-disequilibrium (LD) with the newly arisen expanded $(CAG)_n$ allele. Then, the breakdown of LD over time is mainly due to recombination, even if some recurrent mutations may also arise in SNPs. Typically, recombination between two *loci* increases with physical distance, but patterns of LD are not always constant throughout the chromosomes, with LD blocks and recombination hotspots varying also between populations. The high density of SNPs across the human genome allows, however, the study of virtually any *locus* of interest in great detail.

Based on the analysis of $\underline{A}^{669}TG/\underline{G}^{669}TG$ (rs1048755), $\underline{C}^{987}GG/\underline{G}^{987}GG$ (rs12895357), and $TA\underline{A}^{1118}/TA\underline{C}^{1118}$ (rs7158733), intragenic SNPs in *ATXN3*, it was suggested, for the first time, that not all MJD patients shared the same ancestral mutational origin [43]. Families from 16 different origins presented 4 SNP haplotypes co-segregating with deleterious expanded $(CAG)_n$ alleles: ACA and GGC were observed in the vast majority of the cases. Even if only three SNPs were analysed, the *yin-yang* pattern in these common MJD haplotypes could hardly be explained by a single MJD origin; with $\underline{A}^{669}TG/\underline{G}^{669}TG$ located upstream and $\underline{C}^{987}GG/\underline{G}^{987}GG$ and $TA\underline{A}^{1118}/TA\underline{C}^{1118}$, both downstream the $(CAG)_n$ (at a few 1 and 132 bp distant of the repeat, respectively), the unlikely accumulation of recurrent SNP mutation and/or recombination would be needed to justify a common ancestry for the ACA and GGC lineages. Later, the extension of the analysed haplotype confirmed the two main MJD mutational origins [48]. By including the additional IVS6-30G>T (rs12590497), $GT\underline{T}^{527}/GT\underline{C}^{527}$ (rs16999141) and C^{1178}/A^{1178} (rs3092822) SNPs, in a large study of MJD families from 20 populations, the TTACAC and GTGGCA haplotypes were found to underlie the two major mutational origins, named Joseph and Machado lineages respectively, denoting their predominance in Flores and São Miguel, the Azorean islands home to the Joseph and the Machado families and their descendants (as well as their putative ancestors in the mainland).

On the other hand, the finding of SNP haplotypes different from these two most common could indicate the existence of other, perhaps less frequent and geographically more restricted, independent-origin MJD mutations. In fact, three rare haplotypes have been genotyped in MJD families, TTAGAC, TTGGAC and GTGCCA, but phylogenetic analyses with flanking microsatellites (short tandem repeats, STRs) did not support the hypothesis of independent-origin mutations [43, 48]. Instead, TTAGAC (found in two families from the United States, one from Morocco, one from Ghana and one from the West Indies) seemed to have derived from the ancestral TTACAC lineage, after a mutation in the $\underline{C}^{987}GG/\underline{G}^{987}GG$ SNP, an event followed by a reversion in the $\underline{A}^{669}TG/\underline{G}^{669}TG$ SNP, resulting in the TTGGAC haplotype (present in one family from French Guyana). The haplotype GTGCCA, observed in three Portuguese families, resulted most likely from mutation (again in the $\underline{C}^{987}GG/\underline{G}^{987}GG$ SNP) on a GTGGCA background, since extended STR-haplotypes were shared between them [48].

12.4 Routes of Mutation Spread

When haplotype analyses were not possible or available (as before the discovery of *ATXN3*), some hypotheses of mutational routes for dispersal of MJD have been based on the historical and geographical origin of the families. It has been postulated that an ancestral mutation could have originated among the Sephardic Jewish communities (in the mountainous and relatively inaccessible region of north-eastern Portugal), taken from mainland Portugal to the Azores and then to North America and other regions [17, 49]. Also, assuming a Portuguese origin for the worldwide spread MJD mutation, links with Asia during the Portuguese sea explorations were evoked to explain the presence of MJD in these geographically distant populations via genetic exchange with Portuguese merchants, mercenaries and missionaries, in the 1500–1600s [43, 50–52].

More recent studies followed a strategy based on the same principle applied to the reconstruction of human evolutionary history, which assumes that ancestral populations (in this case, the place-of-birth of a mutation) would have accumulated the highest genetic diversity flanking the *locus* under study. Accordingly, diversity should decrease in populations where the mutation has been more recently introduced, as fewer generations underlie less time to diverge.

Several studies on genetic diversity rely on the analysis of highly informative multi-allelic microsatellites markers (or STRs), which display 1–6 bp tandem repeats, highly variable in length, and widely distributed in the human genome [53]. Their evolution rate at 1×10^{-3} mutations per generation, usually under a stepwise mutation model [54], led in part, to their popularization as an analytic tool for phylogenetic reconstructions of several human genes [55].

Concerns on the analysis of STRs relate to the mutation–migration–drift equilibrium, effective population sizes, and homoplasmy; however, confounding factors may be reduced when STRs are placed on stable SNP backgrounds. When a

narrower time-scale is considered, fewer are the factors that may affect the molecular clock that underlies the evolution of STRs. In the study of disease mutational origins, the analysis of a restricted subpopulation of chromosomes (mutated alleles) allows an accurate approach based on the combined analysis of SNPs and STRs haplotypes.

12.5 The Joseph (Flores) Lineage

The most ancient MJD mutational event arose probably more than 6000 years ago in Asia. The TTACAC SNP background, which was found to segregate with expanded chromosomes in the five continents, is spread worldwide: currently, the Joseph lineage is present in, at least, 19 populations [48]. At the time estimated for the origin of this mutation for MJD, humans experienced the Neolithic culture, a period when domestication of plants and animals occurred together with human settlements. The routes and times of diffusion associated to the New Stone Age do not support simultaneous dispersal of this MJD mutation. After the earliest known development of Neolithic culture in the Middle East (Jericho, Palestine), between 8000 and 6000 BC, further spread of this culture through Europe, the Indus valley (India) and the Huang He valley (North China) seems to have occurred between 6000 BC and 2000 BC. The introduction of Joseph haplotypes in Europe (Germany and France) seemed, nevertheless, to have occurred less than 1500 years ago. This might be a subestimation of the time of presence of the TTACAC mutation in Europe, since other eastern population were not assessed in this study; however, the geographical relative frequency of SCAs have shown, so far, the absence of MJD in the Czech Republic [35], Poland [38], Serbia [40] and Russia [56].

On the other hand, the *Silk Road* trade during the 1st century AD could explain the spread of some MJD founder haplotypes along those routes. Although no single route was taken, different branches are known to have started from Chang'an (the ancient capital of China, actual Xi'an), towards central Asia, northern India, and the Parthian and Roman Empires. Later, trading routes connected the Yellow River valley to the Mediterranean Sea and passed through present-day Iran, Iraq and Syria. As in eastern Europe, reports of MJD are also very rare in central and southwest Asia. In Iran, there are at least two MJD families, molecularly confirmed [57], and one additional large family clinically diagnosed with MJD, including 50 patients and 50 unaffected relatives (Nima Rezaei and Mohammad Najafi, personal communication, 2007).

More recently, the Joseph lineage was introduced from Eastern to Western Europe, through the 10-21-14-15 and 11-21-14-15 founder STR-based haplotypes, respectively in German and French populations, at about the same time, i.e., around 1300 years ago. Later (about 600 years), the French founder haplotype was introduced in mainland Portugal, and from here the mutation migrated to the Azores and to Brazil. In mainland Portugal, this haplotype is observed mainly in the northeast, bordering Spain, from where it must have been introduced in the Azorean island of

Flores, where a founder effect was responsible for the highest prevalence of MJD reported worldwide (835.2 patients per 100,000 inhabitants; Paula Coutinho, personal communication, 2007).

Based on the mutational age estimation, the presence of MJD in North America is most probably explained by more than one introduction of mutations identical by descent, both directly from the ancestral Asian population, and a derived expanded haplotype from Europe.

12.6 The Machado (São Miguel) Lineage

A different de novo expansion occurred at *ATXN3* on the GTGGCA haplotype, the Machado lineage from the Azorean island of São Miguel, where this is the most common MJD haplotype. The Machado lineage predominates also in central mainland Portugal (mainly along the Tagus valley), but is very rare in other parts of the world. Among a total of 264 MJD families, from 20 different populations, only 7 non-Portuguese families carried this haplotype. [48] The scarcity of this lineage has constrained an accurate analysis of its foundation and history; nonetheless, a recent Portuguese origin, less than 2000 years ago, has been suggested based on the flanking haplotype diversity. Phylogenetic relationships among haplotypes from different populations showed more diversity generated from the most common haplotype in mainland Portugal, supporting the hypothesis of its mutational origin in this region, rather than in São Miguel.

12.7 The Azores as a Platform for Dissemination of MJD

Of note is also the fact that all the known MJD families of Azorean ancestry reside in or ultimately originate from Flores (Joseph) or São Miguel (Machado lineage), which are at the extremes of this widely spaced mid-Atlantic archipelago.

Also, the Azores were uninhabited when they were first discovered in 1432. Their colonization began only after 1444, when they became an important platform for the sea fare of the 1500s and, very likely, for the introduction of MJD in other populations, either due to migration over the past 2 centuries (to countries as Brazil, USA and Canada) or even more ancient population exchanges (but always limited to the last 5 centuries).

12.8 Australian Aboriginal Families

In 1980, Kiloh and colleagues reported an endemic neurological complex disorder in 13 (possibly 16) cases in *tribal* Australian aborigines living on Groote Eylandt and adjacent Arnhem Land area of northeast mainland [58]. In the 1960s,

the disease was already known locally and called the *Groote Eylandt syndrome*. Later, Burt and colleagues reported four Australian aboriginal families at Angurugu (on Groote Eylandt) and at Yirrkala (Arnhem Land), with clinical presentation characteristic of cerebellar ataxia, and having a molecular diagnosis of MJD, by 1996 [59].

Archaeological evidence support continuous occupation of Arnhem Land by these communities for many thousand years, preceding recorded history, which indicates an ancient presence of aboriginal people in these territories, prior to the origin of known MJD mutations. A Portuguese ancestry was in fact first suggested (via the Makassar trepangers, from the southwest corner of Sulawesi, formerly the Celebes, a Portuguese possession from 1512 to 1665); later, the alternative hypothesis of a third mutational event at *ATXN3* was also raised.

In 2012, a study based on full-extended haplotypes, including the 6 previously analysed SNPs and 14 additional ones, in aboriginal and 40 other multi-ethnic MJD families, pointed, however, to a different story [50]. The two aboriginal families studied (from Groote and Yirrkala) shared a full-extended SNP background, named *Joseph-derived haplotype*, since a single variant (rs56268847) differed from the Joseph lineage. Other families with this Joseph-derived haplotype included five from Taiwan, three from India and one from Japan, sharing a common ancestor dating back more than 7000 years. It was then proposed that MJD had been introduced in these isolated aboriginal communities of the Northern Territory in Australia via Asia, since an inter-population pairwise analysis of flanking STRs had shown the families with the Joseph-derived lineage to be phylogenetically closer to the Japanese families than to any other MJD population studied.

12.9 African Families

MJD may be underdiagnosed in most African populations, but a few families have already been reported from Morocco, Algeria, Ghana, Ivory Coast, Mali, Nigeria and Somalia, as well as in African-Americans [9, 43, 48, 60–62]. Haplotype studies performed in these populations are thus scarce in order to gain insight into the mutational origins of MJD in this continent; nevertheless, different genetic backgrounds segregating with expanded alleles in some families point to more than one introduction of MJD in Africa. The worldwide spread Joseph lineage (TTACAC) was observed in each of the families described from Algeria, Nigeria and Somalia, as well as in five African-American kindreds; the Joseph-derived TTAGAC haplotype was found in two families, from Morocco and Ghana; and the nuclear haplotype GCC ($A^{669}TG/G^{669}TG$ - $C^{987}GG/G^{987}GG$ - TAA^{1118}/TAC^{1118}) was seen in three African-American families including the first one in which MJD was suspected, as described by Healton and colleagues [43, 48, 61, 62]. The GTGCCA haplotype was found in only three Azorean families, from São Miguel, and suggested to have derived from the Machado lineage (GTGGCA) by mutation in the $C^{987}GG/G^{987}GG$ SNP. Although identity-by-descent of GCC haplotypes shared by

all families could not be assessed due to lack of flanking extended haplotypes in African patients, the hypothesis of an independent-origin event in MJD seems unlikely.

All eight MJD families of African descent described by Subramony and colleagues [61] were diagnosed with the rare MJD subtype 4 (Parkinsonian phenotype), including the Antigua kindred in whom this phenotype was first observed [63], and also the first family of African ancestry suspected to have MJD [9]. Subramony et al. first explained the scarcity of this MJD subtype in patients of European descent with variation in disease expressivity due to different genetic backgrounds [61]. Repeat length did not seem to be responsible for an ethnical bias for Parkinsonism, since correlation between age-at-onset and allele size was similar in both European and Africans; nevertheless, the authors mentioned that the Antigua kindred (having purer Parkinsonian presentation than the other families) had shorter expanded alleles and later onset.

The absence of a common haplotype among African families with the Parkinsonian phenotype of MJD, led the authors to suggest *trans*-acting genetic variation to explain the unusual presentation in a restricted ethnic background [61]. Nevertheless, these ethnic differences in MJD phenotype must be regarded with caution since, e.g., the Antigua family was a branch of a large kindred to whom belonged also the patients reported by Sequeiros and Suite [64], in 1986, with *typical* MJD presentation. After searching the medical archives of the Johns Hopkins Hospital, the authors came to prove this was the same as the first African-American family described by Healton and Brust in 1980 [9], but also the very same family described by Taniguchi & Konigsmark, as early as 1971 [65], as having a *spinopontine atrophy* (one of the many names MJD has assumed along the times).

References

1. Nakano KK, Dawson DM, Spence A (1972) Machado disease. A hereditary ataxia in Portuguese emigrants to Massachusetts. Neurology 22:49–55
2. Woods BT, Schaumburg HH (1972) Nigro-spino-dentatal degeneration with nuclear ophthalmoplegia. A unique and partially treatable clinico-pathological entity. J Neurol Sci 17:149–166
3. Rosenberg RN, Nyhan WL, Bay C, Shore P (1976) Autosomal dominant striatonigral degeneration. A clinical, pathologic, and biochemical study of a new genetic disorder. Neurology 26:703–714
4. IJDF: International Joseph Diseases Newsletter (1978)
5. Coutinho P (1992) Doença de Machado-Joseph- Tentativa de definição. University of Porto Portugal
6. Coutinho P, Andrade C (1978) Autosomal dominant system degeneration in Portuguese families of the Azores Islands. A new genetic disorder involving cerebellar, pyramidal, extrapyramidal and spinal cord motor functions. Neurology 28:703–709
7. Romanul FC, Fowler HL, Radvany J, Feldman RG, Feingold M (1977) Azorean disease of the nervous system. N Engl J Med 296:1505–1508

8. Lima L, Coutinho P (1980) Clinical criteria for diagnosis of Machado-Joseph disease: report of a non-Azorean Portuguese family. Neurology 30:319–322
9. Healton EB, Brust JC, Kerr DL, Resor S, Penn A (1980) Presumably Azorean disease in a presumably non-Portuguese family. Neurology 30:1084–1089
10. Ishino H, Sato M, Mii T, Terano A, Hayahara T (1971) [An autopsy case of Marie's hereditary ataxia]. Seishin shinkeigaku zasshi = Psychiatria et neurologia Japonica 73:747–757
11. Sakai T, Ohta M, Ishino H (1983) Joseph disease in a non-Portuguese family. Neurology 33:74–80
12. Yuasa T, Ohama E, Harayama H, Yamada M, Kawase Y, Wakabayashi M, Atsumi T, Miyatake T (1986) Joseph's disease: clinical and pathological studies in a Japanese family. Ann Neurol 19:152–157
13. Bharucha NE, Bharucha EP, Bhabha SK (1986) Machado-Joseph-Azorean disease in India. Arch Neurol 43:142–144
14. Zhao JB, Wang TL, Wang GX (1994) [The pathology of Joseph's disease in a Chinese family: a report of two autopsy cases]. Zhonghua bing li xue za zhi = Chin J Pathol 23:232–234
15. Burt T, Blumbergs P, Currie B (1993) A dominant hereditary ataxia resembling Machado-Joseph disease in Arnhem Land, Australia. Neurology 43:1750–1752
16. Goldberg-Stern H, D'Jaldetti R, Melamed E, Gadoth N (1994) Machado-Joseph (Azorean) disease in a Yemenite Jewish family in Israel. Neurology 44:1298–1301
17. Sequeiros J, Coutinho P (1993) Epidemiology and clinical aspects of Machado-Joseph disease. Adv Neurol 61:139–153
18. Fergunson F, Critchley M (1929) A clinical study of an heredo-familial disease resembling disseminated sclerosis. Brain 52:203–225
19. Harding AE (1982) The clinical features and classification of the late onset autosomal dominant cerebellar ataxias. A study of 11 families, including descendants of the 'the Drew family of Walworth'. Brain 105:1–28
20. Giunti P, Sweeney MG, Harding AE (1995) Detection of the Machado-Joseph disease/spinocerebellar ataxia three trinucleotide repeat expansion in families with autosomal dominant motor disorders, including the Drew family of Walworth. Brain 118(Pt 5):1077–1085
21. Wang J, Shen L, Lei L, Xu Q, Zhou J, Liu Y, Guan W, Pan Q, Xia K, Tang B et al (2011) Spinocerebellar ataxias in mainland China: an updated genetic analysis among a large cohort of familial and sporadic cases. Zhong nan da xue xue bao Yi xue ban = J Cent S Univ Med Sci 36:482–489
22. de Castilhos RM, Furtado GV, Gheno TC, Schaeffer P, Russo A, Barsottini O, Pedroso JL, Salarini DZ, Vargas FR, de Lima MA et al (2014) Spinocerebellar ataxias in Brazil–frequencies and modulating effects of related genes. Cerebellum 13:17–28
23. Vale J, Bugalho P, Silveira I, Sequeiros J, Guimaraes J, Coutinho P (2010) Autosomal dominant cerebellar ataxia: frequency analysis and clinical characterization of 45 families from Portugal. Eur J Neurol 17:124–128
24. Boonkongchuen P, Pongpakdee S, Jindahra P, Papsing C, Peerapatmongkol P, Wetchaphanphesat S, Paiboonpol S, Dejthevaporn C, Tanprawate S, Nudsasarn A et al (2014) Clinical analysis of adult-onset spinocerebellar ataxias in Thailand. BMC Neurol 14:75
25. Schols L, Amoiridis G, Buttner T, Przuntek H, Epplen JT, Riess O (1997) Autosomal dominant cerebellar ataxia: phenotypic differences in genetically defined subtypes? Ann Neurol 42:924–932
26. Zhao Y, Tan EK, Law HY, Yoon CS, Wong MC, Ng I (2002) Prevalence and ethnic differences of autosomal-dominant cerebellar ataxia in Singapore. Clin Genet 62:478–481
27. Tsai HF, Liu CS, Leu TM, Wen FC, Lin SJ, Liu CC, Yang DK, Li C, Hsieh M (2004) Analysis of trinucleotide repeats in different SCA loci in spinocerebellar ataxia patients and in normal population of Taiwan. Acta Neurol Scand 109:355–360

28. Stevanin G, Durr A, David G, Didierjean O, Cancel G, Rivaud S, Tourbah A, Warter JM, Agid Y, Brice A (1997) Clinical and molecular features of spinocerebellar ataxia type 6. Neurology 49:1243–1246
29. Maruyama H, Izumi Y, Morino H, Oda M, Toji H, Nakamura S, Kawakami H (2002) Difference in disease-free survival curve and regional distribution according to subtype of spinocerebellar ataxia: a study of 1,286 Japanese patients. Am J Med Genet 114:578–583
30. van de Warrenburg BP, Sinke RJ, Verschuuren-Bemelmans CC, Scheffer H, Brunt ER, Ippel PF, Maat-Kievit JA, Dooijes D, Notermans NC, Lindhout D et al (2002) Spinocerebellar ataxias in the Netherlands: prevalence and age at onset variance analysis. Neurology 58:702–708
31. Paradisi I, Ikonomu V, Arias S (2016) Spinocerebellar ataxias in Venezuela: genetic epidemiology and their most likely ethnic descent. J Hum Genet 61:215–222
32. Moseley ML, Benzow KA, Schut LJ, Bird TD, Gomez CM, Barkhaus PE, Blindauer KA, Labuda M, Pandolfo M, Koob MD et al (1998) Incidence of dominant spinocerebellar and Friedreich triplet repeats among 361 ataxia families. Neurology 51:1666–1671
33. Pujana MA, Corral J, Gratacos M, Combarros O, Berciano J, Genis D, Banchs I, Estivill X, Volpini V (1999) Spinocerebellar ataxias in Spanish patients: genetic analysis of familial and sporadic cases (The Ataxia Study Group). Hum Genet 104:516–522
34. Koutsis G, Kladi A, Karadima G, Houlden H, Wood NW, Christodoulou K, Panas M (2014) Friedreich's ataxia and other hereditary ataxias in Greece: an 18-year perspective. J Neurol Sci 336:87–92
35. Bauer PO, Zumrova A, Matoska V, Marikova T, Krilova S, Boday A, Singh B, Goetz P (2005) Absence of spinocerebellar ataxia type 3/Machado-Joseph disease within ataxic patients in the Czech population. Eur J Neurol 12:851–857
36. Votsi C, Zamba-Papanicolaou E, Georghiou A, Kyriakides T, Papacostas S, Kleopa KA, Pantzaris M, Christodoulou K (2012) Investigation of SCA10 in the Cypriot population: further exclusion of SCA dynamic repeat mutations. J Neurol Sci 323:154–157
37. Sumathipala DS, Abeysekera GS, Jayasekara RW, Tallaksen CM, Dissanayake VH (2013) Autosomal dominant hereditary ataxia in Sri Lanka. BMC Neurol 13:39
38. Sulek-Piatkowska A, Zdzienicka E, Raczynska-Rakowicz M, Krysa W, Rajkiewicz M, Szirkowiec W, Zaremba J (2010) The occurrence of spinocerebellar ataxias caused by dynamic mutations in Polish patients. Neurol Neurochir Pol 44:238–245
39. Juvonen V, Hietala M, Kairisto V, Savontaus ML (2005) The occurrence of dominant spinocerebellar ataxias among 251 Finnish ataxia patients and the role of predisposing large normal alleles in a genetically isolated population. Acta Neurol Scand 111:154–162
40. Dragasevic NT, Culjkovic B, Klein C, Ristic A, Keckarevic M, Topisirovic I, Vukosavic S, Svetel M, Kock N, Stefanova E et al (2006) Frequency analysis and clinical characterization of different types of spinocerebellar ataxia in Serbian patients. Mov Disord: Official J Mov Disord Soc 21:187–191
41. Pazarci P, Kasap H, Koc AF, Altunbasak S, Erkoc MA (2015) Mutation analysis of 6 spinocerebellar ataxia (SCA) types in patients from southern Turkey. Turk J Med Sci 45:1228–1233
42. Smith DC, Greenberg LJ, Bryer A (2016) The hereditary ataxias: where are we now? Four decades of local research. S Afr Med J = Suid-Afrikaanse tydskrif vir geneeskunde 106: S38–41
43. Gaspar C, Lopes-Cendes I, Hayes S, Goto J, Arvidsson K, Dias A, Silveira I, Maciel P, Coutinho P, Lima M et al (2001) Ancestral origins of the Machado-Joseph disease mutation: a worldwide haplotype study. Am J Hum Genet 68:523–528
44. Nachman MW, Crowell SL (2000) Estimate of the mutation rate per nucleotide in humans. Genetics 156:297–304
45. Rubinsztein DC, Leggo J, Coetzee GA, Irvine RA, Buckley M, Ferguson-Smith MA (1995) Sequence variation and size ranges of CAG repeats in the Machado-Joseph disease, spinocerebellar ataxia type 1 and androgen receptor genes. Hum Mol Genet 4:1585–1590

46. Limprasert P, Nouri N, Heyman RA, Nopparatana C, Kamonsilp M, Deininger PL, Keats BJ (1996) Analysis of CAG repeat of the Machado-Joseph gene in human, chimpanzee and monkey populations: a variant nucleotide is associated with the number of CAG repeats. Hum Mol Genet 5:207–213
47. Djian P, Hancock JM, Chana HS (1996) Codon repeats in genes associated with human diseases: fewer repeats in the genes of nonhuman primates and nucleotide substitutions concentrated at the sites of reiteration. Proc Natl Acad Sci USA 93:417–421
48. Martins S, Calafell F, Gaspar C, Wong VC, Silveira I, Nicholson GA, Brunt ER, Tranebjaerg L, Stevanin G, Hsieh M et al (2007) Asian origin for the worldwide-spread mutational event in Machado-Joseph disease. Arch Neurol 64:1502–1508
49. Sequeiros J (1989) Análise genética das causas da vairiação fenotípica na doença de Machado-Joseph. Ph.D. thesis, Universidade do Porto
50. Martins S, Soong BW, Wong VC, Giunti P, Stevanin G, Ranum LP, Sasaki H, Riess O, Tsuji S, Coutinho P et al (2012) Mutational origin of Machado-Joseph disease in the Australian Aboriginal communities of Groote Eylandt and Yirrkala. Arch Neurol 69:746–751
51. Chakravarty A, Mukherjee SC (2002) Autosomal dominant cerebellar ataxias in ethnic Bengalees in West Bengal—an Eastern Indian state. Acta Neurol Scand 105:202–208
52. Jayadev S, Michelson S, Lipe H, Bird T (2006) Cambodian founder effect for spinocerebellar ataxia type 3 (Machado-Joseph disease). J Neurol Sci 250:110–113
53. Bhargava A, Fuentes FF (2010) Mutational dynamics of microsatellites. Mol Biotechnol 44:250–266
54. Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462
55. Putman AI, Carbone I (2014) Challenges in analysis and interpretation of microsatellite data for population genetic studies. Ecol Evol 4:4399–4428
56. Illarioshkin SN, Slominsky PA, Ovchinnikov IV, Markova ED, Miklina NI, Klyushnikov SA, Shadrina M, Vereshchagin NV, Limborskaya SA, Ivanova-Smolenskaya IA (1996) Spinocerebellar ataxia type 1 in Russia. J Neurol 243:506–510
57. Saber S, Rostami M, Dehghan MM, Hooshiar KB, Banoie M, Houshmand M (2006) Molecular investigation SCA in 26 patients suspected to SCA in Iran. Eur J Hum Genet 14:275
58. Kiloh LG, Lethlean AK, Morgan G, Cawte JE, Harris M (1980) An endemic neurological disorder in tribal Australian aborigines. J Neurol Neurosurg Psychiatry 43:661–668
59. Burt T, Currie B, Kilburn C, Lethlean AK, Dempsey K, Blair I, Cohen A, Nicholson G (1996) Machado-Joseph disease in east Arnhem Land, Australia: chromosome 14q32.1 expanded repeat confirmed in four families. Neurology 46:1118–1122
60. Buhmann C, Bussopulos A, Oechsner M (2003) Dopaminergic response in Parkinsonian phenotype of Machado-Joseph disease. Mov Disord: Official J Mov Disord Soc 18:219–221
61. Subramony SH, Hernandez D, Adam A, Smith-Jefferson S, Hussey J, Gwinn-Hardy K, Lynch T, McDaniel O, Hardy J, Farrer M et al (2002) Ethnic differences in the expression of neurodegenerative disease: Machado-Joseph disease in Africans and Caucasians. Mov Disord: Official J Mov Disord Soc 17:1068–1071
62. Ogun SA, Martins S, Adebayo PB, Dawodu CO, Sequeiros J, Finkel MF (2015) Machado-Joseph disease in a Nigerian family: mutational origin and review of the literature. Eur J Hum Genet: EJHG 23:271–273
63. Gwinn-Hardy K, Singleton A, O'Suilleabhain P, Boss M, Nicholl D, Adam A, Hussey J, Critchley P, Hardy J, Farrer M (2001) Spinocerebellar ataxia type 3 phenotypically resembling Parkinson disease in a black family. Arch Neurol 58:296–299
64. Sequeiros J, Suite ND (1986) Spinopontine atrophy disputed as a separate entity: the first description of Machado-Joseph disease. Neurology 36:1408
65. Taniguchi R, Konigsmark BW (1971) Dominant spino-pontine atrophy. Report of a family through three generations. Brain 94:349–358

Chapter 13
Clinical Features of Machado-Joseph Disease

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Abstract Machado-Joseph disease (MJD) also known as Spinocerebellar ataxia type 3, is a hereditary neurodegenerative disease associated with severe clinical manifestations and premature death. Although rare, it is the most common autosomal dominant spinocerebellar ataxia worldwide and has a distinct geographic distribution, reaching peak prevalence in certain regions of Brazil, Portugal and China. Due to its clinical heterogeneity, it was initially described as several different entities and as had many designations over the last decades. An accurate diagnosis become possible in 1994, after the identification of the *MJD1* gene. Among its wide clinical spectrum, progressive cerebellar ataxia is normally present. Other symptoms include pyramidal syndrome, peripheral neuropathy, oculomotor abnormalities, extrapyramidal signs and sleep disorders. On the basis of the presence/absence of important extra-pyramidal signs, and the presence/absence of peripheral signs, five clinical types have been defined. Neuroimaging studies like MRI, DTI and MRS, can be useful as they can characterize structural and functional differences in specific subgroups of patients with MJD. There is no effective treatment for MJD. Symptomatic therapies are used to relieve some of the clinical symptoms and physiotherapy is also helpful in improving quality of live. Several clinical trials have been carried out using different molecules like sulfamethoxazole-trimethoprim, varenicline and lithium carbonate, but the results of these trials were negative or showed little benefit. Future studies sufficiently powered and adequately designed are warranted.

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Polyglutamine diseases · Natural history · Pharmacological treatment

13.1 Epidemiology

Globally, spinocerebellar ataxias (SCA)s are considered rare disorders, with prevalence estimates varying from 0.3 to 2.0 per 100,000 [1]. Machado-Joseph disease (MJD) is presently considered the most common form of SCA worldwide [2]. Among SCAs, the relative frequency of MJD is higher in countries such as Brazil (69–92%) [3], Portugal (58–74%) [4, 5], Singapore (53%) [6], China (48–49%) [7], The Netherlands (44%) [8], Germany (42%) [9], and Japan (28–63%) [10, 11]. It is relatively less frequent in Canada (24%) [12], USA (21%) [13], Mexico (12%) [14], Australia (12%) [15], and India (5–14%) [16], and is considered relatively rare in South Africa (4%) [17] and Italy (1%) [18]. Even within each country the geographic distribution pattern of MJD is not homogeneous. Although constituting the most prevalent subtype of SCA in Portugal, for example, MJD is relatively rare in the mainland (1/100,000) [19], with few exceptions such as a small area of the Tagus River Valley (1/1000) [20], but highly prevalent in the Azores Islands, where the highest worldwide prevalence occurs in Flores Island (1/239) [21].

13.2 History

MJD was first described in 1972 among Portuguese immigrants living in New England, United States of America (USA). These immigrants were descendants of William Machado, a native of the Portuguese Azorean islands [22]. A few years later, other cases in families of Portuguese-Azorean extraction were described in the USA (Thomas and Joseph families) [23, 24]. Between 1972 and 1977 the disease was identified in four families, reported as four distinct entities named "Machado disease" [22], "nigrospino-dentatal degeneration" [24], "Joseph disease" [23] and "Azorean disease of the nervous system" [1]. In 1975, Coutinho and Andrade studied 15 families from the Azorean Islands and proposed that the above-mentioned diseases were simply variations of the same clinical disorder [25]. They defined it as "Machado-Joseph disease," a single disorder characterized by an unusually high degree of clinical variability. In 1977, it became commonly accepted that it was a single genetic disease with remarkable phenotypic expression [1, 25]. However, it was only after identification of the *MJD1* gene in 1994 [26] that an accurate diagnosis of MJD becomes available. Furthermore, in 1995, it was observed that an ataxochoreic form of dentatorubral-pallidoluysian atrophy

(DRPLA), spinocerebellar ataxia type 3 (SCA3) and Machado-Joseph disease were in fact the same entity [27]. The ancestral mutation origin and the presence of a founder effect have been a source of much speculation over the years [28–31]. The most accepted theory is that the disease was originated by two distinct mutational events in the affected gene (A-C-A and G-G-C intragenic haplotypes). In MJD gene carriers, the A-C-A haplotype is the most common worldwide, whereas the presence of both haplotypes A-C-A and G-G-C mostly occurs in Portugal (mainland and Azores) and in countries historically related to Portugal (Brazil, Spain, USA) (Gaspar C L.-C. I., [32]. Therefore, it is possible that the mutations were brought from mainland Portugal to Azores (Flores and São Miguel islands) upon colonization in the 15th century [32, 33]; Rosenberg, Autosomal dominant cerebellar phenotypes: the genotype has settled issue, 1995; [29] and worldwide during the Portuguese Age of Discovery in the 15th and 16th century along with the Azorean emigration waves in the 17th century [32, 33]; Rosenberg, Autosomal dominant cerebellar phenotypes: the genotype has settled issue, [29, 34]. The Portuguese sea travels could, therefore, be a possible explanation for the presence of MJD in countries like India, China, Japan, Yemen and parts of Africa [29]. Nevertheless, the mutational event relative to the A-C-A haplotype may have occurred in another ethnic group and later brought to Europe, including Portugal [32]. One possibility is the introduction of large normal alleles with the A-C-A haplotype in Portugal due to intermingling between South Asian and Portuguese populations in the 15th century, which later expanded and originated the MJD mutation [35]. However, the heterogeneity among French and German as compared to Portuguese families does not support the hypothesis of Portugal as the principal source to the establishment of the A-C-A haplotype in Europe [36]. This lineage, the Joseph lineage, seems to have occurred about 7000 years ago, in Asia and is the most common in the vast majority of non-Portuguese. The G-G-C haplotype, or Machado lineage, occurred afterwards and is the most common in mainland of Portugal, the Azores and in other families of proven or suspected Portuguese ancestry and that most probably have a Portuguese origin [32, 36, 37].

13.3 Clinical Symptoms and Natural History

The discovery of MJD illustrates the difficulty of defining a disease as a single entity when various symptoms are hallmarks of the disease.

MJD is, in fact, characterized by a wide range of clinical manifestations. Progressive cerebellar ataxia is normally present, leading to motor incoordination that can affect balance, gait, and speech. In addition, other symptoms may be present, such as: a pyramidal syndrome with brisk deep tendon reflexes, Babinski sign and spasticity; peripheral neuropathy with amyotrophy; oculomotor abnormalities with nystagmus, eyelid retraction, progressive external ophthalmoplegia

(PEO), ophthalmoparesis, bulging eyes; facial and lingual fasciculation; extrapyramidal signs, including dystonia, rigidity and/or bradykynesia; as well as weight loss and sleep disorders (Rosenberg, Machado-Joseph disease: an autosomal dominant motor system degeneration [38]) [29, 39].

A clinical diagnosis of MJD is suspected in individuals with progressive cerebellar ataxia and pyramidal signs, associated with a complex clinical picture extending from extrapyramidal signs to peripheral amyotrophy [40]. Minor, but more specific features such as PEO (especially for upward gaze), dystonia, fasciculation of facial and lingual muscles, as well as bulging eyes, are also of importance in the diagnosis [40]. The mean age at onset is around 40 years, with extremes of 4 [41] and 70 years [19], and a mean survival time of 21 years (ranging from 7 to 29 years) [19] [42]. Gait ataxia is reported as the first symptom in 92.4% [19].

MJD is highly pleomorphic, not only in the variability in the age at onset, but also in the neurological signs presented by different patients as well as in the resulting degree of incapacity. The marked clinical heterogeneity and the progressive nature of MJD rendered its clinical classification difficult and nowadays of limited clinical value to neurologists. The most used classification was created by Coutinho and Andrade [25] who have systematized the disease phenotypes into three main clinical types (see Table 13.1). The authors observed that almost every patient presents with cerebellar signs and PEO, associated with pyramidal signs in variable degrees. Clinical types could, therefore, be distinguished on the basis of the presence/absence of important extra-pyramidal signs, and the presence/absence of peripheral signs. Type 1 (“type Joseph”) is characterized by an early onset (mean of 24.3 years) and a rapid progression of symptoms, which together with cerebellar

Table 13.1 Classification of MJD according to symptoms, prevalence and age of onset

MJD type	Age of onset (years)	Prevalence	Symptoms
I	5–30		Limb and gait ataxia, severe dystonia, pyramidal signs, PEO and a relatively faster progression of symptoms
II	≈36	Most frequent	Ataxia, pyramidal deficits and PEO
III	≈50	Second most frequent	Cerebellar ataxia and PEO, associated with peripheral alterations, may feature pyramidal and extrapyramidal signs
IV	38–47	Typical in patients with short CAG repeat expansions	Slowly progressive parkinsonism, responsive to L-DOPA, fasciculations and peripheral neuropathy
V			Marked spastic paraplegia with or without cerebellar ataxia. Important differential diagnosis with hereditary spastic paraplegia

ataxia and PEO include marked pyramidal and extrapyramidal signs (such as dystonia). Type 2 ("type Thomas") corresponds to presentations with an intermediate onset (mean of 40.5 years), cerebellar ataxia and PEO, with or without pyramidal sings. When present, the extrapyramidal and peripheral signs are tenuous. Patients with type 2 features may maintain these for long periods or evolve (5–10 years later) to type 1 or type 3, by the manifestation of important extrapyramidal or peripheral signs, respectively. Type 3 ("type Machado") presents a later onset (mean of 46.8 years) and is characterized by cerebellar ataxia and PEO, associated with peripheral alterations, with or without slight pyramidal and extrapyramidal signs [19]. These three clinical types can occasionally be present in the same family Table 13.2. Additionally, some authors consider as type 4 a rare presentation with parkinsonian features, mild cerebellar deficits and a distal motor sensory neuropathy or amyotrophy [43]. Furthermore, Sakai and Kawakami [44] observed two siblings that presented spastic paraplegia without cerebellar ataxia and proposed the existence of a fifth type for MJD.

13.4 Non-motor and Extra-Cerebellar Symptoms in MJD

The pathological and neurodegenerative processes in MJD affect a large variety of functional and neurotransmitter systems. This results in a widespread pattern of damage to the central and peripheral nervous system. Thus, several non-motor and extra-cerebellar symptoms may occur and are likely to be under-recognized in clinical practice. Although there is still no disease modifying therapy, many of the extra-cerebellar symptoms are suitable to symptomatic treatment, which can significantly improve the quality of life in patients with MJD [45].

The main non-motor and extra-cerebellar symptoms include sleep disorders, cognitive deficits, olfactory dysfunction, psychiatric symptoms, pain, cramps and fatigue, autonomic dysfunction, nutritional difficulties, neuropathy, movement disorders and autonomic dysfunction [45]. In this chapter, we briefly describe some of the most relevant symptoms.

Sleep disorders frequently occur in the context of neurodegenerative diseases, particularly the alpha-synucleinopathies (e.g. Parkinson Disease, Multiple System Atrophy and Lewy Body Dementia), and are also common in the SCAs. Among all subtypes of SCAs, MJD has the highest incidence of sleep disorders [46]. The main sleep disorders described are restless legs syndrome (RLS), rapid eye movement (REM) sleep behavior disorder (SBD), periodic limb movement during sleep (PLMS), sleep apnea, insomnia, and excessive daytime sleepiness (EDS) [47, 48]. Available evidence demonstrates that the frequency of RLS in MJD is significantly greater than in the general population, affecting up to 55% of patients [49]. It is interesting to note that SBD may precede gait ataxia by several years [50].

Among cognitive and affective disturbances, the so-called cerebellar cognitive affective syndrome (CCAS) has been described in patients with different types of

Table 13.2 Clinical trials in Machado-Joseph disease

Author, year (Refs.)	Study drug	Study design	Number of subjects	Trial duration (weeks)	Primary endpoint	Study results
[127]	Sulfamethoxazole-trimethoprim	Double-blind, placebo-controlled, crossover	22 genetically confirmed SCA3	24	Ataxia ranking Scale (ARS)	Negative
[133]	Fluoxetine	Open-label	13 genetically confirmed SCA3	6	Tended disability status Scale of Kurtzke (18) and the unified Parkinson's disease rating scale (1 tended disability status Scale of Kurtzke (18) and the unified Parkinson's disease rating scale (1 extended disability status Scale of Kurtzke (EDSS) unified Parkinson's disease rating scale (UPDRS)	No evidence of benefit
[134]	Tandospirone citrate (tandospirone) a selective 5HT1A receptor agonist	Open-label	10 genetically confirmed SCA3	7	(ARS and total length traveled (TLT) Self-rating depression scale (SDS)	Some evidence of benefit in ataxic speech

(continued)

Table 13.2 (continued)

Author, year (Refs.)	Study drug	Study design	Number of subjects	Trial duration (weeks)	Primary endpoint	Study results
[123]	Varenicline (partial agonist at $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptors)	Double-blind, placebo-controlled, randomized study	20 genetically confirmed SCA3	8	SARA timed 25-foot walk and 9-hole peg test	Some evidence that varenicline improved the axial functions of gait, stance, and timed 25-foot walk
[128]	Lithium carbonate	randomized, double-blind, parallel, placebo-controlled trial	62 genetically confirmed MJD subjects with a disease duration ≤ 10 years	48	NESSCA and safety Spinocerebellar ataxia (NESSCA)	Lithium was safe and well tolerated, but it had no effect on progression when measured using the NESSCA
[135]	Lamotrigine	open-label	6 genetically confirmed SCA3	7	One leg standing test (OLST) and tandem gait index (TGI)	No evidence of benefit

cerebellar diseases and is possibly related to disruptions of cerebro-cerebellar-cerebral loops [51, 52]. CCAS is characterized by cognitive impairment within the executive and visuospatial domains and by language disturbances and affective symptoms [51]. Executive and visuospatial dysfunctions, together with depression and anxiety, are the main symptoms described [53–57]. On neuropsychological testing, patients with MJD may present executive dysfunction, slowed processing of visual information in complex tasks, abnormal shifting of visual attention, and also verbal and visual memory deficits [53–57]. Nevertheless, although cognitive deficits are frequently reported in patients with MJD, dementia or severe cognitive impairment are rarely described [53–57].

Psychiatric symptoms are also common in patients with MJD, and depressive manifestations are by far the most frequent [58], followed by anxiety. Depressive symptoms are more frequent in patients with MJD than in matched controls [56, 59–61], caregivers, or individuals who are at risk for MJD [62–64]. Despite its importance, depression is probably under diagnosed in SCAs; consequently, a great proportion of patients are not properly treated [59, 62–64].

Olfactory dysfunction is also a very common and early sign in different neurodegenerative diseases and it has been recently described in hereditary ataxias [65–70]. Notably, a recent study described olfactory dysfunction in a large group of patients with MJD (N541), measured through the Sniffin's Sticks (SS-16) odor identification test [57].

Peripheral neuropathy (PN) in MJD has long been known and may be the presenting manifestation of the disease [71–73]. Previous estimates indicate that 60% of all patients ultimately develop clinical signs of peripheral neuropathy [74, 75]. Sensory fibers are the most frequently affected [75]. Damage to motor fibers may cause muscle atrophy, distal weakness with foot drop, and fasciculations, which may further aggravate the functional difficulties [75]. Nerve conduction studies have demonstrated widespread reduction of the amplitudes of motor and especially sensory potentials [74–76]. Needle electromyography (EMG) reveals signs of active and chronic denervation in both proximal and distal muscles. Fasciculation potentials and myokymia are other typical EMG findings [74–76]. Overall, the neurophysiological pattern suggests that PN in MJD is a double neuronopathy in most patients (sensory neuronopathy combined with motor neuron disease). PN is usually found in patients with late disease onset and small CAG expansions [74, 75]. It is interesting to realize, however, that the rate of progression of peripheral nerve damage is higher in patients with larger (CAG) expansions [75].

The frequency of fatigue is greater in MJD patients compared with healthy individuals. Patients with severe fatigue tend to have longer disease duration and are more likely to be wheelchair-bound. There is a significant, positive correlation between Fatigue Severity Scale scores and excessive daytime sleepiness scores. Nonetheless, the frequency of fatigue remained stable across different ataxias, including MJD [77]. The factors that predicted fatigue in patients with ataxia were physical functioning and depression. No correlations with disease duration or the Scale for the Rating and Assessment of Ataxia (SARA) scores were reported [77].

Cramps are a frequent and disabling symptom in MJD [78, 79]. Forty-one of 50 patients presented with muscle cramps in 1 study, whereas cramps were the presenting symptom in 10 patients and the chief complaint in 15 patients [79]. They usually occurred in the lower limbs but also in the arms, trunk, face, and abdominal muscles [78, 79]. No clinical or neurophysiological differences were reported between patients with and without cramps.

There have been at least 4 reports of chronic pain in patients with MJD; however, only 1 was a systematic evaluation [80]. In that study, which included 70 MJD patients, almost half of the patients reported chronic pain and lower back pain was the presenting symptom in 6 patients. Pain was most frequently musculoskeletal; however, in a smaller subset, it was related to dystonia and/or neuropathy. It is noteworthy that patients who had pain had longer CAG expansion [80].

Autonomic dysfunction is another common finding in MJD that is frequently overlooked because of the marked motor impairment [81, 82]. Overall, more than 50% of patients present with at least 1 dysautonomic symptom. Nocturia and urinary incontinence (64%) followed by cold intolerance and disorders of sweating (48%) were the most frequent complaints in 2 studies from Brazil and Taiwan [81, 82]. Gastrointestinal abnormalities (especially constipation) and sexual abnormalities were identified in fewer than 30% of patients. Orthostatic hypotension was much less frequent [81, 82]. Autonomic dysfunction is frequently subclinical in MJD and is only identified through additional investigation [83, 84]. Sudomotor responses were reported as abnormal in 36–73% of patients with MJD [82, 84]. It is likely that the underlying cause for dysautonomia is the damage to autonomic ganglia and central pathways. However, it is not yet clear why some (but not all) patients develop marked autonomic dysfunction.

Movement disorders in MJD include both hypokinetic disorders (parkinsonism) and hyperkinetic disorders (dystonia, myoclonus, chorea, and tremor). They may predominate in some clinical phenotypes and pose a challenge to accurately diagnosing the SCA subtype. Today, it is believed that the presence of movement disorders in patients with SCA is a sign of degeneration of other brain areas in addition to the cerebellum, although some studies have pointed to cerebellar involvement in the origin of some forms of dystonia and myoclonus [85].

Parkinsonism, especially an akinetic-rigid syndrome, has been described in patients with SCA—particularly in those with SCA2, MJD, and SCA17—and is a common condition in Asian patients [85–87]. Some series have reported a particular association of pure parkinsonism with patients of African origin [85, 88–93]. It should be noted that levodopa is a very effective treatment in this group of patients with MJD [94].

Dystonia also has been described in patients with MJD as either focal, segmental, or even a generalized form [85–87]. Patients with early onset of MJD present with a greater frequency of dystonia, which is also associated with larger CAG repeat expansions [94–96]. Schmitz-Hubsch et al. studied several different patients with SCA and observed dystonia in 23.9% of those with MJD, whereas Schols et al. reported dystonia in 10% [86, 87]. Some patients with MJD may have

a clinical presentation that closely resembles dopamine-responsive dystonia with very mild cerebellar signs [40].

Less common manifestations observed in MJD cohorts include myoclonus and chorea, which have been observed in up to 4.4% and up to 10.1% of subjects respectively. [40, 87, 95, 96]. Different forms of tremor can be found in MJD, including titubation and intention tremor that are associated with cerebellar ataxia, and resting tremor which occurs as part of Parkinson disease [85, 86, 87, 95]. Other movement disorders have been more rarely related in patients with MJD, for example, stiff-person syndrome and akathisia [85, 97]. Basal ganglia involvement, substantia nigra degeneration, and a dopaminergic dysfunction are the most likely explanations presumed to be the anatomical basis for the constellation of movement disorders observed in patients with MJD [85, 97].

13.5 Neuroimaging

Magnetic resonance imaging (MRI) has been considered a useful tool to uncover the neuroanatomical substrate of MJD [98–103]. Volumetric analyses of MRI datasets have demonstrated atrophy of both supratentorial and infratentorial structures [104, 105]. Previous studies identified cerebellar (vermian and hemispheric) as well as brainstem volumetric reduction [104, 105, 106]. Interestingly, disease severity correlated with these volumetric measures. Volumetric reduction also extended to the basal ganglia (caudate nuclei, putamen and thalami) and cerebral cortex (particularly at frontal and temporal regions) [104, 105, 106]. Longitudinal assessment of volumetric changes was reported in 2 studies [104, 107]. D'Abreu et al. failed to identify prospective changes after 12 months in a cohort of 30 patients, but Reetz et al. found progressive volumetric reduction at the basal ganglia after 24 months of follow-up. Surprisingly, the rate of atrophy was not correlated with the length of (CAG) expansion.

Diffusion tensor imaging (DTI) is another powerful technique to assess white matter integrity. In MJD, DTI-based analyses revealed microstructural damage to the cerebellum (including the dentate and other nuclei), cerebellar peduncles, and the brain stem, including the midbrain and pons [108].

Magnetic resonance spectroscopy (MRS) studies have also shown abnormalities in MJD [109, 110]. These involved not only the cerebellum and connections, but also the apparently normal deep cerebral white matter [109]. The most conspicuous finding was reduction of N-acetylaspartate/creatine ratio (marker of axonal/neuronal integrity) at the cerebellar hemispheres and deep nuclei [111].

Neuroimaging studies have been lately employed to characterize structural and functional differences in specific subgroups of patients with MJD [112, 113]. Jacobi et al. studied at risk individuals for MJD and found that mutation carriers already have brainstem volumetric reduction when compared to non-mutation

carriers [112]. The extent of central nervous system damage does not seem to be homogeneous in MJD, a finding that helps to explain the remarkable phenotypic heterogeneity of the disease. Nunes et al. have indeed shown that MJD patients with dystonia present different findings in comparison to non-dystonic patients. Dystonic patients have precentral cortical atrophy and more severe volumetric reduction at the basal ganglia (especially the thalami) [113].

Neuroimaging methods other than MRI have also proven useful to investigate cerebral abnormalities in MJD. Brain SPECT demonstrated perfusion abnormalities in the parietal lobes, inferior portion of the frontal lobes, mesial and lateral portions of the temporal lobes, basal ganglia, and cerebellar hemispheres and vermis [114]. The 18F-Dopa uptake in MJD was found to be significantly decreased not only in the regions with apparent pathological involvement such as cerebellum, brainstem and nigro-striatal dopaminergic system, but also in the cerebral cortex and the striatum, where no pathology could be observed using conventional morphological techniques [115]. A PET with fluorine-18-fluorodeoxyglucose (FDG) study in seven asymptomatic MJD patients showed subclinical changes of FDG consumption: decrease in the cerebellar hemispheres, brainstem, as well as occipital, parietal and temporal cortices suggesting preclinical disease activity [116].

13.6 Patient Management

Regarding the treatment of disease, there are yet no effective pharmacologic approaches for MJD. Symptomatic pharmacologic therapeutics have been used to alleviate some of the clinical signs, namely spasticity [117, 118], parkinsonism [88, 119], dystonia [120, 121], and muscle cramps [79].

In addition to pharmacological therapy, physiotherapy may help the patients to cope with the disability associated with gait problems [122]. Physical aids, such as walkers and wheelchairs, can assist the patients in their everyday activities. Furthermore, regular speech therapy for dysarthria and dysphagia as well as occupational therapy may have beneficial effects [122].

Several clinical trials have been carried out, but a critical weakness of many of these studies is the small number of participants. This is typical of trials in rare disorders such as MJD, which makes recruitment for large studies particularly challenging [123].

The first double-blind, placebo-controlled, clinical trials were performed with sulfamethoxazole and trimethoprim, in a small cohort of MJD patients [118, 124–126]. From those studies, encouraging results on spasticity, walker assisted gait [125], and contrast sensitivity were obtained [124]. Mild improvements of hyperreflexia of knee jerks and spasticity of the legs [126], and beneficial effects on gait and coordination were also observed [118]. However, all 4 studies were done with small cohorts and had short if any placebo controlled periods. To clarify the

relevance of this medication a larger, double-blind, placebo-controlled crossover trial was performed in 22 patients with genetically confirmed MJD. The investigators analyzed short- and long-term effects and performed posturography and a computer-based dexterity test and obtained achromatic contrast sensitivity, color discrimination, and clinical scores. Additionally, sub-group analysis using clinical and genetic variables were performed to search for subtypes of MJD responding to trimethoprim-sulfamethoxazole. Unfortunately, the results showed that in a homogenous, genetically determined study population, treated for 6 months, trimethoprim-sulfamethoxazole was not an effective treatment for MJD [127].

Varenicline (Chantix; Pfizer, New York, NY), a partial agonist of $\alpha 4\beta 2$ neuronal nicotinic receptors, that is US Food and Drug Administration–approved as a smoking cessation drug, has also been tried in MJD. The investigators sought to evaluate the efficacy of varenicline in a controlled, multicenter trial. Twenty patients with genetically confirmed MJD were randomly assigned to receive either varenicline (4 weeks for titration and 4 weeks at a dose of 1 mg twice daily) or placebo. The primary efficacy measures included changes in the SARA (Schmitz-Hübsch 2006) subscale scores to assess improvements in axial or appendicular function. Secondary measures included a timed 25-foot walk (axial function), a 9-hole peg test (appendicular function), Beck Depression Inventory, Beck Anxiety Inventory, Clinical Global Impression, Patient Global Impression, and the Short Form 36 to assess activities of daily living. Improvements were noted in the SARA subsections for gait, stance, rapid alternating movements, timed 25-foot walk and Beck Depression Inventory scores in patients taking varenicline compared with those taking placebo at endpoint, with a trend toward improvement in the SARA total score in the varenicline group. Varenicline was well tolerated, and the number of reported adverse events was similar between patients who were treated with varenicline and those who received placebo [123]. There were, however, several weaknesses to the study. Namely, the small number of patients who participated and the high placebo dropout rate (4 of 10 patients). Moreover, the authors cautioned that the effect of nicotinic acetylcholine agonists on spasticity, posterior column disease, or peripheral neuropathy is unclear and that patients who have these conditions or have prolonged timed 25-foot walking scores should not be treated with varenicline until further research is conducted [123].

One of the largest clinical trials in MJD was performed by Jardim et al. The trialists evaluated in a randomized, parallel, placebo-controlled, 48-week trial the safety and efficacy of lithium carbonate in 62 MJD patients [128]. The results showed that lithium was considered safe but resulted in no statistically significant difference in the progression of the primary efficacy outcome the Neurological Examination Score for the Assessment of Spinocerebellar Ataxia (NESSCA) [129]. Furthermore, the investigators studied several secondary outcome measures and, as expected when evaluating multiple outcomes, received mixed results. Whereas no significance was obtained with the SARA [130], some time-based ataxia functional scores and their composite scores SCA Functional Index (SCAFI) [87] and

Composite Cerebellar Functional Score (CCFS) [131] presented less severe progression in the lithium than in the placebo group. Due to the apparent conflicting results in some of the secondary measures the authors suggest that further clarification regarding the putative benefits of lithium is warranted. Furthermore, the investigators published additional observations from the Lithium trial suggesting that future phase 2 clinical trials in MJD should use the SCAFI or CCFS as the primary outcome. They argue that data obtained in a phase 2 study using these composite scales could then allow for sample size estimation for a phase 3 trial, with the same intervention, but where an ataxia scale (e.g. SARA) would be the primary outcome. However, in our view, some caution is needed since changing primary endpoints between phase 2 and phase 3 trials greatly increases the uncertainty of reproducing the phase 2 study results.

The authors also mentioned that although relevant and disabling, extra-cerebellar features of MJD progress slower than ataxia over time and due to that, they should be assessed only as secondary outcomes for disease modifying therapy trials. Moreover, only patients still capable of independent or minimally assisted walking should be included, due to a possible higher sensitivity to change of the clinical scales in this subgroup of patients. Finally, after observing the progression of the SARA in the placebo group, the authors suggest that future phase 3 studies should be planned for 18–24 months duration [132].

As described the information gathered by investigators in all of these past trials will help guide the design of future studies. It is critical that sufficiently powered studies, with adequate duration of treatment, that select the most relevant endpoints and with a design suitable to study rare neurological diseases are performed if we are to detect meaningful effects in disease progression. Future studies should also be based on a deep knowledge of relevant molecular targets in MJD and a clear understanding of the mechanism of action of the experimental compound and backed by solid evidence of target engagement in pre-clinical models.

Conflict of Interest: Nuno Mendonça is a full time AbbVie Deutschland GmbH & Co. KG employee and may hold AbbVie stock and/or stock options.

The content of this work is not associated with the author responsibilities as an AbbVie employee.

References

1. Romanul FC et al (1977) Azorean disease of the nervous system. N Engl J Med 296 (26):1505–1508
2. Schols L et al (2004) Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 3(5):291–304
3. Jardim LB et al (2001) A survey of spinocerebellar ataxia in South Brazil—66 new cases with Machado-Joseph disease, SCA7, SCA8, or unidentified disease-causing mutations. J Neurol 248:870–876

4. Silveira I et al (1998) Analysis of SCA1, DRPLA, MJD, SCA2, and SCA6 CAG repeats in 48 Portuguese ataxia families. Am J Med Genet 81:134–138
5. Vale J et al (2010) Autosomal dominant cerebellar ataxia: frequency analysis and clinical characterization of 45 families from Portugal. Eur J Neurol 17(1):124–128
6. Zhao Y et al (2002) Prevalence and ethnic differences of autosomal-dominant cerebellar ataxia in Singapore. Clin Genet 62:478–481
7. Tang B et al (2000) Frequence of SCA1, SCA2, SCA3/MJD, SCA6, SCA7, and DRPLA CAG trinucleotide repeat expansion in patients with hereditary spinocerebellar ataxia from Chinese kindreds. Arch Neurol 57:540–544
8. van de Warrenburg BP et al (2002) Spinocerebellar ataxias in the Netherlands: prevalence and age at onset variance analysis. Neurology 58:702–708
9. Schols L et al (1997) Autosomal dominant cerebellar ataxia: phenotypic differences in genetically defined subtypes? Ann Neurol 42:924–932
10. Maruyama H et al (2002) Difference in disease-free survival curve and regional distribution according to subtype of spinocerebellar ataxia: a study of 1,286 Japanese patients. Am J Med Genet 114:578–583
11. Shibata-Hamaguchi A et al (2009) Prevalence of spinocerebellar degenerations in the Hokuriku district in Japan. Neuroepidemiology 32:176–183
12. Kraft S et al (2005) Adult onset spinocerebellar ataxia in a Canadian movement disorders clinic. Can J Neurol Sci 32:450–458
13. Moseley ML et al (1998) Incidence of dominant spinocerebellar and Friedreich triplet repeats among 361 ataxia families. Neurology 51:1666–1671
14. Alonso E et al (2007) Distinct distribution of autosomal dominant spinocerebellar ataxia in the Mexican population. Mov Disord 22:1050–1053
15. Storey E et al (2000) Frequency of spinocerebellar ataxia types 1, 2, 3, 6, and 7 in Australian patients with spinocerebellar ataxia. Am J Med Genet 95:351–357
16. Saleem Q et al (2000) Molecular analysis of autosomal dominant hereditary ataxias in the Indian population: high frequency of SCA2 and evidence for a common founder mutation. Hum Genet 106:179–187
17. Bryer A et al (2003) The hereditary adult-onset ataxias in South adult-onset ataxias in South Africa. J Neurol Sci 216:47–54
18. Brusco A et al (2004) Molecular genetics of hereditary spinocerebellar ataxia: mutation analysis of spinocerebellar ataxia genes and CAG/CTG repeat expansion detection in 225 Italian families. Arch Neurol 61:727–733
19. Coutinho P (1992) Doença de Machado-Joseph: Tentativa de definição. Ph.D. Dissertation, Instituto de Ciências Biomédicas Abel Salazar, Porto
20. Maciel P et al (2001) Improvement in the molecular diagnosis of Machado-Joseph disease. Arch Neurol 58:1821–1827
21. Bettencourt C et al (2008) Analysis of segregation patterns in Machado-Joseph disease pedigrees. J Hum Genet 53:920–923
22. Nakano KK et al (1972) Machado disease. A hereditary ataxia in Portuguese emigrants to Massachusetts. Neurology 22:49–55
23. Rosenberg R, Nyhan W, Bay C, Shore P (1976) Autosomal dominant striatonigral degeneration. A clinical, pathologic, and biochemical study of a new genetic disorder. Neurology 26:14–703
24. Woods BT et al (1972) Nigro-spino-dentatal degeneration with nuclearophthalmoplegia. A unique and partially treatable clinico-pathological entity. J Neurol Sci 17:149–166
25. Coutinho P et al (1978) Autosomal dominant system degeneration in Portuguese families of the Azores Islands. A new genetic disorder involving cerebellar, pyramidal, extrapyramidal and spinal cord motor functions. Neurology 28:703–709
26. Kawaguchi Y et al (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8:221–228

27. Cancel G et al (1995) Marked phenotypic heterogeneity associated with expansion of a CAG repeat sequence at the spinocerebellar ataxia 3/Machado-Joseph disease locus. Am J Hum Genet 57:809–816
28. Gaspar C et al (1996) Linkage disequilibrium analysis in Machado-Joseph disease patients of different ethnic origins. Hum Genet 98:620–624
29. Sequeiros J et al (1993) Epidemiology and clinical aspects of Machado-Joseph disease. Adv Neurol 61:139–153
30. Stevanin G et al (1995) Linkage disequilibrium at the Machado-Joseph disease/spinal cerebellar ataxia 3 locus: evidence for a common founder effect in French and Portuguese-Brazilian families as well as a second ancestral Portuguese-Azorean mutation. Am J Hum Genet 57:1247–1250
31. Takiyama Y et al (1995) Evidence for inter-generational instability in the CAG repeat in the MJD1 gene and for conserved haplotypes at flanking markers amongst Japanese and Caucasian subjects with Machado-Joseph disease. Hum Mol Genet 4:1137–1146
32. Gaspar C et al (2001) Ancestral origins of the Machado-Joseph disease mutation: a worldwide haplotype study. Am J Hum Genet 68:523–528
33. Lima M et al (1998) Origins of a mutation: population genetics of Machado-Joseph disease in the Azores (Portugal). Hum Biol 70:1011–1023
34. Rosenberg R (1995) Autosomal dominant cerebellar phenotypes: the genotype has settled issue. Neurology 45:1–5
35. Mittal U et al (2005) Founder haplotype for Machado-Joseph disease in the Indian population: novel insights from history and polymorphism studies. Arch Neurol 62:637–640
36. Martins S et al (2007) Asian origin for the worldwide-spread mutational event in Machado–Joseph disease. Arch Neurol 64:1502–1508
37. Martins S et al (2012) Mutational origin of Machado–Joseph disease in the Australian Aboriginal communities of Groote Eylandt and Yirrkala. Arch Neurol 69:746–751
38. Rosenberg R (1992) Machado-Joseph disease: an autosomal dominant motor system degeneration. Mov Disord 7:193–203
39. Sudarsky LCP (1995) Machado-Joseph disease. Clin Neuro sci 3:17–22
40. Lima L et al (1980) Clinical criteria for diagnosis of Machado-Joseph disease: report of a non-Azorena Portuguese family. Neurology 30:319–322
41. Carvalho DR et al (2008) Homozygosity enhances severity in spinocerebellar ataxia type 3. Pedriatr Neurol 38(4):296–299
42. Kieling C et al (2007) Survival estimates for patients with Machado-Joseph disease (SCA3). Clin Genet 72(6):543–545
43. Suite ND et al (1986) Machado-Joseph disease in a Sicilian-American family. J Neurogenet 2(2):177–182
44. Sakai T et al (1996) Machado-Joseph disease: A proposal of spastic paraplegic subtype. Neurology 46(3):846–847
45. Pedroso JL et al (2013) Nonmotor and extracerebellar features in Machado-Joseph disease: a review. Mov Disord 28:1200–1208
46. Raggi A et al (2010) Sleep disorders in neurodegenerative diseases. Eur J Neurol 17:1326–1338
47. D'Abreu A et al (2009) Sleep symptoms and their clinical correlates in Machado-Joseph disease. Acta Neurol Scand 119:277–280
48. Pedroso JL et al (2011) Sleep disorders in cerebellar ataxias. Arq Neuropsiquiatr 69:253–257
49. Pedroso JL et al (2011) Sleep disorders in Machado-Joseph disease: frequency, discriminative thresholds, predictive values, and correlation with ataxia-related motor and non-motor features. Cerebellum 10:291–295
50. Rub U et al (2008) New insights into the pathoanatomy of spinocerebellar ataxia type 3 (Machado-Joseph disease). Curr Opin Neurol 21:111–116
51. Schmahmann JD et al (2006) Cognition, emotion and the cerebellum. Brain 129:288–292

52. Stoodley CJ et al (2010) Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. Cortex 46:831–844
53. Radvany J et al (1999) Machado Joseph disease of Azorean ancestry in Brazil: the Catarina kindred. Neurological, neuroimaging, psychiatric and neuropsychological findings in the largest known family, the Catarina kindred. Arq Neuropsiquiatr 51:21–30
54. Zawacki TM et al (2002) Executive and emotional dysfunction in Machado-Joseph disease. Mov Disord 17:1004–1010
55. Maruff P et al (1996) Cognitive deficits in Machado-Joseph disease. Ann Neurol 40:421–427
56. Kawai Y et al (2004) Cognitive impairments in Machado-Joseph disease. Arch Neurol 61:1757–1760
57. Braga-Neto P et al (2011) Cerebellar cognitive affective syndrome in Machado Joseph disease: core clinical features. Cerebellum 11:549–556
58. Schmitz-Hubsch T et al (2010) Self-rated health status in spinocerebellar ataxia—results from a European multicenter study. Mov Disord 25:587–595
59. Schmitz-Hubsch T et al (2011) Depression comorbidity in spinocerebellar ataxia. Mov Disord 26:870–876
60. Braga-Neto P et al (2012) Cognitive deficits in Machado-Joseph disease correlate with hypoperfusion of visual system areas. Cerebellum 11:1037–1044
61. Braga-Neto P et al (2012) Cognitive and olfactory deficits in Machado-Joseph disease: a dopamine transporter study. Parkinsonism Relat Disord 18:854–858
62. Cecchin CR et al (2007) Depressive symptoms in Machado-Joseph disease (SCA3) patients and their relatives. Commun Genet 10:19–26
63. Rodrigues CS et al (2002) Presymptomatic testing for neurogenetic diseases in Brazil: assessing who seeks and who follows through with testing. J Genet Couns 21:101–112
64. Saute JA et al (2010) Depressive mood is associated with ataxic and non-ataxic neurological dysfunction in SCA3 patients. Cerebellum 9:603–605
65. Fernandez-Ruiz J et al (2003) Olfactory dysfunction in hereditary ataxia and basal ganglia disorders. Neuroreport 14:1339–1341
66. Connelly T et al (2003) Olfactory dysfunction in degenerative ataxias. J Neurol Neurosurg Psychiatr 74:1435–1437
67. Abele M et al (2003). Olfactory dysfunction in cerebellar ataxia and multiple system atrophy. J Neurol 250:1453–1455
68. Velazquez-Perez L et al (2006) Spinocerebellar ataxia type 2 olfactory impairment shows a pattern similar to other major neurodegenerative diseases. J Neurol 253:1165–1169
69. Moscovich M et al (2012) Olfactory impairment in familial ataxias. J Neurol Neurosurg Psychiatry 83:970–974
70. Braga-Neto P et al (2011) Clinical correlates of olfactory dysfunction in spinocerebellar ataxia type 3. Parkinsonism Relat Disord 17:353–356
71. Coutinho P et al (1986) The peripheral neuropathy in Machado-Joseph disease. Acta Neuropathol (Berl) 71:119–124
72. Kinoshita A et al (1995) Clinicopathological study of the peripheral nervous system in Machado-Joseph disease. J Neurol Sci 130:48–58
73. Pedroso JL et al (2011) Is neuropathy involved with restless legs syndrome in Machado-Joseph disease? Eur Neurol 66:200–203
74. van de Warrenburg BPC et al (2004) Peripheral nerve involvement in spinocerebellar ataxias. Arch Neurol 61:257–261
75. Franca MC Jr et al (2009) Prospective study of peripheral neuropathy in Machado-Joseph disease. Muscle Nerve 40:1012–1018
76. Klockgether T et al (1999) Age related axonal neuropathy in spinocerebellar ataxia type 3/ Machado-Joseph disease (SCA3/MJD). J Neurol Neurosurg Psychiatry 66:222–224
77. Friedman JH et al (2008) Fatigue and daytime somnolence in Machado-Joseph disease (spinocerebellar ataxia type 3). Mov Disord 23:1323–1324

78. Franca MC Jr et al (2008) Muscle excitability abnormalities in Machado-Joseph disease. Arch Neurol 65:525–529
79. Kanai K et al (2003) Muscle cramp in Machado-Joseph disease: altered motor axonal excitability properties and mexiletine treatment. Brain 126:965–973
80. Franca MC Jr et al (2007) Chronic pain in Machado-Joseph disease: a frequent and disabling symptom. Arch Neurol 64:1767–1770
81. Yeh TH et al (2005) Autonomic dysfunction in Machado-Joseph disease. Arch Neurol 62:630–636
82. Franca MC Jr et al (2010) Clinical correlates of autonomic dysfunction in patients with Machado-Joseph disease. . Acta Neurol Scand 121:422–425
83. Pradhan C et al (2008) Spinocerebellar ataxias type 1, 2 and 3: a study of heart rate variability. Acta Neurol Scand 117:337–342
84. Yamanaka Y et al (2012) Cutaneous sympathetic dysfunction in patients with Machado-Joseph disease. Cerebellum 11:1057–1060
85. van Gaalen J et al (2011) Movement disorders in spinocerebellar ataxias. Mov Disord 26:792–800
86. Schols L et al (2000) Extrapyramidal motor signs in degenerative ataxias. Arch Neurol 57:1495–1500
87. Schmitz-Hubsch T et al (2008) Spinocerebellar ataxia types 1, 2, and 6. Disease severity and nonataxia symptoms. Neurology 71:982–989
88. Tuite PJ et al (1995) Dopa- responsive parkinsonism phenotype of Machado-Joseph disease: confirmation of 14q CAG expansion. Ann Neurol 38:684–687
89. Bettencourt C et al (2011) Parkinsonism pheno- type in Machado-Joseph disease (MJD/SCA3): a two-case report [serial online]. BMC Neurol 11:131
90. Socal MP et al (2009) Intrafamilial variability of Parkinson phenotype in SCAs: novel cases due to SCA2 and SCA3 expansions. Parkinsonism Relat Disord 15:374–378
91. Gwinn-Hardy K et al (2001) Spinocerebellar ataxia type 3 phenotypically resembling Parkinson disease in a black family. Arch Neurol 58:296–299
92. Subramony SH et al (2002) Ethnic differences in the expression of neurodegenerative disease: Machado–Joseph disease in Africans and Caucasians. Mov Disord 17:1068–1071
93. Lu CS et al (2004) The parkinsonian phenotype of spinocerebellar ataxia type 3 in a Taiwanese family. Parkinsonism Relat Disord 10:369–373
94. Pedroso JL et al (2012) Transcranial sonography: Brazilian experience. Arq Neuropsiquiatr 70:313–314
95. Teive HAG et al (2012) Spinocerebellar ataxias—Genotype-phenotype correlation in 104 Brazilian families. Clinics (Sao Paulo) 67:443–449
96. Siebert M et al (2012) Glucocerebrosidase gene variants in parkinsonism patients with Machado-Joseph/spinocerebellara ataxia 3. Parkinsonism Relat Disord 18:185–190
97. Pedroso JL et al (2011) Akathisia: an unusual movement disorder in Machado-Joseph disease. Parkinsonism Relat Disord 17:12–13
98. Horimoto Y et al (2011) Longitudinal study on MRI intensity changes of Machado-Joseph disease: correlation between MRI findings and neuropathological changes. J Neurol:1657–1664
99. Imon Y et al (1998) A necropsied case of Machado-Joseph disease with a hyperintense signal of transverse pontine fibres on long TR sequences of magnetic resonance images. J Neurol Neurosurg Psychiatry 64:140–141
100. Murata Y et al (1998) Characteristic magnetic resonance imaging findings in Machado-Joseph disease. Arch Neurol 55:33–37
101. Yamada S et al (2005) Linear high intensity area along the medial margin of the internal segment of the globus pallidus in Machado-Joseph disease patients. J Neurol Neurosurg Psychiatry 76:573–575
102. Lee YC et al (2009) The 'hot cross bun' sign in the patients with spinocerebellar ataxia. Eur J Neurol 16:513–516

103. De Oliveira MS et al (2010) MRI-texture analysis of corpus callosum, thalamus, putamen, and caudate in Machado-Joseph disease. J Neuroimaging 22:46–52
104. D'Abreu A et al (2012) Neocortical atrophy in Machado-Joseph disease: a longitudinal neuroimaging study. J Neuroimaging 22:285–291
105. de Rezende TJ et al (2015) Cerebral cortex involvement in Machado-Joseph disease. Eur J Neurol 11:277– 83 (e23–24)
106. Schulz JB et al (2010) Visualization, quantification and correlation of brain atrophy with clinical symptoms in spinocerebellar ataxia types 1, 3 and 6. Neuroimage 49:158–68
107. Reetz K et al (2013) Ataxia Study Group Investigators. Genotype-specific patterns of atrophy progression are more sensitive than clinical decline in SCA1, SCA3 and SCA6. Brain 136:905–17
108. Guimarães RP (2013) A multimodal evaluation of microstructural white matter damage in spinocerebellar ataxia type 3. Mov Disord 28:1125–1132
109. D'Abreu A et al (2009) Axonal dysfunction in the deep white matter in Machado-Joseph disease. J Neuroimaging 19:9–12
110. Wang PS et al (2012) Association between proton magnetic resonance spectroscopy measurements and CAG repeat number in patients with spinocerebellar ataxias 2, 3, or 6. PLoS One 7:e47479
111. Lirng JF et al (2012) Differences between spinocerebellar ataxias and multiple system atrophy-cerebellar type on proton magnetic resonance spectroscopy. PLoS One 7:e47925
112. Jacobi H et al (2013) Biological and clinical characteristics of individuals at risk for spinocerebellar ataxia types 1, 2, 3, and 6 in the longitudinal RISCA study: analysis of baseline data. Lancet Neurol 12:630
113. Nunes MB, Martinez AR, Rezende TJ et al (2015) Dystonia in Machado–Joseph disease: Clinical profile, therapy and anatomical basis. Parkinsonism Relat Disord 21:1441–1447
114. Etchebehere EC et al (2001) Brain single- photon emission computed tomography and magnetic resonance imaging in Machado-Joseph disease. Arch Neurol 58:1257–1263
115. Taniwaki T et al (1997) Positron emission tomography (PET) in Machado-Joseph disease. J Neurol Sci 145:63–67
116. Soong BW et al (1998) Positron emission tomography in asymptomatic gene carriers of Machado-Joseph disease. J Neurol Neurosurg Psychiatry 64:499–504
117. Ogawa M (2004) Pharmacological treatments of cerebellar ataxia. Cerebellum 3:107–111
118. Correia M et al (1995) Evaluation of the effect of sulphametoxazole and trimethoprim in patients with Machado-Joseph disease. Rev Neurol 23:632–634
119. Buhmann C et al (2003) Dopaminergic response in Parkinsonian phenotype of Machado–Joseph disease. Mov Disord 18:219–221
120. Wilder-Smith E et al (2003) Spinocerebellar ataxia type 3 presenting as an L-DOPA responsive dystonia phenotype in a Chinese family. J Neurol Sci 213:25–28
121. Nandagopal R et al (2004) Dramatic levodopa responsiveness of dystonia in a sporadic case of spinocerebellar ataxia type 3. Postgrad Med J 80:363–365
122. D'Abreu A et al (2010) Caring for Machado- Joseph disease: current understanding and how to help patients. Relat Disord 16:2–7
123. Zesiewicz TA et al (2012) A randomized trial of varenicline (Chantix) for the treatment of spinocerebellarataxia type 3. Neurology 78:545–550
124. Azulay JP et al (1994) Contrast sensitivity improvement with sulfamethoxazole and trimethoprim in a patient with Machado-Joseph disease without spasticity. J Neurol Sci 123:95–99
125. Mello KA et al (1988) Effect of sulfamethoxazole and trimethoprim on neurologic dysfunction in a patient with Joseph's disease. Arch Neurol 45:210–213
126. Sakai T et al (1995) Sulfamethoxazoletrimethoprim double-blind, placebo-controlled, crossover trial in Machado-Joseph disease: sulfamethoxazole-trimethoprim increases cerebrospinal fluid level of biopterin. J Neural Transm Gen Sect 102:159–172

127. Thorsten Schulte RM (2001) Double-blind crossover trial of trimethoprim-sulfamethoxazole in spinocerebellar ataxia type 3/Machado-Joseph disease. Arch Neurol 58:1451–1457
128. Saute JA et al (2014) A Randomized, phase 2 clinical trial of lithium carbonate in Machado-Joseph disease. Mov Disord 29:568–573
129. Kieling C et al (2008) A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur J Neurol 15:371–376
130. Schmitz-Hübsch T et al (2006) Scale for the assessment and rating of ataxia. Neurology 66:1717–1720
131. Du Montcel ST et al (2008) Composite cerebellar functional severity score: Validation of a quantitative score of cerebellar impairment. Brain 131:1352–1361
132. Saute JA et al (2015) Planning future clinical trials in Machado Joseph disease: Lessons from a phase 2 trial. J Neurol Sci 358:72–76
133. Monte TL et al (2003) Use of fluoxetine for treatment of Machado-Joseph disease: an open-label study. Acta Neurol Scand 107:207–210
134. Takei A et al (2004) Effects of tandospirone on "5-HT1A receptor-associated symptoms" in patients with Machado-Josephe disease: an open-label study. Clin Neuropharmacol 27:9–13
135. Liu C-S et al (2005) Clinical and molecular events in patients with Machado–Joseph disease underlamotrigine therapy. Acta Neurol Scand 111:385–390

Chapter 14
Polyglutamine-Independent Features in Ataxin-3 Aggregation and Pathogenesis of Machado-Joseph Disease

Ana Luisa Carvalho, Alexandra Silva and Sandra Macedo-Ribeiro

Abstract The expansion of a trinucleotide (CAG) repeat, translated into a polyglutamine expanded sequence in the protein encoded by the MJD gene, was identified over 20 years ago as the causative mutation in a severe neurodegenerative disorder originally diagnosed in individuals of Portuguese ancestry. This incapacitating disease, called Machado-Joseph disease or spinocebellar ataxia type 3, is integrated into a larger group of neurodegenerative disorders—the polyglutamine expansion disorders—caused by extension of a CAG repeat in the coding sequence of otherwise unrelated genes. These diseases are generally linked with the appearance of intracellular inclusions, which despite having a controversial role in disease appearance and development represent a characteristic common fingerprint in all polyglutamine-related disorders. Although polyglutamine expansion is an obvious trigger for neuronal dysfunction, the role of the different domains of these complex proteins in the function and aggregation properties of the carrier proteins is being uncovered in recent studies. In this review the current knowledge about the structural and functional features of full-length ataxin-3 protein will be discussed. The intrinsic conformational dynamics and interplay between the globular and

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intrinsically disordered regions of ataxin-3 will be highlighted, and a perspective picture of the role of known ataxin-3 post-translational modifications on regulating ataxin-3 aggregation and function will be drawn.

Keywords Amino acid repeats · Amyloid · Conformational plasticity
Post-translational modifications

14.1 Ataxin-3: A Multidomain Protein with Considerable Conformational Plasticity

Ataxin-3 (Atx3) is a modular protein, containing a globular N-terminal domain (Josephin domain, JD) and a flexible C-terminal tail containing ubiquitin interaction motifs (UIM) and a polyglutamine (polyQ)-rich region [1] (Fig. 14.1). Mutations leading to expansion of the repeated polyQ sequence represent the major trigger for

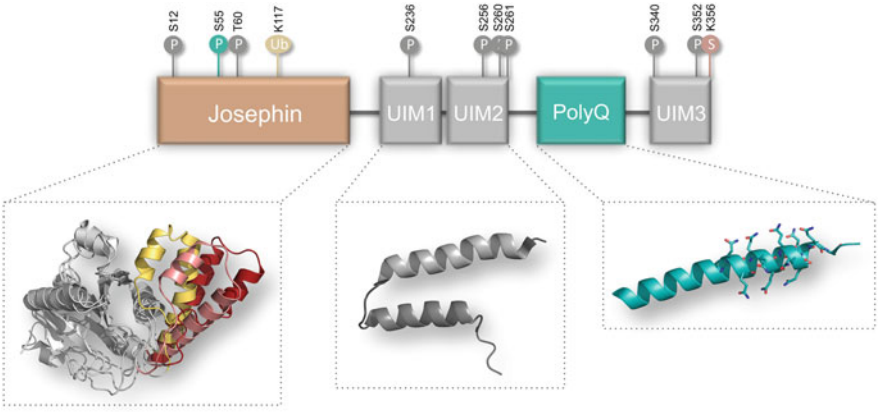

Fig. 14.1 Domain architecture of ataxin-3. Schematic representation of the modular structure of Atx3 composed of a globular domain [the Josephin domain (JD)] and a flexible tail containing two or three ubiquitin interaction motifs (UIMs) and the expandable polyQ stretch. The JD is formed by a catalytic subdomain and a flexible helical hairpin. The cartoon representation of the JD shows two of the structures determined by nuclear magnetic resonance depicting the closed (PDB accession 2aga, catalytic subdomain in grey and helical hairpin in yellow), half-closed (PDB accession 2dos, catalytic subdomain in grey and helical hairpin in pink) and open (PDB accession 1yzb, catalytic subdomain in grey and helical hairpin in red) conformations of the mobile hairpin. UIMs 1 and 2 adopt a predominantly helical conformation that is more compact in the presence of ubiquitin (PDB accession 2klz). The polyQ segment is polymorphic and can adopt a helical conformation (PDB accession 4wth) stabilized by interactions with a neighbor molecule in the crystal lattice. Known phosphorylation sites of Atx3 are represented as grey circles (S12 and T60 at the JD and S236, S256, S260, S261, S340 and S352 at the UIMs) or as a green circle (S55 at the JD, phosphorylation is more pronounced in expanded Atx3). Ubiquitination (K117 at the JD) and SUMOylation sites (K356 at the UIM3) of Atx3 are represented as yellow and pink circles, respectively. Figure prepared with Pymol (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.)

the development of spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD), a neurodegenerative disease for which no pharmacological therapies are available so far [2, 3].

Early bioinformatic studies predicted that Atx3 shared several signatures typically found in ubiquitin-interacting proteins and ubiquitin hydrolases [4, 5]. In agreement, both normal and polyQ-expanded Atx3 were shown to be able to cleave standard ubiquitin hydrolase substrates, an enzymatic activity that was critically dependent on the conserved catalytic residue cysteine 14 [6]. Follow-up studies with the isolated JD corroborated its identity as Atx3 catalytic domain [7], and the link between Atx3 and the ubiquitin-proteasome pathway was further established [8, 9]. Investigation of Atx3 biochemical properties and enzyme specificity showed its preference for K63-linked or heterotypic linkage (mixed K63 and K48 linkages) polyubiquitin chains with four or more ubiquitins [10]. While the JD plays a role in defining the cleavage properties and linkage specificity [11], polyQ expansion does not seem to impose significant differences in enzyme activity or specificity [10]. Accordingly, normal and expanded Atx3 bind to K48-linked tetraubiquitin chains with equivalent affinities [12]. Covalent modification of Atx3 by ubiquitination partly increments its proteolytic activity towards polyubiquitin chains [13], whereas Atx3 phosphorylation decreases its catalytic activity [14] (see below).

The first structural insights of the domain architecture of Atx3 in solution resulted from combined studies using circular dichroism and nuclear magnetic resonance (NMR) spectroscopy [1]. Later, the solution structure of the JD was determined, unveiling a canonical cysteine protease fold [15–18] with a distinctive helical hairpin, whose conformation is variable in the several solution and crystallographic structures determined thus far. This α2/α3 hairpin samples multiple conformations and can be found in a closed (PDB accession 2aga, [15]), half-closed (PDB accession 2dos, [18]) and open conformation (PDB accession 1yzb, [17]), a dynamic behavior that has been linked with ubiquitin substrate recognition and binding [18–21]. The JD encloses two central ubiquitin-binding sites: a proximal site (a.k.a. site 1), located close to the catalytic triad, and a distal site (a.k.a. site 2) including an extended hydrophobic patch, located at the back of this globular domain [18, 19]. The binding region of the ubiquitin-like domain of HHR23B also maps to the distal ubiquitin-binding site [18, 19], a region that modulates Atx3 cellular turnover [22].

The first two UIMs of Atx3 are structurally similar to related motifs in different proteins and form two α-helices connected by a short linker, adopting a more compact structure upon addition of ubiquitin [23]. The crystal structures of a small C-terminal fragment of Atx3 containing the nuclear localization sequence [24] and a polyQ region of 14 glutamines, interrupted by a single lysine residue, were recently solved in fusion with maltose-binding protein (MBP) [25]. The polyQ region was also shown to display conformational plasticity sampling multiple conformations that range from random coil to α-helical structures, the latter stabilized by interactions with neighbouring MBP molecules in the crystal lattice [25]. The essentially polymorphic nature of Atx3 polyQ region is not unusual, and is also common to polyQ segments in other polyQ disease-related proteins [26, 27].

Contrary to what was originally postulated, the expansion of the polyQ region in disease-related Atx3 is not accompanied by global structural changes [28, 29], and has no relevant impact on polyubiquitin binding [12] and hydrolysis [10]. Recent data from ion mobility mass spectrometry revealed that normal and polyQ-expanded Atx3 adopt a wide range of conformational states imparted by the intrinsic plasticity of the C-terminal tail, containing the UIMs and the polyQ stretch, and the α2/α3 hairpin of the globular JD [29–31].

14.2 The Complex Pathway of Ataxin-3 Self-assembly into Amyloid-like Fibrils

Expansion of Atx3 polyQ repeat sequence is directly correlated with the appearance of ubiquitinated intracellular inclusions containing the mutated protein [32]. Early evidence, where expression of an expanded polyQ-containing sequence stimulated recruitment of full-length Atx3 into insoluble inclusions, suggested a central role for the expanded polyQ sequences in protein aggregation and disease pathogenesis. In vitro studies with the full-length protein, with variable polyQ tract sizes, revealed that polyQ expansion promoted protein self-assembly into insoluble SDS-resistant aggregates [28, 33]. These aggregates had fibrillar morphology, were enriched in β-structure, and displayed the capacity to bind the amyloid dye Congo Red, suggesting that they have amyloid-like properties [33]. Interestingly, non-pathological Atx3 is also found in intracellular inclusions under stress-induced conditions [34, 35].

Follow-up studies with recombinant Atx3 demonstrated that the non-expanded protein as well as the isolated JD could also self-assemble into insoluble β-rich structures upon protein destabilization/unfolding [36–39], but also under near-physiological conditions [40]. The modulation of protein aggregation by regions outside the polyQ tract is a common feature in several proteins involved in polyQ expansion diseases [41–44]. The JD has a number of aggregation-prone regions [7, 38, 40, 45] and plays a central role in the early aggregation steps of both normal and polyQ-expanded Atx3 [40, 46, 47]. Small oligomers and protofibrils from non-pathological Atx3 and JD were efficiently detected by a specific antibody, which universally recognizes toxic amyloid oligomeric structures from unrelated amyloid-forming proteins [40, 48]. Those findings suggest that studying the "slower" aggregation pathway of normal Atx3 or the JD will provide clues to identify early intermediates with relevance for neuronal degeneration. Native mass spectrometry data has contributed for the delineation of the early molecular events underlying JD-mediated aggregation: self-association of the JD is initiated through dimerization, followed by a conformational change of the dimer, which can be recognized by a conformation-specific antibody [29, 40]. The more compact dimer further sustains linear oligomer assembly by sequential monomer addition [29].

Current experimental data support a two-step model for Atx3 aggregation as proposed by Ellisdon and coworkers [47]: the first step leads to the formation of SDS-sensitive protofibrils, while the second step generates long-straight and

SDS-resistant mature fibrils (Fig. 14.2). The first aggregation step is mediated by self-association of the JD and occurs in both expanded and non-pathological Atx3, being independent of the polyQ tract. The second step is observed only for Atx3 with a pathological polyQ tract size and leads to the polyQ-dependent formation of SDS-resistant fibrils, as initially reported by Bevivino and Loll [33]. A combination of mass spectrometry and limited proteolysis studies identified the region encompassing helix α4 from the JD as the core of the protofibrils formed in the JD-mediated aggregation step [29, 49]. Additionally, the fibrils formed by polyQ-expanded Atx3 contained two structured regions, the protofibril core and a polyQ-containing core [29]. These results denote that the polyQ stretch is directly involved in the maturation into SDS-resistant fibrils [29], which are stabilized by hydrogen bonds mediated by the glutamine side-chains [50]. However, although both the isolated JD as well as non-pathological and polyQ-expanded protein can self-assemble into amyloid-like fibrils, the in vitro aggregation rates are different for the different constructs [28–30, 33]. This implies that further mechanistic details are required to completely comprehend the complexity of Atx3 aggregation pathway(s).

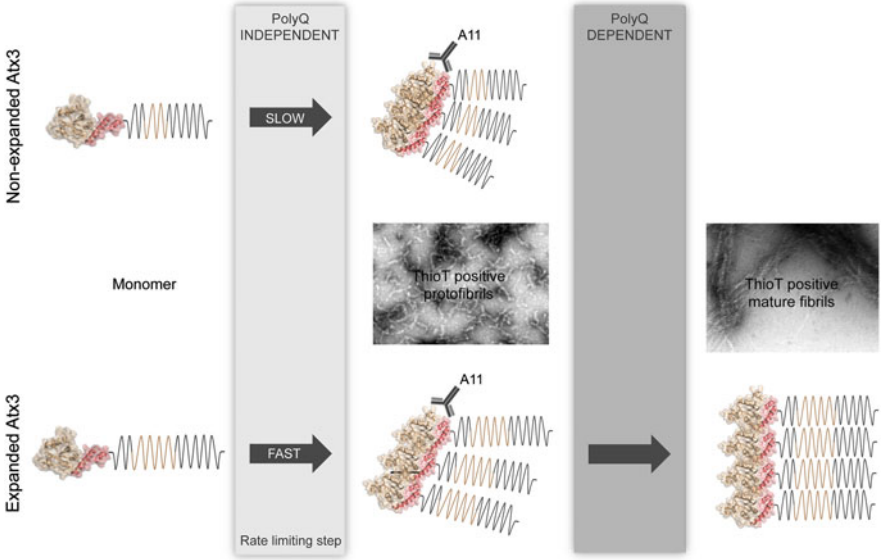

Fig. 14.2 Two-step model of ataxin-3 aggregation. The first step of ataxin-3 self-assembly, common to non-pathological and polyQ-expanded Atx3, is centered on the Josephin domain. The oligomers and small protofibrils formed are sensitive to SDS and can be recognized by the anti-oligomer specific antibody A11 [40, 48]. A second step, exclusively dependent on the polyQ expansion, leads to the generation of mature and SDS-resistant Atx3 fibrils

14.3 Polyglutamine Sequence Expansion and the Conformational Dynamics of the Josephin Domain

Expansion of the polyQ tract has been consistently demonstrated to accelerate Atx3 fibrillation, although multiple studies have demonstrated that the JD and the C-terminal tail are not directly involved in a stable interaction [28, 29, 31, 38] and polyQ expansion does not modify Atx3 overall stability or folding/unfolding [28]. Experimental data from different research groups have demonstrated that polyQ expansion induces local conformational fluctuations in the aggregation-prone regions of the globular JD, exposing them more frequently and thereby increasing the rates of aberrant self-assembly events [29, 49]. PolyQ expansion affects in particular helix α4, identified as part of the protofibrillar core, and helix α1, containing the catalytic residue C14 [49] (Fig. 14.3). This region is adjacent to the mobile α2/α3 hairpin. Interestingly, those aggregation-prone and mobile surfaces of the catalytic JD overlap with the functionally relevant Ub binding sites [51]. In agreement, JD-mediated interactions set off the oligomerization of both normal Atx3 and its expanded counterpart in vitro [31, 40, 45, 47], and interaction with ubiquitin delays JD aggregation [51]. The key role of JD-mediated protein interactions as regulators of Atx3 self-assembly is further reinforced by the findings that a molecular chaperone, transiently interacting with JD catalytic subdomain, significantly reduces aggregation [52]. Those studies expose a role for protein interactions as modulators of the conformational plasticity and self-assembly properties of

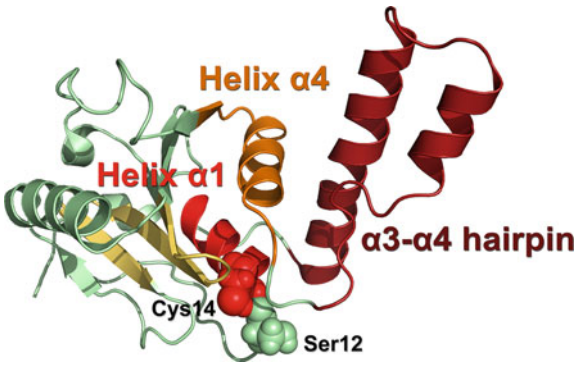

Fig. 14.3 Cartoon representation of the JD structure depicting the aggregation-prone stretch forming the core of the protofibril (orange) and the regions showing structural perturbations characterized by increased solvent exposure upon expansion of the polyQ segment (helix α1, red; helix α4, orange; central β3–β5 strand, yellow). The helical hairpin is shown in dark red. Phosphorylation site serine 12, close to the ubiquitin-binding site of JD and with an impact in Atx3 aggregation, and cysteine 14, the nucleophile of the catalytic triad, are represented as red and green spheres, respectively. Figure prepared with Pymol (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC.)

full-length Atx3 (for a recent review on Atx3 interacting proteins see [53]), providing an attractive link between cellular context and Atx3 aggregation and toxicity.

14.4 Ataxin-3 Post-translational Modifications as Modulators of Protein Aggregation and Function

A striking feature of MJD and other polyQ diseases is the selective vulnerability of specific brain regions to the expanded protein, despite its ubiquitous expression [3]. The disease features reflect the loss or dysfunction of specific neuron populations that are affected by expanded Atx3. These observations motivated a series of studies addressing Atx3 regulation by post-translational modifications (which could be cell type-specific) as an attractive mechanism for modulating protein aggregation and toxicity. Important insights have been provided by studying Atx3 regulation by phosphorylation, ubiquitination and SUMOylation (Table 14.1).

14.4.1 Ataxin-3 Phosphorylation

Atx3 is phosphorylated at both the JD and the UIMs (Fig. 14.1), in some cases with consequences on protein aggregation and toxicity. In the JD, Atx3 is phosphorylated at serine 12 [14] (Fig. 14.3), a residue located in proximity to structural elements associated with substrate binding and hydrolysis. Serine 12 is in the loop preceding the α1 helix where the C14 nucleophile of the catalytic triad is located, near Q9, a residue that may contribute to the stabilization of the negatively charged transition state during peptide bond cleavage, and in the vicinity of the loop centered on S27, close to the docking site for the ubiquitin substrate [19]. In fact, mimicking constitutive phosphorylation of Atx3 at residue 12 decreases its deubiquitinase (DUB) activity [14]. Additionally, in cortical neurons phosphorylation of S12 reduces dendritic tract and synapse (excitatory and inhibitory) loss caused by expression of expanded Atx3, and reduces expanded Atx3 aggregation. In a lentiviral rat MJD model, expanded Atx3 phosphorylation at this position is associated with decreased formation of Atx3 aggregates, and decreased neuronal and synapse loss [14], suggesting that modifications targeting S12 may reduce the toxicity of expanded Atx3, and be protective. The effect of S12 phosphorylation on Atx3 aggregation may be related to its proximity to the proximal ubiquitin-binding site in the JD (a.k.a. site 1), which has been described to overlap with regions that take part in JD self-assembly [19, 23, 51]. In addition to this phosphorylation site, S55 and T60 at the JD were also recently described to undergo phosphorylation [54] (Fig. 14.1), but the functional consequences of these phosphorylation events are so far unknown. Interestingly, phosphorylation at S55 is increased in expanded Atx3 compared to the non-expanded protein [54].

Table 14.1 Post-translational modifications in ataxin-3

Modification	Residue in Atx3	Enzyme	Protein domain	Functional effect	References
Phosphorylation	S12	Unknown	JD	Phosphorylation decreases DUB activity; prevents dendritic shrinkage and synapse loss caused by expression of expanded Atx3; decreases expanded Atx3 aggregation	[14]
	S55	Unknown	JD	Unknown	[54]
	T60	Unknown	JD	Unknown	[54]
	S236	Unknown	UIM1	Phosphorylation enhances nuclear localization and transcription repression activity	[56]
	S256	GSK3β	UIM2	Preventing phosphorylation increases expanded Atx3 aggregation	[55]
	S260/261	Unknown	UIM2	Unknown	[56]
	S340	Unknown	UIM3	Phosphorylation enhances nuclear localization and transcription repression activity	[56]
	S352	Unknown	UIM3	Phosphorylation enhances nuclear localization and transcription repression activity	[56]
Ubiquitination	K117	CHIP/E4B	JD	Ubiquitination enhances Atx3 catalytic activity	[13, 58]
SUMOylation	K356	Unknown	UIM3	SUMOylation increases affinity for p97/VCP	[64]

Six other Atx3 phosphorylation sites have been described, localized in the UIMs of the protein (Fig. 14.1). Glycogen synthase kinase 3β (GSK3β) phosphorylates Atx3 in vitro at S256 in the second UIM [55]; preventing Atx3 phosphorylation at S256 by replacing the serine residue by alanine increased Atx3 aggregation, which was prevented by overexpression of the Hsp70 chaperone [55]. Serine 236 in UIM1, serines 260 and 261 in UIM2, as well as serines 340 and 352 in UIM3 are also phosphorylation substrates [56]. Phosphorylation of S236 or of S340 and S352 enhances Atx3 nuclear localization and repression of Atx3-regulated transcription, whereas inhibition of casein kinase 2 (CK2), a kinase that binds to Atx3 [56, 57], leads to cytoplasmatic accumulation of the protein [56].

14.4.2 Ataxin-3 Ubiquitination

Atx3 is ubiquitinated at lysine 117 (Fig. 14.1), near the DUB active site [58]. Ubiquitination enhances the catalytic activity of the protein [13, 58], without affecting its preference for K63-linked ubiquitin chains [13]. A recent study addressed the consequences of Atx3 ubiquitination at K117 at the structural level, and found that K117-covalently linked mono-ubiquitin binds to the proximal ubiquitin-binding site 1, suggesting that the observed increase in catalytic activity could result from locking Atx3 in an active state [59]. Interestingly, regulation of Atx3 DUB activity by ubiquitination is enhanced upon proteasome inhibition or activation of the unfolded protein response [13], suggesting that cellular protein turnover control by Atx3 is regulated by its ubiquitination.

The co-chaperone C-terminal Hsp70-interacting protein (CHIP) as well as the mammalian E4B (UFD2a), a ubiquitin chain assembly factor (E4), interact with Atx3 and promote its ubiquitination and degradation [60–62]. However, cellular degradation of Atx3 does not require its ubiquitination, rather is dependent on binding of Rad23 to its distal ubiquitin-binding site, which rescues it from proteasomal degradation [22]. In a *Drosophila* model of MJD, expression of E4B suppressed the neurodegeneration induced by expanded Atx3 [61], suggesting that enhancing Atx3 ubiquitination is neuroprotective. Accordingly, it was more recently found that ubiquitination of wild-type Atx3 at K117 enhances its protective effect against expanded Atx3-induced degeneration in *Drosophila* [63].

14.4.3 Ataxin-3 SUMOylation

Covalent attachment of small ubiquitin-like modifier (SUMO) protein to lysine residues in target proteins can influence their localization, interactions, activity and stability, and has been shown for several polyQ-containing proteins, including Atx3 [64, 65]. Atx3 is SUMOylated by SUMO1 and SUMO2 at K356 in UIM3 (Fig. 14.1), which decreases Atx3 fibril formation in vitro, and increases Atx3 affinity for the ATPase p97/VCP [64]. P97/VCP is involved in retrotranslocation of misfolded proteins from the endoplasmic reticulum to the cytosol, for degradation by the proteasome, and Atx3 is known to participate in efficient elimination of retrotanslocated misfolded proteins [66, 67]. On the other hand, p97/VCP influences the assembly of Atx3 protein aggregates in vitro, in a concentration-dependent manner, and suppresses Atx3-induced neurodegeneration in *Drosophila* [67]. Atx3 SUMOylation, by impacting Atx3 affinity for p97/VCP, may affect the p97/VCP-mediated ER-associated degradation, as well as the modulation of Atx3 aggregation by VCP.

14.4.4 Concluding Remarks

The modular structure of Atx3, containing a globular domain and an intrinsically disordered flexible tail, outlines its polymorphic character and multifaceted self-assembly process. Multiple studies have delineated the relevance of the regions and domains flanking the polyQ segment in the regulation of Atx3 function and in modulating its aggregation behavior. In particular, the globular JD assumes a central role for Atx3 function and self-assembly, properties that can be partly modulated by post-translational modifications and protein-protein interactions. The identification of a globular region whose conformational dynamics is influenced by polyQ expansion, and is directly involved in the establishment of the protofibrillar core, suggests that JD can constitute a valuable target for rational design of anti-Atx3 aggregation molecules.

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References

1. Masino L, Musi V, Menon RP, Fusi P, Kelly G, Frenkiel TA, Trottier Y, Pastore A (2003) Domain architecture of the polyglutamine protein ataxin-3: a globular domain followed by a flexible tail. FEBS Lett 549:21–25
2. Costa Mdo C, Paulson HL (2012) Toward understanding Machado-Joseph disease. Prog Neurobiol 97:239–257
3. Matos CA, de Macedo-Ribeiro S, Carvalho AL (2011) Polyglutamine diseases: the special case of ataxin-3 and Machado-Joseph disease. Prog Neurobiol 95:26–48
4. Hofmann K, Falquet L (2001) A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. Trends Biochem Sci 26:347–350
5. Scheel H, Tomiuk S, Hofmann K (2003) Elucidation of ataxin-3 and ataxin-7 function by integrative bioinformatics. Hum Mol Genet 12:2845–2852
6. Burnett B, Li F, Pittman RN (2003) The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. Hum Mol Genet 12:3195–3205

7. Chow MK, Mackay JP, Whisstock JC, Scanlon MJ, Bottomley SP (2004) Structural and functional analysis of the Josephin domain of the polyglutamine protein ataxin-3. Biochem Biophys Res Commun 322:387–394
8. Berke SJ, Chai Y, Marrs GL, Wen H, Paulson HL (2005) Defining the role of ubiquitin-interacting motifs in the polyglutamine disease protein, ataxin-3. J Biol Chem 280:32026–32034
9. Schmitt I, Linden M, Khazneh H, Evert BO, Breuer P, Klockgether T, Wuellner U (2007) Inactivation of the mouse Atxn3 (ataxin-3) gene increases protein ubiquitination. Biochem Biophys Res Commun 362:734–739
10. Winborn BJ, Travis SM, Todi SV, Scaglione KM, Xu P, Williams AJ, Cohen RE, Peng J, Paulson HL (2008) The deubiquitinating enzyme ataxin-3, a polyglutamine disease protein, edits Lys63 linkages in mixed linkage ubiquitin chains. J Biol Chem 283:26436–26443
11. Nicastro G, Todi SV, Karaca E, Bonvin AM, Paulson HL, Pastore A (2010) Understanding the role of the Josephin domain in the PolyUb binding and cleavage properties of ataxin-3. PLoS ONE 5:e12430
12. Chai Y, Berke SS, Cohen RE, Paulson HL (2004) Poly-ubiquitin binding by the polyglutamine disease protein ataxin-3 links its normal function to protein surveillance pathways. J Biol Chem 279:3605–3611
13. Todi SV, Winborn BJ, Scaglione KM, Blount JR, Travis SM, Paulson HL (2009) Ubiquitination directly enhances activity of the deubiquitinating enzyme ataxin-3. EMBO J 28:372–382
14. Matos CA, Nobrega C, Louros SR, Almeida B, Ferreiro E, Valero J, Pereira de Almeida L, Macedo-Ribeiro S, Carvalho AL (2016) Ataxin-3 phosphorylation decreases neuronal defects in spinocerebellar ataxia type 3 models. J Cell Biol 212:465–480
15. Mao Y, Senic-Matuglia F, Di Fiore PP, Polo S, Hodsdon ME, De Camilli P (2005) Deubiquitinating function of ataxin-3: insights from the solution structure of the Josephin domain. Proc Natl Acad Sci U S A 102:12700–12705
16. Nicastro G, Habeck M, Masino L, Svergun DI, Pastore A (2006) Structure validation of the Josephin domain of ataxin-3: conclusive evidence for an open conformation. J Biomol NMR 36:267–277
17. Nicastro G, Menon RP, Masino L, Knowles PP, McDonald NQ, Pastore A (2005) The solution structure of the Josephin domain of ataxin-3: structural determinants for molecular recognition. Proc Natl Acad Sci U S A 102:10493–10498
18. Satoh T, Sumiyoshi A, Yagi-Utsumi M, Sakata E, Sasakawa H, Kurimoto E, Yamaguchi Y, Li W, Joazeiro CA, Hirokawa T, Kato K (2014) Mode of substrate recognition by the Josephin domain of ataxin-3, which has an endo-type deubiquitinase activity. FEBS Lett 588:4422–4430
19. Nicastro G, Masino L, Esposito V, Menon RP, De Simone A, Fraternali F, Pastore A (2009) Josephin domain of ataxin-3 contains two distinct ubiquitin-binding sites. Biopolymers 91:1203–1214
20. Sanfelice D, De Simone A, Cavalli A, Faggiano S, Vendruscolo M, Pastore A (2014) Characterization of the conformational fluctuations in the Josephin domain of ataxin-3. Biophys J 107:2932–2940
21. Weeks SD, Grasty KC, Hernandez-Cuebas L, Loll PJ (2011) Crystal structure of a Josephin-ubiquitin complex: evolutionary restraints on ataxin-3 deubiquitinating activity. J Biol Chem 286:4555–4565
22. Blount JR, Tsou WL, Ristic G, Burr AA, Ouyang M, Galante H, Scaglione KM, Todi SV (2014) Ubiquitin-binding site 2 of ataxin-3 prevents its proteasomal degradation by interacting with Rad23. Nat Commun 5:4638
23. Song AX, Zhou CJ, Peng Y, Gao XC, Zhou ZR, Fu QS, Hong J, Lin DH, Hu HY (2010) Structural transformation of the tandem ubiquitin-interacting motifs in ataxin-3 and their cooperative interactions with ubiquitin chains. PLoS ONE 5:e13202
24. Macedo-Ribeiro S, Cortes L, Maciel P, Carvalho AL (2009) Nucleocytoplasmic shuttling activity of ataxin-3. PLoS ONE 4:e5834

25. Zhemkov VA, Kulminskaya AA, Bezprozvanny IB, Kim M (2016) The 2.2-Angstrom resolution crystal structure of the carboxy-terminal region of ataxin-3. FEBS open bio 6:168–178
26. Kim M (2013) Beta conformation of polyglutamine track revealed by a crystal structure of Huntingtin N-terminal region with insertion of three histidine residues. Prion 7:221–228
27. Kim MW, Chelliah Y, Kim SW, Otwinowski Z, Bezprozvanny I (2009) Secondary structure of Huntingtin amino-terminal region. Structure 17:1205–1212
28. Chow MK, Ellisdon AM, Cabrita LD, Bottomley SP (2004) Polyglutamine expansion in ataxin-3 does not affect protein stability: implications for misfolding and disease. J Biol Chem 279:47643–47651
29. Scarff CA, Almeida B, Fraga J, Macedo-Ribeiro S, Radford SE, Ashcroft AE (2015) Examination of ataxin-3 (atx-3) aggregation by structural mass spectrometry techniques: a rationale for expedited aggregation upon polyglutamine (polyQ) expansion. Mol Cell Proteomics 14:1241–1253
30. Santambrogio C, Frana AM, Natalello A, Papaleo E, Regonesi ME, Doglia SM, Tortora P, Invernizzi G, Grandori R (2012) The role of the central flexible region on the aggregation and conformational properties of human ataxin-3. FEBS J 279:451–463
31. Scarff CA, Sicorello A, Tomé RJL, Macedo-Ribeiro S, Ashcroft AE, Radford SE (2013) A tale of a tail: structural insights into the conformational properties of the polyglutamine protein ataxin-3. Int J Mass Spectrom 345–347:63–70
32. Paulson HL, Perez MK, Trottier Y, Trojanowski JQ, Subramony SH, Das SS, Vig P, Mandel JL, Fischbeck KH, Pittman RN (1997) Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. Neuron 19:333–344
33. Bevivino AE, Loll PJ (2001) An expanded glutamine repeat destabilizes native ataxin-3 structure and mediates formation of parallel β-fibrils. Proc Natl Acad Sci U S A 98:11955–11960
34. Fujigasaki H, Uchihara T, Koyano S, Iwabuchi K, Yagishita S, Makifuchi T, Nakamura A, Ishida K, Toru S, Hirai S, Ishikawa K, Tanabe T, Mizusawa H (2000) Ataxin-3 is translocated into the nucleus for the formation of intranuclear inclusions in normal and Machado-Joseph disease brains. Exp Neurol 165:248–256
35. Kettner M, Willwohl D, Hubbard GB, Rub U, Dick EJ Jr, Cox AB, Trottier Y, Auburger G, Braak H, Schultz C (2002) Intranuclear aggregation of nonexpanded ataxin-3 in marinesco bodies of the nonhuman primate substantia nigra. Exp Neurol 176:117–121
36. Chow MK, Paulson HL, Bottomley SP (2004) Destabilization of a non-pathological variant of ataxin-3 results in fibrillogenesis via a partially folded intermediate: a model for misfolding in polyglutamine disease. J Mol Biol 335:333–341
37. Marchal S, Shehi E, Harricane MC, Fusi P, Heitz F, Tortora P, Lange R (2003) Structural instability and fibrillar aggregation of non-expanded human ataxin-3 revealed under high pressure and temperature. J Biol Chem 278:31554–31563
38. Masino L, Nicastro G, Menon RP, Dal Piaz F, Calder L, Pastore A (2004) Characterization of the structure and the amyloidogenic properties of the Josephin domain of the polyglutamine-containing protein ataxin-3. J Mol Biol 344:1021–1035
39. Shehi E, Fusi P, Secundo F, Pozzuolo S, Bairati A, Tortora P (2003) Temperature-dependent, irreversible formation of amyloid fibrils by a soluble human ataxin-3 carrying a moderately expanded polyglutamine stretch (Q36). Biochemistry 42:14626–14632
40. Gales L, Cortes L, Almeida C, Melo CV, Costa MC, Maciel P, Clarke DT, Damas AM, Macedo-Ribeiro S (2005) Towards a structural understanding of the fibrillization pathway in Machado-Joseph's disease: trapping early oligomers of non-expanded ataxin-3. J Mol Biol 353:642–654
41. de Chiara C, Pastore A (2014) Kaleidoscopic protein-protein interactions in the life and death of ataxin-1: new strategies against protein aggregation. Trends Neurosci 37:211–218
42. Eftekharzadeh B, Piai A, Chiesa G, Mungianu D, Garcia J, Pierattelli R, Felli IC, Salvatella X (2016) Sequence context influences the structure and aggregation behavior of a PolyQ tract. Biophys J 110:2361–2366

43. Menon RP, Soong D, de Chiara C, Holt M, McCormick JE, Anilkumar N, Pastore A (2014) Mapping the self-association domains of ataxin-1: identification of novel non overlapping motifs. PeerJ 2:e323
44. Monsellier E, Redeker V, Ruiz-Arlandis G, Bousset L, Melki R (2015) Molecular interaction between the chaperone Hsc70 and the N-terminal flank of huntingtin exon 1 modulates aggregation. J Biol Chem 290:2560–2576
45. Masino L, Nicastro G, De Simone A, Calder L, Molloy J, Pastore A (2011) The Josephin domain determines the morphological and mechanical properties of ataxin-3 fibrils. Biophys J 100:2033–2042
46. Ellisdon AM, Pearce MC, Bottomley SP (2007) Mechanisms of ataxin-3 misfolding and fibril formation: kinetic analysis of a disease-associated polyglutamine protein. J Mol Biol 368:595–605
47. Ellisdon AM, Thomas B, Bottomley SP (2006) The two-stage pathway of ataxin-3 fibrillogenesis involves a polyglutamine-independent step. J Biol Chem 281:16888–16896
48. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300:486–489
49. Lupton CJ, Steer DL, Wintrode PL, Bottomley SP, Hughes VA, Ellisdon AM (2015) Enhanced molecular mobility of ordinarily structured regions drives polyglutamine disease. J Biol Chem 290:24190–24200
50. Natalello A, Frana AM, Relini A, Apicella A, Invernizzi G, Casari C, Gliozzi A, Doglia SM, Tortora P, Regonesi ME (2011) A major role for side-chain polyglutamine hydrogen bonding in irreversible ataxin-3 aggregation. PLoS ONE 6:e18789
51. Masino L, Nicastro G, Calder L, Vendruscolo M, Pastore A (2011) Functional interactions as a survival strategy against abnormal aggregation. FASEB J 25:45–54
52. Robertson AL, Headey SJ, Saunders HM, Ecroyd H, Scanlon MJ, Carver JA, Bottomley SP (2010) Small heat-shock proteins interact with a flanking domain to suppress polyglutamine aggregation. Proc Natl Acad Sci U S A 107:10424–10429
53. Almeida B, Fernandes S, Abreu IA, Macedo-Ribeiro S (2013) Trinucleotide repeats: a structural perspective. Front Neurol 4:76
54. Kristensen LV, Oppermann FS, Rauen MJ, Hartmann-Petersen R, Thirstrup K (2017) Polyglutamine expansion of ataxin-3 alters its degree of ubiquitination and phosphorylation at specific sites. Neurochemistry International (in press)
55. Fei E, Jia N, Zhang T, Ma X, Wang H, Liu C, Zhang W, Ding L, Nukina N, Wang G (2007) Phosphorylation of ataxin-3 by glycogen synthase kinase 3β at serine 256 regulates the aggregation of ataxin-3. Biochem Biophys Res Commun 357:487–492
56. Mueller T, Breuer P, Schmitt I, Walter J, Evert BO, Wullner U (2009) CK2-dependent phosphorylation determines cellular localization and stability of ataxin-3. Hum Mol Genet 18:3334–3343
57. Tao RS, Fei EK, Ying Z, Wang HF, Wang GH (2008) Casein kinase 2 interacts with and phosphorylates ataxin-3. Neurosci Bull 24:271–277
58. Todi SV, Scaglione KM, Blount JR, Basrur V, Conlon KP, Pastore A, Elenitoba-Johnson K, Paulson HL (2010) Activity and cellular functions of the deubiquitinating enzyme and polyglutamine disease protein ataxin-3 are regulated by ubiquitination at lysine 117. J Biol Chem 285:39303–39313
59. Faggiano S, Menon RP, Kelly GP, Todi SV, Scaglione KM, Konarev PV, Svergun DI, Paulson HL, Pastore A (2015) Allosteric regulation of deubiquitylase activity through ubiquitination. Front Mol Biosci 2:2
60. Jana NR, Dikshit P, Goswami A, Kotliarova S, Murata S, Tanaka K, Nukina N (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. J Biol Chem 280:11635–11640
61. Matsumoto M, Yada M, Hatakeyama S, Ishimoto H, Tanimura T, Tsuji S, Kakizuka A, Kitagawa M, Nakayama KI (2004) Molecular clearance of ataxin-3 is regulated by a mammalian E4. EMBO J 23:659–669

62. Miller VM, Nelson RF, Gouvion CM, Williams A, Rodriguez-Lebron E, Harper SQ, Davidson BL, Rebagliati MR, Paulson HL (2005) CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. Journal Neurosci Official J Soc Neurosci 25:9152–9161
63. Tsou WL, Burr AA, Ouyang M, Blount JR, Scaglione KM, Todi SV (2013) Ubiquitination regulates the neuroprotective function of the deubiquitinase ataxin-3 in vivo. J Biol Chem 288:34460–34469
64. Almeida B, Abreu IA, Matos CA, Fraga JS, Fernandes S, Macedo MG, Gutierrez-Gallego R, Pereira PJ, Carvalho AL, Macedo-Ribeiro S (2015) SUMOylation of the brain-predominant ataxin-3 isoform modulates its interaction with p97. Biochem Biophys Acta 1852:1950–1959
65. Zhou YF, Liao SS, Luo YY, Tang JG, Wang JL, Lei LF, Chi JW, Du J, Jiang H, Xia K, Tang BS, Shen L (2013) SUMO-1 modification on K166 of polyQ-expanded ataxin-3 strengthens its stability and increases its cytotoxicity. PLoS ONE 8:e54214
66. Wang Q, Li L, Ye Y (2006) Regulation of retrotranslocation by p97-associated deubiquitinating enzyme ataxin-3. J Cell Biol 174:963–971
67. Zhong X, Pittman RN (2006) Ataxin-3 binds VCP/p97 and regulates retrotranslocation of ERAD substrates. Hum Mol Genet 15:2409–2420

Chapter 15
Animal Models of Machado-Joseph Disease

Jana Schmidt and Thorsten Schmidt

Abstract Animal models are an important tool to study the pathophysiology of Machado-Joseph Disease (MJD). So far, animal models using simple organisms (like the round worm *Caenorhabditis elegans* or the fruit fly *drosophila*) but also mammalian models (mouse and even a non-human primate model) have been generated to study MJD. While simple organisms made an important contribution to the identification of pathophysiological mechanisms in MJD and were further used for modifier and screening purposes, mammalian models recapitulate major disease features of MJD in humans and are therefore a highly valuable tool for e.g. the validation of mechanisms or for pre-clinical validation of treatment approaches. Here we give an overview about the strategies which were used to model MJD and about the different models generated so far. We further highlight advantages of specific model organisms and describe the new findings which were made employing these animal models of MJD.

Keywords Animal models · *Drosophila* · *C. elegans* · *Mus musculus* Transgenic

15.1 Introduction

Animal models are indispensable especially for a late-onset disease like Machado-Joseph Disease (MJD), as they allow analysing pathogenic mechanisms and aspects of the disease which obviously cannot be studied in human MJD patients. For example, histopathological analyses of *post-mortem* human brain samples can be for obvious reasons only conducted at the end stage of a disease and therefore cannot reveal details about different disease stages. Sections and brain samples over the pathophysiological course of MJD can therefore only be gathered

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from animal models. In addition to this, animal models allow the assessment of disease modifiers or treatment approaches. Most commonly used model organisms contain an endogenous orthologue of the human *ATXN3* gene (Table 15.1).

No naturally occurring animal models of MJD or expansions of the endogenous polyglutamine repeats beyond a disease-causing threshold have been reported in animals yet [1]. Animal models of MJD were therefore either generated by transfection with a viral construct, classical transgenic approaches or by the modification (knock-in) of the endogenous *ATXN3* orthologue via the integration of an expanded polyglutamine repeat with surrounding sequence. Transgenic lines (in mice, flies, worms and even non-human primates) comprise of either the full-length ATXN3 cDNA, a fragment of it or even a large genomic YAC construct containing major parts of the *ATXN3* locus.

15.2 Non-mammalian Models of MJD

Simple organisms, like the roundworm (*Caenorhabditis elegans*) or the fruit fly (*Drosophila melanogaster*) appear on the first sight not that much suitable to model a neurodegenerative disorder, like MJD. However during the recent years, a number of highly relevant studies were performed employing either *C. elegans* or *Drosophila m.* thereby demonstrating their high suitability for research in MJD.

15.2.1 Caenorhabditis elegans *(Roundworm) Models of MJD*

The round worm (*Caenorhabditis elegans*) is especially suitable to serve as disease model due to its size (about 1 mm in length), short generation time (about 4 days), and especially its transparency. This allows an easy visualization (and counting) of living (or degenerating) cells in vivo. *C. elegans* are in most cases self-fertilizing hermaphrodites or (rarely) males. It is known that *C. elegans* hermaphrodites consists of about 1000 cells in total from which 302 are neurons (divided into 118 distinct classes) and 56 are glial cell [2]. *C. elegans* is considered to be a "good compromise between complexity of vertebrates… and extreme simplicity of yeast" [2].

About 65% of human disease genes have homologs in *C. elegans* [3] including *ATXN3*. The endogenous worm ortholog of human *ATXN3* (called atx-3) consists of four exons and does not contain a CAG repeat [4]. A detailed characterization of the *C. elegans* orthologue of *ATXN3* revealed ubiquitously expression starting in late embryonic development and retaining during adulthood. Strong expression can be detected in the spermatheca (i.e. the organ that produces sperm in hermaphrodites) and vulval muscle as well as in neuronal dorsal and ventral cord and neurons of the head and tail [4]. As human ATXN3, *C. elegans* atx-3 possesses deubiquitinase activity in vitro against polyubiquitin chains with a minimum length of four ubiquitin residues. The knock-out of the gene does not induce an obvious

Table 15.1 Orthologues of the ATXN3 protein in major model organisms

Organisms	NCBI Reference Sequence	UniProt	Protein size	PolyQ repeat	Josephin	UIM1	UIM2	PolyQ	UIM3
Human (*Homo sapiens*)	NP_004984	P54252-2	361 aa	variable	1–180	224–243	244–263	292–305	335–354
Rhesus macaque (*Macaca mulatta*)	XP_014999873	H9FUL0	360 aa	7 Q	1–180	224–243	244–263	298–304	334–353
Rat (*Rattus norvegicus*)	NP_067734	O35815	355 aa	3 Q	1–180	224–243	244–263	295–297	329–348
Mouse (*Mus musculus*)	NP_083981	O17850	355 aa	6 Q	1–180	224–243	244–263	292–297	329–348
Zebrafish (*Danio rerio*)	NP_001307461	Q1XA84	311 aa	–	1–175	219–234	239–258	–	–
Fly (*Drosphila melanogaster*)	no orthologue	–	–	–	–	–	–	–	–
Worm (*Caenorhabditis elegans*)	NP_506873	O17850	317 aa	–	7–178	219–239	247–264	–	–

Orthologues according to HomoloGene (www.ncbi.nlm.nih.gov/homologene). Protein domains according to UniProtKB (www.uniprot.org) and Prosite (prosite.expasy.org). Amino acid positions of domains within ATXN3 are listed. *UIM* Ubiquitin-interacting motif; – not existing/included

phenotype in worms under normal growth conditions but lead to differential expression of genes involved in cell structural/motility, signal transduction and the ubiquitin proteasome system [4]. Known interactions in humans (e.g. between ATXN3 and p97/VCP), likewise occur in *C. elegans* (i.e. between atx-3 and the *C. elegans* homologs of p97/VCP namely CDC-48.1 and CDC-48.2) [5]. Interestingly, under challenging growth conditions *atx-3* knock-out worms proved to have an enhanced stress response and display a better survival and tolerance against heat shock conditions possibly explained by the (pre-)activation of molecular chaperones upon the loss of *atx-3* (dependent on DAF-16) and thereby a more effective activation of this process upon heat shock [6].

In order to induce aggregation and an MJD-like phenotype in worms, transgenic constructs (under control of the pan-neuronal *unc-119* promoter) with either an extremely long polyglutamine repeat (130Q) within full-length ATXN3 or a C-terminal fragment of ATXN3 (aa284 ff with at least 63Q) are required. Full-length ATXN3 with 91Q or less, however, did not aggregate ([7], Table 15.2).

C-terminal fragments of ATXN3 induces neuronal dysfunction and behavioural symptoms (including uncoordinated locomotion and egg laying defects), interrupts synaptic transmission and causes morphological abnormalities of neuronal processes [7]. The fact that C-terminal fragments of ATXN3 induce a stronger phenotype than full-length ATXN3 has likewise previously been observed in mice [12]. Worms expressing full-length ATXN3 with an extremely long repeat (130Q) likewise developed movement deficits however not before reaching mid-age.

In another study, full-length ATXN3 with 130 glutamine repeats was able to induce an earlier phenotype in worms [8] including aggregation foci, lethargy and slightly reduced lifespan. This may be either due to the use of a different promoter (F25B3.3 promoter instead of unc-119) or a different isoform of ATXN3: While Khan et al. [7] employed the 1a short isoform of ATXN3 [10] (containing two ubiquitin-interacting motifs), Teixeira-Castro et al. [8] used the 3c isoform of ATXN3 (MJD1-1, [11]) harbouring an additional, third Ubiquitin-interacting motif.

As already observed in the study mentioned before, worms expressing a C-terminal fragment of ATXN3 exhibit a more severe phenotype than worms expressing the full-length protein. Motor dysfunction was highly associated with the aggregation of ATXN3: Likewise, the C-terminal fragment of ATXN3 was more aggregation prone than the full-length protein i.e. 75 glutamines induced aggregation in a C-terminal fragment of ATXN3 only but not in the full-length protein. Interestingly, aggregation of expanded ATXN3 occurred in a neuron-subtype-specific pattern rather than randomly indicating that the sequence surrounding the polyglutamine repeat confers a kind of cell-type specificity on ATXN3's toxicity [8]. Furthermore, it was shown that the insulin/insulin growth-factor-1 (*IGF-1*)-like signalling pathway facilitated by heat-shock factor-1 (HSF-1) together with aging modify the proteotoxicity of expanded ATXN3 [8]. This age-dependency seems to be neuron-specific as the aggregation and toxicity of a comparable C-terminal fragment of ATXN3 turned out not to be age-dependent when expressed in the *C. elegans* body wall muscle cells instead of neurons [9]. A special strength of *C. elegans* as a small model organism is its suitability for

Table 15.2 *C. elegans* models of MJD

Designation	Promoter	Full-length ATXN3/ fragment	Tag	Isoform	Repeat length	Included point mutations	Reference
MJD1-17Q-GFP MJD1-91Q-GFP MJD1-130Q-GFP	unc-119 (pan-neuronal)	full-length	GFP (C-term)	1a short	17/91/ 130Q	N/A	[7]
19Q-GFP 33Q-GFP 63Q-GFP 127Q-GFP	unc-119 (pan-neuronal)	C-term frag (aa 283ff)	GFP (C-term)	1a short	19/33/63/ 127Q	N/A	[7]
19Q-CFP 127Q-CFP	unc-119 (pan-neuronal)	C-term frag (aa 283ff)	CFP (C-term)	1a short	19/127Q	N/A	[7]
19Q-RFP 127Q-RFP	unc-119 (pan-neuronal)	C-term frag (aa 283ff)	RFP (C-term)	1a short	19/127Q	N/A	[7]
unc-47-19Q-GFP unc-47-127Q-GFP	unc-47 (GABAergic neurons)	C-term frag (aa 283ff)	GFP (C-term)	1a short	19/127Q	N/A	[7]
unc-47-19Q-RFP unc-47-127Q-RFP	unc-47 (GABAergic neurons)	C-term frag (aa 283ff)	RFP (C-term)	1a short	19/127Q	N/A	[7]
AT3q14 AT3q75 AT3q130	F25B3.3 (pan-neuronal)	full-length	YFP (C-term)	3c	14/75/ 130Q	N/A	[8]
257cAT3q14 257cAT3q75 257cAT3q80 257cAT3q128	F25B3.3 (pan-neuronal)	C-term frag (aa 258ff)	YFP (C-term)	3c	14/75/80/ 128Q	N/A	[8]
AT3CT(Q45) AT3CT(Q63)	unc-54 (body wall muscle)	C-term frag (aa 258ff)	YFP (C-term)	3c	45/63Q	N/A	[9]

The designation (according to the original description), the employed promoter, as well as the protein expressed from the transgene are listed. Isoform: The 1a short isoform of ATXN3 (MJD1a, [10]) contains two UIM (ubiquitin-interacting motifs) and a premature stop. The 1a long isoform contains two UIM as well but 16 additional amino acids at its C-terminus (MJD2-1, [11]; GenBank: U64821) and the 3c isoform contains an alternative C-terminus including a third UIM (MJD5-1, [11]; GenBank: U64820)

screening procedures. While large genetic screens for disease modifiers of MJD have been conducted in drosophila (see below), *C. elegans* models of MJD turned out to be especially useful for an effective screening of compound libraries and validation of small molecules alleviating locomotive defects induced by mutant ATXN3. Using this approach, the serotonin reuptake inhibitor citalopram was identified and later even validated in a transgenic mouse model for MJD [13].

15.2.2 Drosophila melanogaster *(Fly) Models of MJD*

The fruit fly (*Drosophila melanogaster*) is a small organisms of about three mm in length. One invaluable advantage of using *Drosophila m*. as model organism is the availability of fly stocks allowing practically an infinite number of genetic manipulations (e.g. overexpression or downregulation of genes using transgenic flies or flies containing genomic duplications or deletions). The availability of suitable promoter (driver) lines, allow in addition goal-directed targeting of the expression of the gene of interest.

Although about 75% of all human disease genes have related sequences in *drosophila* [14], the fruit fly does not contain a natural orthologue of *ATXN3*. The lack of an endogenous copy of the *ATXN3* gene excludes any knock-in and knock-out approaches in flies and may question the suitability of flies to model MJD as relevant target or binding partners of ATXN3 may be missing as well. Nevertheless, drosophila models expressing either a C-terminal fragment [15] or full-length [16] expanded human ATXN3 mimic some features of the disease in humans including intranuclear inclusions and neuronal degeneration. So far only flies expressing the short variant of the 1a isoform (containing a premature stop codon) are available and no flies expressing the 3c isoform (Table 15.3). The stability of the CAG repeat within a transgenic *ATXN3* depends on the activity of the CREB-binding protein (CBP) in flies [17].

A severe and progressive neurodegeneration with adult onset is induced upon expression of ATXN3 (either in the flies' eye or on the nervous system) with an expanded polyglutamine repeat (Q78 or Q84) only but not with a normal repeat (Q27) [15, 16]. On the contrary, co-expression of normal ATXN3 (Q27) delayed or even protected against the toxicity induced by either full-length or C-terminal expanded ATXN3 (Q78 or Q84) and normal ATXN3 further protected against the toxicity of expanded huntingtin and ataxin-1 [16]. This protective effect of normal ATXN3 depended on both its Ubiquitin-interactive motifs (UIM) and its deubiquitinase activity [16]. Interestingly, full-length ATXN3 even with an expanded repeat turned out to alleviate the toxicity of the C-terminal fragment of expanded ATXN3 [16]. In order to prevent the generation of ATXN3 fragments in vivo, Jung et al. [19] generated flies expressing full-length ATXN3 with six mutated putative Caspase cleavage sites (D171/208/217/223/225/228 N). The six mutations indeed prevented the cleavage of ATXN3 and mitigated its neurotoxicity, but without influencing its aggregation and formation of neuronal intranuclear inclusion [19].

Table 15.3 *Drosophila* models of MJD

Designation	Driver	Full-length ATXN3/fragment	Tag	Isoform	Repeat length	Included point mutations	Reference
MJDtr-Q27 MJDtr-Q78	gmr-GAL4 (eye)	C-term frag (aa 281ff)	HA (N-term)	1a short	27/78Q	N/A	[15]
MJDtr-Q27 MJDtr-Q78	elav-GAL4 (pan-neuronal)	C-term frag (aa 281ff)	HA (N-term)	1a short	27/78Q	N/A	[15]
MJDtr-Q27 MJDtr-Q78	elav, Appl-X, and Appl-III (neuronal)	C-term frag (aa 281ff)	HA (N-term)	1a short	27/78Q	N/A	[18]
MJDtr-Q27 MJDtr-Q78	M1B, loco-II, and loco-III (glial)	C-term frag (aa 281ff)	HA (N-term)	1a short	27/78Q	N/A	[18]
SCA3-Q27 SCA3-Q84	gmr-GAL4 (eye) elav-GAL4 (pan-neuronal)	full-length	myc (N-term)	1a short	27Q/84Q	N/A	[16]
SCA3-Q78	gmr-GAL4 (eye)	full-length	HA (N-term)	1a short	78Q	N/A	[16]
SCA3-Q27-UIM* SCA3-Q80-UIM*	gmr-GAL4 (eye)	full-length	myc (N-term)	1a short	27Q/80Q	S236A & S256A	[16]
SCA3-Q27-C14A SCA3-Q88-C14A	gmr-GAL4 (eye)	full-length	myc (N-term)	1a short	27Q/88Q	C14A	[16]
SCA3-delta	gmr-GAL4 (eye)	N-term frag (aa 1-287)	myc (N-term)	N/A	N/A	N/A	[16]
Myc-Atx3Q84-Flag	elav-GAL4 (neuronal) rh1-GAL4 (photoreceptor) gmr-GAL4 (eye)	full-length	myc (N-term) FLAG (C-term)	1a short	84Q	N/A	[19]
Myc-Atx3Q84-Flag (6 M)	elav-GAL4 (neuronal) rh1-GAL4 (photoreceptor) gmr-GAL4 (eye)	full-length	myc (N-term) FLAG (C-term)	1a short	84Q	D171/208/217/223/225/228 N	[19]

The designation, the employed driver, as well as the protein expressed from the transgene are listed. Isoform: see legend to Table 15.2

Noteworthy, expanded C-terminal ATXN3 induce the formation of aggregates and behavioral changes not only when expressed in neuronal cells but comparable effects were likewise be observed upon its expression in glial cells [18]. In the search for factors alleviating the phenotype in flies, it was observed in a candidate-gene-based approach that the toxicity of ATXN3 is diminished by the co-expression of chaperones, like HSP70 [20, 21]. Large genome-wide screens further revealed that several chaperones and components of the ubiquitin pathway alleviated the phenotype of MJD flies induced by expanded ATXN3 by reducing its aggregation and thereby its toxicity [22]. Additional modifiers identified are implicated in nuclear export and transcription [22]. Interestingly, some of these modifiers not only impacted the toxicity of expanded ATXN3 but of mutant Tau as well. This finding was later confirmed in an independent modifier screen [23] and indicates their relevance for a broader range of neurodegenerative disorders beyond MJD [22].

The identification of another modifying pathway in drosophila models of MJD shed light on a novel component of the pathophysiology in MJD: The upregulation of muscleblind (mbl), a modifier of CUG repeat RNA toxicity, increased the toxicity of expanded *ATXN3* thereby indicating that the CAG expansion within *ATXN3* does not only lead to toxicity mediated by the (translated) polyglutamine repeat but by the (transcribed) CUG mRNA as well [24]. Importantly, the authors further demonstrated that an *ATXN3* transgene loses partly its toxicity if a pure CAG repeat is interrupted by CAA although both CAG and CAA encode for glutamine and therefore the identical protein is encoded. Muscleblind seems to "act on the RNA to enhance polyQ toxicity" thereby accelerating the aggregation of ATXN3 [24].

ATXN2 is another highly interesting modifier identified in flies, as it causes (in its expanded form) another type of Spinocerebellar Ataxia: SCA2. The affected protein in SCA2, ATXN2, enhances in its normal state (when upregulated) the toxicity of ATXN3 and likewise mitigates (when downregulated) the neurodegeneration induced by expanded ATXN3 [25].

15.3 Mammalian (Mouse) Models of MJD

To learn more about the physiological function of ATXN3 in mammals, knock-out mice were generated by targeted mutagenesis [26]. *ATXN3* knock-out mice had normal viability and fertility and showed no gross locomotor abnormalities, but contained a higher level of ubiquitinated proteins, underlining a deubiquitinating function of ATXN3 [26]. Attempting to create a MJD knock-in model, Switonski et al. [27] generated a MJD knock-out model due to alternative splicing events. Also these MJD knock-out mice were viable, fertile with no reduced life span or neurological abnormalities, but showed no higher amounts of ubiquitinated proteins [27].

The first mouse model to study MJD pathology was generated by targeting the expression of full-length human ATXN3 containing an expanded polyglutamine stretch (79Q) to Purkinje cells (L7 promoter, [12], Table 15.4). Up to an observation time of 23 weeks, these mice did not demonstrate any phenotype. Mice

Table 15.4 Mouse models of MJD

Designation	Mouse strain	Promoter	ATXN3 protein (isoform, repeats)	Onset of symptoms (details)	Phenotype progression	Premature death	Used in modifier/ treatment study	Reference
MJD79	ns	L7	Full-length (1a short, 79Q)	No	No	No		[12]
$Q_{35}C$	ns	L7	C-term frag (aa290 ff.) (1a short, 35Q, N-term HA)	No	No	No		[12]
Q79	ns	L7	PolyQ frag (79Q, N-term HA)	4 w (gait, frequent falling)	Fast	ns		[12]
$Q_{79}C$	ns	L7	C-term frag (aa290 ff.) (1a short, 35Q, N-term HA)	4 w (gait, frequent falling)	Fast	ns		[12]
MJD15.1 MJD15.4	C57BL/6 J	ATXN3	Full-length (human locus, 15Q)	No	No	No		[28]
MJD64-84 (various)	C57BL/6 J	ATXN3	Full-length (human locus, 64-84Q)	4 w (gait & pelvic elevation)	Slow	No	[29–31]	[28]
Q20-A Q20-B	C57BL/6 J	*PRNP* (mouse)	Full-length(1a short, 20Q)	No	No	No		[32]
Q71-A Q71-B Q71-C	C57BL/6 J	*PRNP* (mouse)	Full-length (1a short, 71Q)	2 mo (hom only; tremor, hunchback)	Fast	Yes (hom)		[32]
deltaQ20	C57BL/6 J	*PRNP* (mouse)	Full-length (1a short, 20Q, Δaa190-220)	No	No	No		[33]
deltaQ71	C57BL/6 J	*PRNP* (mouse)	Full-length (1a short, 71Q, Δaa190-220)	1 mo (rotarod, weight)	Fast	Yes		[33]
15Q	C57BL/6 N	*PRNP* (mouse)	Full-length (3c, 15Q)	No	No	No		[34]

(continued)

Table 15.4 (continued)

Designation	Mouse strain	Promoter	ATXN3 protein (isoform, repeats)	Onset of symptoms (details)	Phenotype progression	Premature death	Used in modifier/ treatment study	Reference
70Q	C57BL/6 N	*PRNP* (mouse)	Full-length (3c, 70Q)	3 mo (tremor, wide based hindlimbs)	Fast	No	[35]	[34]
148Q	C57BL/6 N	*PRNP* (mouse)	Full-length (3c, 148Q)	2 mo (tremor, wide based hindlimbs)	Fast	Yes		[34]
148Q.NES	C57BL/6 N	*PRNP* (mouse)	Full-length (3c, 148Q, NES)	No	No	No		[34]
148Q.NLS	C57BL/6 N	*PRNP* (mouse)	Full-length (3c, 148Q, NLS)	1 mo (tremor, wide based hindlimbs)	Fast	Yes		[34]
ataxin-3-Q22	FVB/N	*PRNP* (mouse)	Full-length (1a short, 22Q)	No	No	No		[36]
ataxin-3-Q79	FVB/N	*PRNP* (mouse)	Full-length (1a short, 79Q)	5–6 mo (forelimb clasping, weight)	Slow	ns	[37–41]	[36]
ataxin-3[Q69]	C57BL/6 J	L7	C-term frag (HA-tagged) (aa 287 ff, 1a long, 35Q)	3 w (rotarod)	Fast	ns	[42–44]	[45]
Prp/MJD77	C57BL/6 N	*PRNP* (hamster)	Full-length (3c, 77Q)	9 w (rotarod)	Slow	No	[46]	[47]
HDPromMJD148	C57BL/6 N	huntingtin	Full-length (3c, 148Q)	12 mo (rotarod)	Slow	No		[48]
CMVMJD83	C57BL/6 bc FVB/N	CMV	Full-length (3c, 83Q)	No	No	No		[49]
CMVMJD94	C57BL/6 bc FVB/N	CMV	Full-length (3c, 94Q)	4 months (rotarod)	Slow	No		[49]

(continued)

Table 15.4 (continued)

Designation	Mouse strain	Promoter	ATXN3 protein (isoform, repeats)	Onset of symptoms (details)	Phenotype progression	Premature death	Used in modifier/ treatment study	Reference
CMVMJD135	C57BL/6	CMV	Full-length (3c, 135Q)	4 mo (gait)	Slow	No	[50–52, 13]	[53]
ataxin-3$^{(wt/gt)}$	C57BL/6 bc CD1	*ATXN3*	ATXN3 (aa 1-259)/lacZ-neoR	9 mo (clasping, gait, weight)	Fast	Yes		[54]
ataxin-3$^{(gt/gt)}$	C57BL/6 bc CD1	*ATXN3*	ATXN3 (aa 1-259)/lacZ-neoR	9 mo(clasping, gait, weight)	Fast	Yes		[54]
Ki91	C57BL/6 J	ATXN3	Mouse ex 1-6 mouse/human ex 7 human ex 8-11 with 91Q	90 w (rotaord)	Slow	No		[55]
ATXN3Q82/Q6	C57BL/6	ATXN3	Full length mouse ATXN3 (82Q)	No	Slow	No		[56]
ATXN3Q82/Q82	C57BL/6	ATXN3	Full length mouse ATXN3 (82Q)	No	Slow	No		[56]

The designation and further details about the employed construct, the protein expressed from the transgene and the induced phenotype are listed. Isoform: see legend to Table 15.2. *bc* backcrossed from strain; *hom* homozygotes; *PRNP* prion protein gene; *w* weeks; *mo* months; *ns* not specified

expressing a C-terminal ATXN3/polyglutamine fragment of 35 repeats ($Q_{35}C$) in Purkinje cells as well did not develop a phenotype. Only when the polyglutamine repeat number was extended to 79, mice developed an ataxic phenotype starting at four weeks of age with wide-based hindlimb stance and falling down when moving [12]. Neuropathologically, $Q_{79}C$ mice presented with cerebellar atrophy with all three layers affected by neuronal cell loss [12]. Ikeda et al. concluded that the truncated ATXN3 protein is more potent in inducing Purkinje cell pathology than the full-length protein [12].

In order to generate a model including all regulatory elements, Cemal et al. [28] used a yeast artificial chromosome (YAC) spanning 250 kb of the human *ATXN3* locus. Several different lines were established with 15 CAG repeats as control line (MJD15) and between 64 and 84 repeats resembling MJD disease lines (MJD64-84). Transgene expression levels equaled that of endogenous mouse *ATXN3*. Both the 1a long and the 3c isoforms of *ATXN3* [11] are expressed in these mice, while 3c is the predominant isoform detected on protein level [57]. The observed behavioral phenotype of affected mice included wide gait, lowered pelvis, tremor, hypoactivity, body clasping and loss of weight. Neuropathological analyses revealed cell loss in several brain regions also primarily affected in humans like pons, deep cerebellar nuclei and cerebellum. Transgenic ATXN3 is predominately localized in the nucleus and accumulates in neuronal nuclear inclusion bodies (NIIs) in MJD disease lines. Severity of disease symptoms was increased with higher copy number and repeat length [28]. Dantrolene, a stabilizer of intracellular calcium signaling, was able to improve the motor coordinative performance of MJD84.2 mice and reduced neuronal cell loss in this model [29]. Using this mouse line it was also shown that Purkinje cell dysfunction in these mice is associated with alterations in the physiology of voltage-activated potassium channels. The administration of SKA-31, an activator of calcium-activated potassium channels, was able to correct Purkinje cell firing and improve motor function of SCA3/MJD84.2 mice [31]. A RNA interference approach using microRNA was able to reduce soluble levels as well as abnormal neuronal intranuclear accumulation of mutant ATXN3 protein in MJD84.2 mice [30].

Goti et al. [32] generated MJD transgenic mice using full length human *ATXN3* cDNA containing either 20 CAG repeats for control lines (Q20) or 71 CAG repeats for disease lines (Q71). Transgene expression was controlled by the prion protein promoter from mouse. Starting at the age of 2 months homozygous Q71 mice developed a behavioral phenotype with tremor, ataxic limbs, abnormal posture (hunchback with low pelvic elevation and muscle wasting), impaired grip strength of fore- and hindlimbs, impaired rotarod performance, deteriorating footprint pattern and body weight loss. ATXN3 immunostaining was enriched in nuclei in several brain regions both in Q71 heterozygous and homozygous mice, but only the latter presented with large intranuclear inclusions. Furthermore the authors were able to detect a C-terminal ATXN3 cleavage fragment with high abundance in phenotypic Q71 mice and in affected brain regions of MJD patients [32]. Heterozygous Q71 mice bred poorly and homozygous mice were infertile due to gonadal failure secondary to a neuroendocrine dysfunction [58]. The generation of a mouse model lacking amino acids 190-220 (deltaQ71) narrowed a putative

proteolytic cleavage site to a region N-terminal of amino acids 190 within ATXN3 [33]. Genetic reduction of the co-chaperone and ubiquitin ligase CHIP (C-terminus of Hsp70-interacting protein) in Q71 mice resulted in an increase of ATXN3 microaggregates and worsening of the neurological phenotype leading the authors to suggest that enhancing CHIP activity could be therapeutically beneficial in MJD [59].

In 2007, Bichelmeier and colleagues [34] not only demonstrated that the severity of a MJD phenotype increases with increasing CAG repeat number, but also that nuclear localization of mutant ATXN3 is a prerequisite for the development of symptoms: Transgenic mice expressing full-length ATXN3 with 70 CAG repeats under the control of the murine prion protein promoter (70Q) developed a strong neurological phenotype with tremor, wide based hind limbs, reduced activity, body weight and grooming. Purkinje cells in this model appeared shrunken and with an increased electron density. Expanding the CAG repeat size to 148 (148Q), mice showed comparable symptoms earlier in life leading to premature death already at three months of age. Transgenic mice in which ATXN3 with 148 CAG repeats was kept in the nucleus by attaching a nuclear localization signal (148Q.NLS) were even more severely and earlier affected. On the other hand, keeping ATXN3 out of the nucleus using a nuclear export signal (148Q.NES) led to only very mild or even missing behavioral symptoms. Moreover, inclusion bodies abundant in several brain regions in transgenic mice with 70, 148 and 148 polyglutamines with NLS were highly diminished in the 148Q.NES model [34]. Pharmaceutical upregulation of autophagy resulted in decrease of soluble mutant ATXN3 and number of inclusion bodies as well as in an improvement of rotarod performance in 70Q mice [35].

Another model using the mouse prion protein promoter and an ATXN3 construct with 22 and 79 polyglutamines (ataxin-3-Q22 and ataxin-3-Q79), respectively was generated by Chou et al. [36]. Transgenic ataxin-3-Q79 mice developed a progressive neurological phenotype with forelimb clasping, impaired motor coordination, reduced locomotor activity and ataxic wide-based gait. Symptoms deteriorated with age leading to reduced pelvic elevation and hunch back posture at around one year of age. Purkinje cells exhibited morphological characteristics of degeneration. Intranuclear inclusion bodies were observed in neurons of the dentate nucleus, pontine nucleus and substantia nigra [36]. The authors identified transcriptional dysregulation of genes involved in glutamatergic neurotransmission, intracellular calcium signaling, MAP kinase pathways, regulators of neuronal survival or differentiation of $GABA_{A/B}$ receptor subunits and heat shock response in ataxin-3-Q79 mice before ataxic symptoms became evident. These findings led to hypothesize that the ataxin-3-Q79 transgene causes transcriptional downregulation by the induction of hypoacetylation and that the HDAC inhibitor sodium butyrate is able to reverse this transcriptional repression and thereby alleviates ataxic symptoms observed in ataxin-3-Q79 mice. In follow up studies the authors were able to proof the efficacy of sodium butyrate in reversing transcriptional downregulation in the cerebellum of ataxin-3-Q79 mice. Furthermore, the onset and severity of ataxic symptoms in these mice were delayed and ameliorated, respectively [37, 39]. Based

on upregulated mRNA expression profiles of different apoptotic pathways, the authors also tested a second hypothesis: Ataxin-3-Q79 mice were treated with a pro-apototic gene inhibitor leading to significantly ameliorated neuronal cell death in pontine nuclei [38]. In a third attempt, the same authors therapeutically targeted the ubiquitin-proteasome system. Transgenic mice were treated with an adenosine A_{2A} receptor agonist leading to significantly increased chymotrypsin-like activity of the proteasome. This treatment resulted in reduced protein level of mutant ATXN3 and alleviated neuronal cell death and ataxic symptoms in treated ataxin-3-Q79 mice [40].

To test whether a newly identified guanosine triphosphatase (CRAG) has the potential to be therapeutically effective in the treatment of polyglutamine diseases Torashima et al. [45] generated a new mouse model. In these mice the Purkinje-cell-specific promoter L7 controlled the expression of a truncated form of human ATXN3 starting at amino acid 287 with 69 glutamines (ataxin-3[Q69]). Transgenic mice developed an ataxic phenotype with early onset, reduced weight, impaired rotarod performance and severe cerebellar atrophy. Inclusion bodies were present in the cytoplasm or extracellular of Purkinje cells [45]. Lentiviral vector-mediated expression of CRAG in Purkinje cells of this model led to clearance of inclusions and rescue of the ataxic phenotype [45]. Konno et al. [43] further analyzed this model and another study proved that Neuropeptide Y was able to alleviate motor coordinative abnormalities and neuropathological parameters in these mice [42].

The first conditional mouse model for MJD was generated by Boy et al. in 2009 [47]. Mice were generated employing the Tet-off system [60]. A promoter mouse line containing the prion protein promoter from hamster was cross bred to a responder mouse line expressing *ATXN3* with 77 CAG repeats. In double transgenic mice containing both transgene constructs (Prp/MJD77) a factor (tTA) transcribed by the promoter construct is able to bind to the responder construct, thereby activating the expression of transgenic ATXN3. In the presence of tetracycline, this antibiotic binds to the tTA factor and thus inhibits its binding to the responder. In this way the expression of mutant ATXN3 can be turned off. Double transgenic Prp/MJD77 mice developed a progressive neurological phenotype characterized by deficits in motor coordination and balance, hyperactivity, reduced anxiety and reduced gain in body weight. ATXN3 positive intranuclear inclusion bodies were detected in brains of double transgenic mice and Purkinje cells presented with higher electron density indicating dark cell degeneration. Turning the expression of mutant ATXN3 off resulted in a complete reversal of the observed rotarod impairment as well as the reduced body weight gain proving that MJD symptoms although already manifested can be reversed [47]. Employing this model the suitability of riluzole as treatment for MJD has been assessed [46].

In 2010, Boy et al. [48] published a late onset MJD mouse model, using an *ATXN3* construct with 148 CAG repeats under the control of a fragment of the huntingtin promoter (HDPromMJD148). Mutant ATXN3 is ubiquitiously expressed throughout the whole brain. Several main characteristics of the MJD disease in humans, like the late onset as well as intergenerational CAG repeat instability, are

resembled in this model. Rotarod impairment as sign of decline of motor coordination occurs at around one year of age, whereas inclusion bodies are not detectable before 18 months of age, stressing that neuronal dysfunction is independent from the formation of inclusions. Electron microscopical analysis revealed that Purkinje cells, also in this MJD model, show dark cell degeneration [48].

Using the CMV promoter to control the expression of an *ATXN3* construct with 83 and 94 CAG repeats, respectively, was the strategy of Silva-Fernandes et al. [49] to generate two more mouse models for MJD. CMVMJD94 mice presented with CAG repeat instability, neuronal dysfunction and impairment of motor function, whereas no phenotype was detectable in the CMVMJD83 model [49]. Using the CMVMJD94 model mitochondrial DNA damage and its suitability as a biomarker of initial stages of the SCA3 disease was dissected [61, 62]. Silva-Fernanades et al. also generated a third model in which the CAG stretch was expanded to 135 repeats (CMVMJD135) [53]. In these mice main characteristics of the human MJD disease, like intergenerational repeat length variation, progressive neurological deficits, reduced gain of body weight and formation of intranuclear inclusion bodies in different brain regions are recapitulated [53]. Hsp90 inhibition by administration of 17-DMAG resulted in reduced levels of ATXN3 protein and lower numbers of intranuclear inclusions in CMVMJD135 mice [53]. Using these mice the efficacy of valproic acid and citalopram as treatment for MJD was assessed [52, 13].

In 2011, Hübener et al. [54] reported on a MJD mouse model generated by gene trap integration, leading to an ATXN3 fusion protein containing 259 N-terminal amino acids, but lacking the C-terminal polyglutamine stretch and other regulatory regions. Initially ataxin-$3^{(gt/gt)}$ mice develop without gross abnormalities. However, with 9 months of age a severe deterioration with impaired motor coordination and body weight loss was observed, leading to premature death with about 12 months of age. Phenotypic mice presented with extranuclear inclusion bodies and neuronal cell death of Purkinje cells and cells of the molecular layer of the cerebellum. Authors concluded that the N-terminal region of ATXN3 can contribute to MJD symptoms [54].

Knock-in technology presents a method to generate a model which expresses the mutant gene under control of the endogenous promoter in the proper genomic context including all regulatory elements. 2015 two different knock-in mouse models for MJD were published [56, 55]. In the first model a CAG repeat expansion was inserted into the murine *ATXN3* locus by homologous recombination generating heterozygous Atxn3Q82/Q6 and homozygous Atxn3Q82/Q82 mice. Early accumulation of ATXN3 protein in intranuclear puncta as well as large neuritic inclusions in older mice and altered splicing of mutant *ATXN3* were characteristics observed in this knock-in model. However, behavioral deficits or neuropathological changes are missing in these mice, leading the authors to promote this model as being especially suitable for analyses of pathogenic mechanisms preceding neuronal dysfunction or cell loss [56].

The second humanized knock-in model was generated by exchanging exons 7 through 11 of the mouse *ATXN3* locus with human exon 7 through 11 containing 91 CAG repeats. This resulted in intergenerational CAG repeat

instability, loss of Purkinje cells and impairment of motor coordination with late onset in Ki91 mice [55].

Injection of lentiviral vectors encoding transgenic *ATXN3* constructs presents an alternative, fast and cost effective approach to generate MJD models. First studies using this technique involved rats as a MJD model organism [63, 64]. Lentiviral vectors expressing mutant ATXN3 constructs were injected in different brain regions led to behavioral deficits, neuronal cell loss and inclusion body formation [63]. Delivery of allele-specific siRNA via lentiviral vectors was able to silence mutant *ATXN3* expression and decreased neuropathological abnormalities [64, 65, 44]. Targeting the mouse cerebellum with lentiviral-mediated expression of mutant *ATXN3* resulted in the development of a behavioral phenotype, intranuclear inclusions and neuronal cell death abnormalities [66].

15.4 Non-human Primate Model of MJD

Very recently, the first transgenic non-human primate i.e. marmoset model of MJD has been generated [67]. Transgenic marmosets were generated using a lentiviral approach based on a construct containing 120 CAG interrupted by CAA (coding for glutamine as well) every 30 repeats within the full-length *ATXN3* cDNA (3c isoform). However, studies employing non-human primates are expensive and time-consuming and only three monkeys of the founder generation reaching a certain expression level of the transgene (likely due to multiple insertions of the transgene in different chromosomes) developed symptoms and could be analysed yet. This model displays protein aggregates, neurodegeneration and degeneration of skeletal muscles as well as varying neurological symptoms (including grip strength deficits and reduced body weight gain) within 3–4 months of age. Although transmission of the transgene was confirmed, a stable transgenic line has not been generated yet, possibly complicated by mosaicism in the founder marmoset and integration into multiple and different chromosomes [67].

References

1. Matos CA, de Macedo-Ribeiro S, Carvalho AL (2011) Polyglutamine diseases: the special case of ataxin-3 and Machado-Joseph disease. Prog Neurobiol 95(1):26–48
2. Bessa C, Maciel P, Rodrigues AJ (2013) Using *C. elegans* to decipher the cellular and molecular mechanisms underlying neurodevelopmental disorders. Mol Neurobiol 48(3):465–489
3. Sonnhammer EL, Durbin R (1997) Analysis of protein domain families in *Caenorhabditis elegans*. Genomics 46(2):200–216
4. Rodrigues AJ, Coppola G, Santos C, Costa Mdo C, Ailion M, Sequeiros J, Geschwind DH, Maciel P (2007) Functional genomics and biochemical characterization of the *C. elegans* orthologue of the Machado-Joseph disease protein ataxin-3. FASEB J 21(4):1126–1136

5. Rodrigues AJ, Neves-Carvalho A, Ferro A, Rokka A, Corthals G, Logarinho E, Maciel P (2009) ATX-3, CDC-48 and UBXN-5: a new trimolecular complex in *Caenorhabditis elegans*. Biochem Biophys Res Commun 386(4):575–581
6. Rodrigues AJ, Neves-Carvalho A, Teixeira-Castro A, Rokka A, Corthals G, Logarinho E, Maciel P (2011) Absence of ataxin-3 leads to enhanced stress response in *C. elegans*. PLoS One 6(4):e18512
7. Khan LA, Bauer PO, Miyazaki H, Lindenberg KS, Landwehrmeyer BG, Nukina N (2006) Expanded polyglutamines impair synaptic transmission and ubiquitin-proteasome system in *Caenorhabditis elegans*. J Neurochem 98(2):576–587
8. Teixeira-Castro A, Ailion M, Jalles A, Brignull HR, Vilaça JL, Dias N, Rodrigues P, Oliveira JF, Neves-Carvalho A, Morimoto RI, Maciel P (2011) Neuron-specific proteotoxicity of mutant ataxin-3 in *C. elegans*: rescue by the DAF-16 and HSF-1 pathways. Hum Mol Genet 20(15):2996–3009
9. Christie NT, Lee AL, Fay HG, Gray AA, Kikis EA (2014) Novel polyglutamine model uncouples proteotoxicity from aging. PLoS One 9(5):e96835
10. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, Katayama S, Kawakami H, Nakamura S, Nishimura M, Akiguchi I, Kimura J, Narumiya S, Kakizuka A (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8 (3):221–228
11. Goto J, Watanabe M, Ichikawa Y, Yee SB, Ihara N, Endo K, Igarashi S, Takiyama Y, Gaspar C, Maciel P, Tsuji S, Rouleau GA, Kanazawa I (1997) Machado-Joseph disease gene products carrying different carboxyl termini. Neurosci Res 28(4):373–377
12. Ikeda H, Yamaguchi M, Sugai S, Aze Y, Narumiya S, Kakizuka A (1996) Expanded polyglutamine in the Machado-Joseph disease protein induces cell death in vitro and in vivo. Nat Genet 13(2):196–202
13. Teixeira-Castro A, Jalles A, Esteves S, Kang S, da Silva Santos L, Silva-Fernandes A, Neto MF, Brielmann RM, Bessa C, Duarte-Silva S, Miranda A, Oliveira S, Neves-Carvalho A, Bessa J, Summavielle T, Silverman RB, Oliveira P, Morimoto RI, Maciel P (2015) Serotonergic signalling suppresses ataxin 3 aggregation and neurotoxicity in animal models of Machado-Joseph disease. Brain 138(Pt 11):3221–3237
14. Bier E (2005) Drosophila, the golden bug, emerges as a tool for human genetics. Nat Rev Genet 6:9–23
15. Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, Pittman RN, Bonini NM (1998) Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. Cell 93(6):939–949
16. Warrick JM, Morabito LM, Bilen J, Gordesky-Gold B, Faust LZ, Paulson HL, Bonini NM (2005) Ataxin-3 suppresses polyglutamine neurodegeneration in *Drosophila* by a ubiquitin-associated mechanism. Mol Cell 18(1):37–48
17. Jung J, Bonini N (2007) CREB-binding protein modulates repeat instability in a *Drosophila* model for polyQ disease. Science 315(5820):1857–1859
18. Kretzschmar D, Tschäpe J, Bettencourt Da Cruz A, Asan E, Poeck B, Strauss R, Pflugfelder GO (2005) Glial and neuronal expression of polyglutamine proteins induce behavioral changes and aggregate formation in *Drosophila*. Glia 49(1):59–72
19. Jung J, Xu K, Lessing D, Bonini NM (2009) Preventing Ataxin-3 protein cleavage mitigates degeneration in a *Drosophila* model of SCA3. Hum Mol Genet 18(24):4843–4852
20. Chan HY, Warrick JM, Gray-Board GL, Paulson HL, Bonini NM (2000) Mechanisms of chaperone suppression of polyglutamine disease: selectivity, synergy and modulation of protein solubility in *Drosophila*. Hum Mol Genet 9(19):2811–2820
21. Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM (1999) Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone HSP70. Nat Genet 23(4):425–428
22. Bilen J, Bonini NM (2007) Genome-wide screen for modifiers of ataxin-3 neurodegeneration in *Drosophila*. PLoS Genet 3(10):1950–1964

23. Voßfeldt H, Butzlaff M, PrüSSing K, Ní Chárthaigh RA, Karsten P, Lankes A, Hamm S, Simons M, Adryan B, Schulz JB, Voigt A (2012) Large-scale screen for modifiers of ataxin-3-derived polyglutamine-induced toxicity in *Drosophila*. PLoS One 7(11):e47452
24. Li LB, Yu Z, Teng X, Bonini NM (2008) RNA toxicity is a component of ataxin-3 degeneration in *Drosophila*. Nature 453(7198):1107–1111
25. Lessing D, Bonini NM (2008) Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in *Drosophila*. PLoS Biol 6(2):e29
26. Schmitt I, Linden M, Khazneh H, Evert BO, Breuer P, Klockgether T, Wuellner U (2007) Inactivation of the mouse Atxn3 (ataxin-3) gene increases protein ubiquitination. Biochem Biophys Res Commun 362(3):734–739
27. Switonski PM, Fiszer A, Kazmierska K, Kurpisz M, Krzyzosiak WJ, Figiel M (2011) Mouse ataxin-3 functional knock-out model. Neuromolecular Med 13(1):54–65
28. Cemal CK, Carroll CJ, Lawrence L, Lowrie MB, Ruddle P, Al-Mahdawi S, King RH, Pook MA, Huxley C, Chamberlain S (2002) YAC transgenic mice carrying pathological alleles of the MJD1 locus exhibit a mild and slowly progressive cerebellar deficit. Hum Mol Genet 11(9):1075–1094
29. Chen X, Tang TS, Tu H, Nelson O, Pook M, Hammer R, Nukina N, Bezprozvanny I (2008) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. J Neurosci 28(48):12713–12724
30. Rodríguez-Lebrón E, Costa MD, Luna-Cancalon K, Peron TM, Fischer S, Boudreau RL, Davidson BL, Paulson HL (2013) Silencing mutant ATXN3 expression resolves molecular phenotypes in SCA3 transgenic mice. Mol Ther 21(10):1909–1918
31. Shakkottai VG, do Carmo Costa M, Dell'Orco JM, Sankaranarayanan A, Wulff H, Paulson HL (2011) Early changes in cerebellar physiology accompany motor dysfunction in the polyglutamine disease spinocerebellar ataxia type 3. J Neurosci 31(36):13002–13014
32. Goti D, Katzen SM, Mez J, Kurtis N, Kiluk J, Ben-Haïem L, Jenkins NA, Copeland NG, Kakizuka A, Sharp AH, Ross CA, Mouton PR, Colomer V (2004) A mutant ataxin-3 putative-cleavage fragment in brains of Machado-Joseph disease patients and transgenic mice is cytotoxic above a critical concentration. J Neurosci 24(45):10266–10279
33. Colomer Gould VF, Goti D, Pearce D, Gonzalez GA, Gao H, Bermudez de Leon M, Jenkins NA, Copeland NG, Ross CA, Brown DR (2007) A mutant ataxin-3 fragment results from processing at a site N-terminal to amino acid 190 in brain of Machado-Joseph disease-like transgenic mice. Neurobiol Dis 27(3):362–369
34. Bichelmeier U, Schmidt T, Hübener J, Boy J, Rüttiger L, Häbig K, Poths S, Bonin M, Knipper M, Schmidt WJ, Wilbertz J, Wolburg H, Laccone F, Riess O (2007) Nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3: in vivo evidence. J Neurosci 27(28):7418–7428
35. Menzies FM, Huebener J, Renna M, Bonin M, Riess O, Rubinsztein DC (2010) Autophagy induction reduces mutant ataxin-3 levels and toxicity in a mouse model of spinocerebellar ataxia type 3. Brain 133(Pt 1):93–104
36. Chou AH, Yeh TH, Ouyang P, Chen YL, Chen SY, Wang HL (2008) Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. Neurobiol Dis 31(1):89–101
37. Chou AH, Chen SY, Yeh TH, Weng YH, Wang HL (2011) HDAC inhibitor sodium butyrate reverses transcriptional downregulation and ameliorates ataxic symptoms in a transgenic mouse model of SCA3. Neurobiol Dis 41(2):481–488
38. Chou AH, Lin AC, Hong KY, Hu SH, Chen YL, Chen JY, Wang HL (2011) p53 activation mediates polyglutamine-expanded ataxin-3 upregulation of Bax expression in cerebellar and pontine nuclei neurons. Neurochem Int 58(2):145–152
39. Chou AH, Chen YL, Hu SH, Chang YM, Wang HL (2014) Polyglutamine-expanded ataxin-3 impairs long-term depression in Purkinje neurons of SCA3 transgenic mouse by inhibiting HAT and impairing histone acetylation. Brain Res 1583:220–229
40. Chou AH, Chen YL, Chiu CC, Yuan SJ, Weng YH, Yeh TH, Lin YL, Fang JM, Wang HL (2015) T1-11 and JMF1907 ameliorate polyglutamine-expanded ataxin-3-induced

neurodegeneration, transcriptional dysregulation and ataxic symptom in the SCA3 transgenic mouse. Neuropharmacology 99:308–317
41. Wang HL, Hu SH, Chou AH, Wang SS, Weng YH, Yeh TH (2013) H1152 promotes the degradation of polyglutamine-expanded ataxin-3 or ataxin-7 independently of its ROCK-inhibiting effect and ameliorates mutant ataxin-3-induced neurodegeneration in the SCA3 transgenic mouse. Neuropharmacology 70:1–11
42. Duarte-Neves J, Gonçalves N, Cunha-Santos J, Simões AT, den Dunnen WF, Hirai H, Kügler S, Cavadas C, Pereira de Almeida L (2015) Neuropeptide Y mitigates neuropathology and motor deficits in mouse models of Machado-Joseph disease. Hum Mol Genet 24(19):5451–5463
43. Konno A, Shuvaev AN, Miyake N, Miyake K, Iizuka A, Matsuura S, Huda F, Nakamura K, Yanagi S, Shimada T, Hirai H (2014) Mutant ataxin-3 with an abnormally expanded polyglutamine chain disrupts dendritic development and metabotropic glutamate receptor signaling in mouse cerebellar Purkinje cells. Cerebellum 13(1):29–41
44. Nóbrega C, Nascimento-Ferreira I, Onofre I, Albuquerque D, Hirai H, Déglon N, de Almeida LP (2013) Silencing mutant ataxin-3 rescues motor deficits and neuropathology in Machado-Joseph disease transgenic mice. PLoS One 8(1):e52396
45. Torashima T, Koyama C, Iizuka A, Mitsumura K, Takayama K, Yanagi S, Oue M, Yamaguchi H, Hirai H (2008) Lentivector-mediated rescue from cerebellar ataxia in a mouse model of spinocerebellar ataxia. EMBO Rep 9(4):393–399
46. Schmidt J, Schmidt T, Golla M, Lehmann L, Weber JJ, Hübener-Schmid J, Riess O (2016) In vivo assessment of riluzole as a potential therapeutic drug for spinocerebellar ataxia type 3. J Neurochem 138(1):150–162
47. Boy J, Schmidt T, Wolburg H, Mack A, Nuber S, Böttcher M, Schmitt I, Holzmann C, Zimmermann F, Servadio A, Riess O (2009) Reversibility of symptoms in a conditional mouse model of spinocerebellar ataxia type 3. Hum Mol Genet 18(22):4282–4295
48. Boy J, Schmidt T, Schumann U, Grasshoff U, Unser S, Holzmann C, Schmitt I, Karl T, Laccone F, Wolburg H, Ibrahim S, Riess O (2010) A transgenic mouse model of spinocerebellar ataxia type 3 resembling late disease onset and gender-specific instability of CAG repeats. Neurobiol Dis 37(2):284–293
49. Silva-Fernandes A, Costa Mdo C, Duarte-Silva S, Oliveira P, Botelho CM, Martins L, Mariz JA, Ferreira T, Ribeiro F, Correia-Neves M, Costa C, Maciel P (2010) Motor uncoordination and neuropathology in a transgenic mouse model of Machado-Joseph disease lacking intranuclear inclusions and ataxin-3 cleavage products. Neurobiol Dis 40(1):163–176
50. Duarte-Silva S, Neves-Carvalho A, Soares-Cunha C, Teixeira-Castro A, Oliveira P, Silva-Fernandes A, Maciel P (2014) Lithium chloride therapy fails to improve motor function in a transgenic mouse model of Machado-Joseph disease. Cerebellum 13(6):713–727
51. Duarte-Silva S, Silva-Fernandes A, Neves-Carvalho A, Soares-Cunha C, Teixeira-Castro A, Maciel P (2016) Combined therapy with m-TOR-dependent and -independent autophagy inducers causes neurotoxicity in a mouse model of Machado-Joseph disease. Neuroscience 313:162–173
52. Esteves S, Duarte-Silva S, Naia L, Neves-Carvalho A, Teixeira-Castro A, Rego AC, Silva-Fernandes A, Maciel P (2015) Limited effect of chronic valproic acid treatment in a mouse model of Machado-Joseph Disease. PLoS One 10(10):e0141610
53. Silva-Fernandes A, Duarte-Silva S, Neves-Carvalho A, Amorim M, Soares-Cunha C, Oliveira P, Thirstrup K, Teixeira-Castro A, Maciel P (2014) Chronic treatment with 17-DMAG improves balance and coordination in a new mouse model of Machado-Joseph disease. Neurotherapeutics 11(2):433–449
54. Hübener J, Vauti F, Funke C, Wolburg H, Ye Y, Schmidt T, Wolburg-Buchholz K, Schmitt I, Gardyan A, Driessen S, Arnold HH, Nguyen HP, Riess O (2011) N-terminal ataxin-3 causes neurological symptoms with inclusions, endoplasmic reticulum stress and ribosomal dislocation. Brain 134(Pt 7):1925–1942

55. Switonski PM, Szlachcic WJ, Krzyzosiak WJ, Figiel M (2015) A new humanized ataxin-3 knock-in mouse model combines the genetic features, pathogenesis of neurons and glia and late disease onset of SCA3/MJD. Neurobiol Dis 73:174–188
56. Ramani B, Harris GM, Huang R, Seki T, Murphy GG, Costa Mdo C, Fischer S, Saunders TL, Xia G, McEachin RC, Paulson HL (2015) A knockin mouse model of spinocerebellar ataxia type 3 exhibits prominent aggregate pathology and aberrant splicing of the disease gene transcript. Hum Mol Genet 24(5):1211–1224
57. Harris GM, Dodelzon K, Gong L, Gonzalez-Alegre P, Paulson HL (2010) Splice isoforms of the polyglutamine disease protein ataxin-3 exhibit similar enzymatic yet different aggregation properties. PLoS One 5(10):e13695
58. Colomer Gould VF, Goti D, Kiluk J (2006) A neuroendocrine dysfunction, not testicular mutant ataxin-3 cleavage fragment or aggregate, causes cell death in testes of transgenic mice. Cell Death Differ 13(3):524–526
59. Williams AJ, Knutson TM, Colomer Gould VF, Paulson HL (2009) In vivo suppression of polyglutamine neurotoxicity by C-terminus of Hsp70-interacting protein (CHIP) supports an aggregation model of pathogenesis. Neurobiol Dis 33(3):342–353
60. Gossen M, Bujard H (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci U S A 89(12):5547–5551
61. Kazachkova N, Raposo M, Montiel R, Cymbron T, Bettencourt C, Silva-Fernandes A, Silva S, Maciel P, Lima M (2013) Patterns of mitochondrial DNA damage in blood and brain tissues of a transgenic mouse model of Machado-Joseph disease. Neurodegener Dis 11 (4):206–214
62. Ramos A, Kazachkova N, Silva F, Maciel P, Silva-Fernandes A, Duarte-Silva S, Santos C, Lima M (2015) Differential mtDNA damage patterns in a transgenic mouse model of Machado-Joseph disease (MJD/SCA3). J Mol Neurosci 55(2):449–453
63. Alves S, Régulier E, Nascimento-Ferreira I, Hassig R, Dufour N, Koeppen A, Carvalho AL, Simões S, de Lima MC, Brouillet E, Gould VC, Déglon N, de Almeida LP (2008) Striatal and nigral pathology in a lentiviral rat model of Machado-Joseph disease. Hum Mol Genet 17 (14):2071–2083
64. Alves S, Nascimento-Ferreira I, Auregan G, Hassig R, Dufour N, Brouillet E, Pedroso de Lima MC, Hantraye P, Pereira de Almeida L, Déglon N (2008) Allele-specific RNA silencing of mutant ataxin-3 mediates neuroprotection in a rat model of Machado-Joseph disease. PLoS One 3(10):e3341
65. Alves S, Nascimento-Ferreira I, Dufour N, Hassig R, Auregan G, Nóbrega C, Brouillet E, Hantraye P, Pedroso de Lima MC, Déglon N, de Almeida LP (2010) Silencing ataxin-3 mitigates degeneration in a rat model of Machado-Joseph disease: no role for wild-type ataxin-3? Hum Mol Genet 19(12):2380–2394
66. Nóbrega C, Nascimento-Ferreira I, Onofre I, Albuquerque D, Conceição M, Déglon N, de Almeida LP (2013) Overexpression of mutant ataxin-3 in mouse cerebellum induces ataxia and cerebellar neuropathology. Cerebellum 12(4):441–455
67. Tomioka I, Ishibashi H, Minakawa EN, Motohashi HH, Takayama O, Saito Y, Popiel HA, Puentes S, Owari K, Nakatani T, Nogami N, Yamamoto K, Noguchi S, Yonekawa T, Tanaka Y, Fujita N, Suzuki H, Kikuchi H, Aizawa S, Nagano S, Yamada D, Nishino I, Ichinohe N, Wada K, Kohsaka S, Nagai Y, Seki K (2017) Transgenic monkey model of the polyglutamine diseases recapitulating progressive neurological symptoms. eNeuro 4(2). pii: ENEURO.0250-16.2017

Chapter 16
Towards the Identification of Molecular Biomarkers of Spinocerebellar Ataxia Type 3 (SCA3)/Machado-Joseph Disease (MJD)

Manuela Lima and Mafalda Raposo

Abstract Whereas spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD) remains an untreatable disorder, disease-modifying compounds have begun being tested in the context of clinical trials; their success is dependent on the sensitivity of the methods used to measure subtle therapeutic benefits. Thus, efforts are being made to propose a battery of potential outcome measures, including molecular biomarkers (MBs), which remain to be identified; MBs are particularly pertinent if SCA3 trials are expected to enroll preataxic subjects. Recently, promising candidate MBs of SCA3 have emerged from gene expression studies. In this chapter we provide a synthesis of the cross-sectional and pilot longitudinal studies of blood-based transcriptional biomarkers conducted so far. Other alterations with potential to track the progression of SCA3, such as those involving mitochondrial DNA (mtDNA) are also referred. It is expected that a set of molecular biomarkers can be identified; these will be used in complementarity with clinical and imaging markers to fully track SCA3, from its preataxic phase to the disease stage.

Keywords Polyglutamine disorders · Biochemical markers · Transcriptional dysregulation · RNA · Trait biomarkers · State biomarkers

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16.1 Spinocerebellar Ataxia Type 3 (SCA3): Overview

Spinocerebellar ataxia type 3 (SCA3)/Machado-Joseph disease (MJD) is the most frequent of the autosomal dominant ataxias, displaying a worldwide prevalence of 1.5 cases per 100,000 inhabitants [1]. In the Portuguese Azores, to where the first descriptions of SCA3 trace (revised in [2]), an homogeneous cohort of patients has been described [3–5], whose importance for the understanding of several aspects of this disease has been reinforced by Raposo and collaborators [6]. A recent longitudinal epidemiological study of SCA3 in the Azores Islands reported an overall prevalence of 1 in each 2544 for the total archipelago, and of 1 in each 158 for the island of Flores [7].

SCA3 involves predominantly the cerebellar, pyramidal, extrapyramidal, motor neuron and oculomotor systems. Although the average age for the appearance of first symptoms is around 40 years, extremes from 4 to 70 years have been reported (revised in [8]). As a clinical entity, SCA3 is recognized as highly pleomorphic; pleomorphism is reflected on the marked variation of age at onset, the heterogeneity of the clinical features displayed by the different patients, as well as on the existence of particular/atypical clinical presentations (see, for example, [9]). Clinical heterogeneity is further evidenced by inter-patient differences in the rate of disease progression and magnitude of the associated disability.

SCA3 causative gene displays almost complete penetrance ($\sim$98%, in accordance with [10]), following an age-dependent pattern. The probability of being a mutation carrier and consequently a posteriori risk diminishes with age of asymptomatic at risk individuals, reaching approximately zero in 70 years old subjects [11].

ATXN3, SCA3 causative gene, was described as containing a polymorphic expanded and unstable CAG tract at exon 10 [12], consensually ranging from 14 to 42 repeats in normal chromosomes and from 52 to 91 repeats in chromosomes harboring its mutated form [13, 14]. *ATXN3* encodes for the ubiquously expressed ataxin-3, a cysteine protease whose main native role is that of a deubiquitinating enzyme (DUB) in the ubiquitin-proteasome pathway. Expansion of the polyglutamine (polyQ) tract above the pathological threshold initiates a cascade of pathogenic events that have been extensively studied (revised in [15, 16]). Aggregation of mutant ataxin-3, proteolytic cleavage, transcription dysregulation, mitochondrial dysfunction, axonal transport impairment, dysregulation of intracellular Ca^{2+} homeostasis and impairment of protein degradation are considered the main mechanisms implicated in SCA3 pathogenesis [16].

Despite the progresses made in the understanding of the underlying molecular mechanisms, disease-modifying therapies for SCA3 are still lacking, and only symptomatic approaches are currently available. So far interventional studies undertaken have failed to demonstrate an impact of compounds tested on disease progression. Therefore, despite corresponding to the SCA for which the highest number of interventional studies has been conducted, the large majority has been clearly power-limited; the lack of randomization, the absence of a placebo group

and the short duration of the trials are some of the limitations pinpointed (revised in [17]). Based on their own previous experience with an interventional study, Saute and collaborators [18] have provided insights on potential pitfalls in SCA3 clinical trials, drawing attention to the importance of improving their efficacy [18].

Empowering clinical trials emerges as a major goal of current SCA3 research, given the previously described scenario, in which anticipated trials should be lengthy and require large numbers of patients. Part of such empowerment is related with the increase in sensitivity of the instruments used to measure disease progression and detect subtle therapeutic benefits; it is therefore expected that molecular biomarkers (MBs) will have the potential to provide a crucial contribution to the quality of SCA3 interventional studies.

16.2 Molecular Biomarkers of SCA3

A biomarker is understood as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [19]. Biomarkers are frequently subdivided into "trait biomarkers" if they provide indications as to the presence/absence of disease and "state biomarkers" if they can inform about the severity of a certain pathology [20]. Biomarkers are expected to be useful in diagnostic, staging and prognosis of disease, as well as to aid in the prediction and monitoring of intervention [19].

As it would be expectable, clinical biomarkers correspond to the most investigated markers so far in SCA3. Several standardized clinical tests, including rating scales, have been developed to measure different aspects of the SCA3 phenotype. Such measures have been validated in studies of the natural history of the disease, targeting different cohorts of patients. Advantages pinpointed for the widespread use of clinical markers are the relatively low requirement in time and their reduced cost, as well as the fact that they can be obtained without the need for any sophisticated equipment [20]. Regardless of the progresses made with the development of more objective clinical scales, it is assumed that clinical measures are to a certain extent subjective (the complexity of the SCA3 phenotype further aggravates this limitation), insensitive to subtle changes in small periods of time, as well as potentially subjected to observational bias [20]. Also, current clinical measures are limited as to their usefulness in the preataxic stage of the disease, a phase that should be extremely important on what concerns the development of therapeutics.

Neuroimaging information holds the promise of sensitivity and informativity; neuroimaging indicators, such as specific volumetric alterations, are already being used as primary endpoint in clinical trials of neurodegenerative diseases similar to SCA3 (www.clinicaltrials.gov—clinical trial NCT02336633). Notwithstanding, it has to be acknowledged that although neuroimaging alterations in SCA3 are well documented in cross-sectional studies, longitudinal data is still scarce, a limitation

that will be necessary to overcome, if such type of biomarker is to be considered as a primary endpoint in future interventional trials of SCA3.

Because in slow progressing disorders, such as SCA3, the capture of small effects remains a challenge, the development of MBs is urgently needed. MBs should be crucial to improve sensitivity, when used in complement to clinical endpoints; they are also crucial because they can be applied to the presymptomatic stage, thus resolving one of the limitations of clinical markers. Furthermore, when ameliorating drugs will be available MBs being able to precociously detect pathogenic alterations will be of use to optimize therapeutics efficiency, since such compounds are expected to be more efficient if administrated to mutation carriers before disease.

16.2.1 Molecular Trait Biomarkers of SCA3

The CAG repeat at the *ATXN3* locus constitutes the primary trait biomarker of SCA3. In fact, the identification of the *ATXN3* gene opened prospects for the direct detection of the mutation, providing the grounds for molecular diagnosis and allowing presymptomatic (predictive) testing. The identification of carriers of the mutation before onset offers the possibility of understanding the full process of progression of the disease (Fig. 16.1), which in turn will be of major importance at

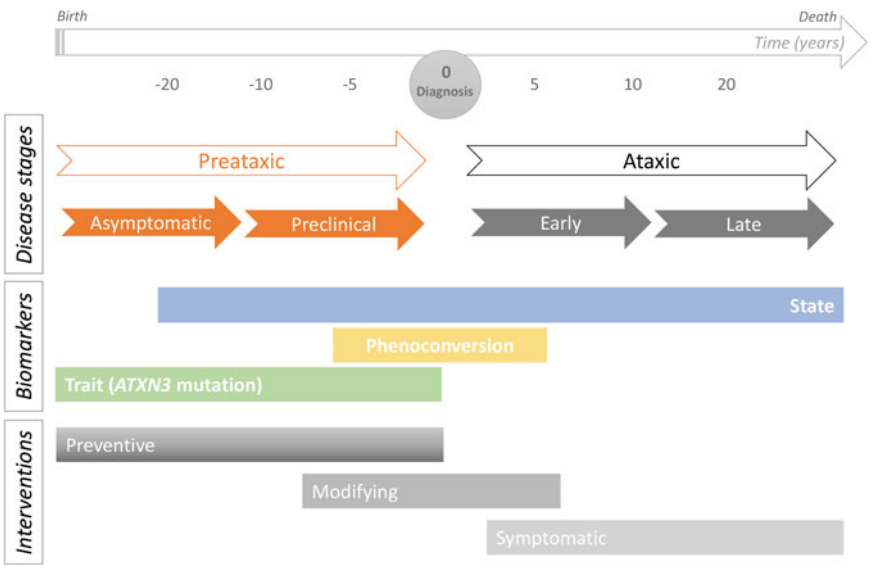

Fig. 16.1 Stages, type of biomarkers and interventional studies which can be performed during the natural course of SCA3

a stage where therapeutic offers will be available, as previously referred. In SCA3 patients, a negative correlation between the number of CAG repeats in the expanded allele and age at onset has been widely reported, the number of CAGs accounting for nearly 50–75% of the variation in the age of appearance of first symptoms (revised in [8]); in the Azorean SCA3 cohort, for example, the number of CAG repeats in the expanded allele explains 68% of the onset variance [21]. In addition to the size of the CAG tract, age at onset, specific symptoms, rate of progression as well as degree of severity are notably modifiable by other factors. Such factors could theoretically be environmental or genetic; probably both contribute to the specific final phenotype. Although research on genetic modifiers of SCA3 is still at an incipient stage, several genetic factors have already been investigated as modifiers of SCA3: the number of CAG repeats at several expansion loci [21–23]; allelic variants at the interleukine 6 (*IL6*; [24]), apolipoprotein E (*APOE*) [25, 26] and glucosylceramidase beta (*GBA*) [27] genes; variation in the 3'UTR at the *ATXN3* gene [28] and size of the normal SCA3 allele [29]. Moreover, for some of these genes/variants a possible cumulative effect has also been tested; in fact, Azorean SCA3 patients carrying the APOE*e2 and the IL6*C allele presented an onset which was anticipated by an average of 10 years [24]. More recently, exome sequencing has been proposed to study genetic modifiers of SCA3 (Manuela Lima, personal communication). The identification of genetic modifiers would allow: (1) targeting particular pathways/mechanisms for development of therapeutic interventions; (2) stratifying patients based on their genotype and incorporating this knowledge into the design of clinical trials. This type of stratification will empower clinical trials by controlling for the effect of the genetic background of participants [30]; and (3) improving the prediction of age at onset, aiming to provide better genetic counseling.

16.2.2 Molecular State Biomarkers of SCA3

16.2.2.1 Transcriptional biomarkers

As previously referred, abnormal conformation of mutated ataxin-3 promotes a gain of a toxic function compromising several cellular mechanisms, namely transcription. Transcriptional regulation seems to be affected by mutated ataxin-3 via two processes: (1) recruitment of transcription factors into polyQ-rich inclusions [31–33]; and (2) abnormal interactions with transcription factors and co-activators [34–38]. Transcriptional dysregulation was initially studied in cellular and animal models of SCA3 [35, 39, 40]. In such models, transcriptional alterations of genes involved in inflammatory processes, cell signaling and encoding cell-surface associated proteins has been described [35, 39, 40]. Based on this previous evidence Raposo and colleagues [6] hypothesized that in SCA3 patients the analysis of disease-specific

transcriptional changes in blood, a peripheral tissue, had the potential to allow the identification of novel biomarkers. A cross-sectional study with SCA3 patients and controls, using the Illumina Human V4-HT12 array, confirmed the presence of differences in expression between the two groups [6]. Twenty six genes, found to be up-regulated in patients, were selected for a first step of validation by quantitative real-time PCR (technical validation). From these 21 genes, fourteen were subsequently selected for validation by qPCR in a new set of SCA3 patients and controls. In this second validation step, the expression levels of *FCGR3B* (Fc fragment of IgG, low-affinity IIIb, receptor (CD16b)), *CSF2RA* (Colony-stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage), *CLC* (Charcot-Leyden crystal protein), *FPR2* (Formyl peptide receptor 2), *SLA* (Src-like-adaptor), *GPR97* (G protein-coupled receptor 97), *P2RY13* (Purinergic receptor P2Y, G-protein coupled, 13), *TNFSF14* (Tumor necrosis factor (ligand) superfamily, member 14), *SELPLG* (Selectin P ligand) and *YIPF6* (Yip1 domain family, member 6) were found to be 1.11–2.60-fold higher in patients when compared to controls. Noteworthy, *FCGR3B, P2RY13* and *SELPLG* genes were significantly up-regulated. Raposo and colleagues [6] further shown that, particularly for *FCGR3B* and *CLC*, patients with shorter disease duration tended to have higher expression levels when compared with patients with longer disease duration [6].

In a second approach Raposo and colleagues [41] listed molecules whose levels had been previously described in the literature as altered in SCA3 patients. The goal was to verify if expression levels of the selected genes *HSPB1* (heat shock 27 kDa protein 1), *DNAJB1* (DnaJ (Hsp40) homolog, subfamily B, member 1), *DNAJB12* (DnaJ (Hsp40) homolog, subfamily B, member 12), *DNAJB14* (DnaJ (Hsp40) homolog, subfamily B, member 14), *BAX* (BCL2-associated X protein), *BCL2* (B-cell CLL/lymphoma 2), *SOD2* (superoxide dismutase 2, mitochondrial), *IL1B* (interleukin 1, beta) and *IL6* (interleukin 6) correlated with disease. The authors concluded that *HSPB1* and *BCL2* were significantly down-regulated in patients compared to controls. Given the previously highlighted importance of the preclinical stage of SCA3 Raposo and colleagues [41] expanded this analysis to include samples from preataxic SCA3 subjects. mRNA levels adjusted for age at blood collection were obtained for a set of premanifest SCA3 subjects, patients and controls. *BCL2* levels were distinct in SCA3 subjects as compared to controls, although not being able to differentiate between premanifest carriers and patients. Moreover, lower levels of *IL6* mRNA were also found in preataxic carriers.

In the quest for SCA3 biomarkers, longitudinal studies should provide the best quality data; such studies, however, represent an important effort for researchers and patients and therefore are lacking for SCA3. Due to the availability of a homogeneous subset of SCA3 patients with several blood collection

points, Raposo and colleagues [41] investigated the behavior of *HSPB1* and *BCL2* mRNA levels in a longitudinal setup. Blood samples from SCA3 patients were collected at the baseline of the study and at a second moment. *BCL2* and *HSPB1* mRNA adjusted levels were found to be significantly different between the baseline and the second observational moment; during disease progression, the mRNA levels of *BCL2* and *HSPB1* increased.

16.2.2.2 Biomarkers of mitochondrial (mtDNA) depletion and damage

Amongst the several cellular pathways shown to be altered in the presence of mutated ataxin-3 are those associated with mitochondrial integrity and function. Because different compounds which improve energy metabolism defects or reduce oxidative stress (such as creatine and coenzyme Q10, amongst others) have been proposed as having the potential to ameliorate polyQ diseases, the investigation of the potential of mitochondrial alterations as biomarkers is pertinent. Metabolic alterations linked with mitochondrial function in SCA3 have been widely reported [42–44]. Furthermore, data on mtDNA depletion and increased damage (higher frequency of mutations, namely deletions) has also been produced [44–48]. Some of these previous reports indicated that alterations in the leukocytes mtDNA content had the potential to be used as biomarkers of SCA3; results from the several studies, however, were not consensual. Aiming to confirm previous findings of increased mtDNA depletion and damage, analysing a larger and independent set of patients, Raposo and colleagues [49] measured the levels of mitochondrial encoded NADH dehydrogenase 1 *(MT-ND1)*, NADH dehydrogenase 4 *(MT-ND4)* and ribonuclease P RNA component H1 *(RPPH1)* genes, which enabled the determination of the mtDNA content and the evaluation of the common deletion (m.8470_13446del4977 deletion, which is considered a marker of age). In this study, along with blood samples collected from SCA3 patients and community controls, samples from preataxic carriers were also analysed. Although differences in mtDNA content were not evidenced in carriers of the mutation, as compared to controls, the common deletion was significantly more frequent in patients and preataxic subjects than in controls, after adjusting for age at collection. In preataxic subjects, moreover, a significant correlation between the number of CAG repeats in expanded allele and the frequency of common deletion was obtained; the frequency of common deletion was lower in preataxic subjects carrying a higher number of CAGs [49].

Alterations in several molecules related with other SCA3-associated mechanisms, quantified either at the mRNA or protein level have been reported and are synthetized in Table 16.1.

Table 16.1 Candidate biomarkers linked to several SCA3-associated mechanisms, either identified using cross-sectional and longitudinal analysis

Mechanism	Type of study	Gene/ protein	Subjects	Main results	Reference
Autophagy	Cross-sectional	*BECN1*	Controls Patients	Levels of *BECN1* mRNA were 1.4 times higher in SCA3 patients compared to controls	[50]
	Cross-sectional	*SIRT1*/ SIRT1	Controls Patients	mRNA levels of *SIRT1* as well as protein levels were severely decreased in human fibroblasts of SCA3 patients in comparison with controls	[51]
Inflammation	Cross-sectional	Eotaxin	Controls Preataxic subjects patients	Eotaxin levels was found to be higher in SCA3 asymptomatic carriers and in patients	[52]
	Longitudinal		Patients	Eotaxin levels decreased after 360 days	
Insulin system	Cross-sectional	IGFBP3 IGF1 IGFBP1	Controls Patients	Low levels of insulin and IGFBP3 and high levels of insulin sensitivity (HOMA2), free IGFI, and IGFBP1 where obtained in SCA3 patients	[53]

16.3 Conclusions

The quest for MBs of SCA3 has started; although studies are presently in an exploratory phase, longitudinal studies have begun to aid in the validation of results from cross-sectional analysis. Candidate transcriptional biomarkers evaluated so far will need to be further tested to warrant their reliability; this means that their behavior should be independent from inter-individual variation and comorbidities, for example. Similar to what is expected in similar neurodegenerative diseases, it is likely that for SCA3 not only a single biomarker, but a set of different molecules, will have to be identified. This set of MBs will be use, in complementarity with clinical and imaging markers, to fully track SCA3, from its presymptomatic phase to the disease stage.

References

1. Ruano L, Melo C, Silva MC, Coutinho P (2014) The global epidemiology of hereditary ataxia and spastic paraplegia: a systematic review of prevalence studies. Neuroepidemiology 42 (3):174–183
2. Lima M, Bruges-Armas J, Bettencourt C (2012) Non-mendelian genetic aspects in spinocerebellar ataxias (scas): the case of Machado-Joseph disease (MJD). Spinocerebellar Ataxia, InTech
3. Coutinho P (1992) Doença de Machado-Joseph: Tentativa de definição. PhD dissertation, Universidade do Porto
4. Lima M, Mayer F, Coutinho P, Abade A (1997) Prevalence, geographic distribution, and genealogical investigation of Machado-Joseph disease in the Azores (Portugal). Hum Biol 69 (3):383–391
5. Lima M, Mayer FM, Coutinho P, Abade A (1998) Origins of a mutation: population genetics of Machado-Joseph disease in the Azores (Portugal). Hum Biol Int Rec Res 70(6):1011–1023
6. Raposo M, Bettencourt C, Maciel P, Gao F, Ramos A et al (2015) Novel candidate blood-based transcriptional biomarkers of Machado-Joseph disease. Mov Disord 30(7):968–975
7. de Araújo M, Raposo M, Kazachkova N, Vasconcelos J, Kay T, Lima M (2016) Trends in the epidemiology of spinocerebellar ataxia type 3/Machado-Joseph disease in the Azores Islands, Portugal. JSM Brain Sci 1(1):1001
8. Bettencourt C, Lima M (2011) Machado-Joseph disease: from first descriptions to new perspectives. Orphanet J Rare Dis 6:35
9. Bettencourt C, Raposo M, Kazachkova N, Cymbron T, Santos C et al (2011) The APOE ε2 allele increases the risk of earlier age at onset in Machado-Joseph disease. Arch Neurol 68 (12):1580–1583
10. Sequeiros J, Coutinho P (1993) Epidemiology and clinical aspects of Machado-Joseph disease. Adv Neurol 61:139–153
11. Bettencourt C, Santos C, Kay T, Vasconcelos J, Lima M (2008) Analysis of segregation patterns in Machado-Joseph disease pedigrees. J Hum Genet 53(10):920–923
12. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M et al (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8(3):221–228
13. Maciel P, Costa MC, Ferro A, Rousseau M, Santos CS et al (2001) Improvement in the molecular diagnosis of Machado-Joseph disease. Arch Neurol 58(11):1821–1827
14. Souza GN, Kersting N, Krum-Santos AC, Santos ASP, Furtado GV et al (2016) Spinocerebellar ataxia type 3/Machado-Joseph disease: segregation patterns and factors influencing instability of expanded CAG transmissions. Clin Genet 90(2):134–140
15. Evers MM, Toonen LJA, van Roon-Mom WMC (2014) Ataxin-3 protein and RNA toxicity in spinocerebellar ataxia type 3: current insights and emerging therapeutic strategies. Mol Neurobiol 49(3):1513–1531
16. do Costa MC, Paulson HL (2012) Toward understanding Machado-Joseph disease. Prog Neurobiol 97(2):239–257
17. Kazachkova N, Lima M (2015) Towards a therapeutic intervention in polyglutamine ataxias: from models to clinical trials. In: Atta-ur-Rahman (ed) Frontiers in clinical drug research - CNS and neurological disorders, pp. 77–130
18. Saute JAM, Rieder CRM, Castilhos RM, Monte TL, Schumacher-Schuh AF et al (2015) Planning future clinical trials in Machado Joseph disease: lessons from a phase 2 trial. J Neurol Sci 358(1–2):72–76
19. Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95
20. Weir DW, Sturrock A, Leavitt BR (2011) Development of biomarkers for Huntington's disease. Lancet Neurol 10(6):573–590

21. Raposo M, Ramos A, Bettencourt C, Lima M (2015) Replicating studies of genetic modifiers in spinocerebellar ataxia type 3: can homogeneous cohorts aid? Brain 138(Pt 12):e398
22. Jardim L, Silveira I, do Pereira ML, Céu Moreira M, Mendonça P et al (2003) Searching for modulating effects of SCA2, SCA6 and DRPLA CAG tracts on the Machado-Joseph disease (SCA3) phenotype. Acta Neurol Scand 107(3):211–214
23. Tezenas du Montcel S, Durr A, Bauer P, Figueroa K, Ichikawa Y et al (2014) Modulation of the age at onset in spinocerebellar ataxia by CAG tracts in various genes. Brain 137(Pt 9):2444–2455
24. Raposo M, Bettencourt C, Ramos A, Kazachkova N, Vasconcelos J et al (2016) Promoter variation and expression levels of inflammatory genes IL1A, IL1B, IL6 and TNF in blood of spinocerebellar ataxia type 3 (SCA3) patients. Neuromolecular Med 19(1):41–45
25. Bettencourt C, Santos C, Coutinho P, Rizzu P, Vasconcelos J et al (2011) Parkinsonian phenotype in Machado-Joseph disease (MJD/SCA3): a two-case report. BMC Neurol 11:131
26. Peng H, Wang C, Chen Z, Sun Z, Jiao B et al (2014) APOE ε2 allele may decrease the age at onset in patients with spinocerebellar ataxia type 3 or Machado-Joseph disease from the Chinese Han population. Neurobiol Aging 35(9):2179.e15–2179.e18
27. Siebert M, Donis KC, Socal M, Rieder C, Emmel VE et al (2012) Glucocerebrosidase gene variants in parkinsonian patients with Machado Joseph/spinocerebellar ataxia 3. Park Relat Disord 18(2):185–190
28. Long Z, Chen Z, Wang C, Huang F, Peng H et al (2015) Two novel SNPs in ATXN3 3' UTR may decrease age at onset of SCA3/MJD in Chinese patients. PLoS One 10(2):e0117488
29. França MC, Emmel VE, D'Abreu A, Maurer-Morelli CV, Secolin R et al (2012) Normal ATXN3 allele but not CHIP polymorphisms modulates age at onset in Machado-Joseph disease. Front Neurol 3:164
30. Gusella JF, MacDonald ME (2009) Huntington's disease: the case for genetic modifiers. Genome Med 1(8):80
31. Chai Y, Wu L, Griffin JD, Paulson HL (2001) The role of protein composition in specifying nuclear inclusion formation in polyglutamine disease. J Biol Chem 276(48):44889–44897
32. Perez MK, Paulson HL, Pendse SJ, Saionz SJ, Bonini NM, Pittman RN (1998) Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. J Cell Biol 143 (6):1457–1470
33. Takahashi J, Tanaka J, Arai K, Funata N, Hattori T et al (2001) Recruitment of nonexpanded polyglutamine proteins to intranuclear aggregates in neuronal intranuclear hyaline inclusion disease. J Neuropathol Exp Neurol 60(4):369–376
34. Evert BO, Araujo J, Vieira-Saecker AM, de Vos RAI, Harendza S et al (2006) Ataxin-3 represses transcription via chromatin binding, interaction with histone deacetylase 3, and histone deacetylation. J Neurosci 26(44):11474–11486
35. Evert BO, Vogt IR, Vieira-Saecker AM, Ozimek L, de Vos RAI et al (2003) Gene expression profiling in ataxin-3 expressing cell lines reveals distinct effects of normal and mutant ataxin-3. J Neuropathol Exp Neurol 62(10):1006–1018
36. Li F, Macfarlan T, Pittman RN, Chakravarti D (2002) Ataxin-3 is a histone-binding protein with two independent transcriptional corepressor activities. J Biol Chem 277(47):45004–45012
37. Nicastro G, Menon RP, Masino L, Knowles PP, McDonald NQ, Pastore A (2005) The solution structure of the Josephin domain of ataxin-3: structural determinants for molecular recognition. Proc Natl Acad Sci USA 102(30):10493–10498
38. Rodrigues A-J, Coppola G, Santos C, do Costa MC, Ailion M et al (2007) Functional genomics and biochemical characterization of the *C. elegans* orthologue of the Machado-Joseph disease protein ataxin-3. FASEB J. 21(4):1126–1136
39. Chou A-H, Yeh T-H, Ouyang P, Chen Y-L, Chen S-Y, Wang H-L (2008) Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. Neurobiol Dis 31(1):89–101

40. Evert BO, Vogt IR, Kindermann C, Ozimek L, de Vos RA et al (2001) Inflammatory genes are upregulated in expanded ataxin-3-expressing cell lines and spinocerebellar ataxia type 3 brains. J Neurosci 21(15):5389–5396
41. Raposo M (2017) Predicting and tracking Machado-Joseph disease: biomarkers of diagnosis and prognosis. PhD dissertation, Universidade dos Açores
42. Matsuishi T, Sakai T, Nagamitsu S, Shoji H, Ueda N et al (1996) Decreased cerebrospinal fluid levels of substance P in Machado-Joseph disease. J Neurol Sci 142(1–2):107–110
43. Pacheco LS, da Silveira AF, Trott A, Houenou LJ, Algarve TD et al (2013) Association between Machado-Joseph disease and oxidative stress biomarkers. Mutat Res 757(2):99–103
44. Yu Y-C, Kuo C-L, Cheng W-L, Liu C-S, Hsieh M (2009) Decreased antioxidant enzyme activity and increased mitochondrial DNA damage in cellular models of Machado-Joseph disease. J Neurosci Res 87(8):1884–1891
45. Kazachkova N, Raposo M, Montiel R, Cymbron T, Bettencourt C et al (2013) Patterns of mitochondrial DNA damage in blood and brain tissues of a transgenic mouse model of Machado-Joseph disease. Neurodegener Dis 11(4):206–214
46. Liu C-S, Cheng W-L, Kuo S-J, Li J-Y, Soong B-W, Wei Y-H (2008) Depletion of mitochondrial DNA in leukocytes of patients with poly-Q diseases. J Neurol Sci 264 (1–2):18–21
47. Ramos A, Kazachkova N, Silva F, Maciel P, Silva-Fernandes A et al (2015) Differential mtDNA damage patterns in a transgenic mouse model of Machado-Joseph disease (MJD/SCA3). J Mol Neurosci 55(2):449–453
48. Zeng A, Liu X, Shen L, Li W, Ding Z et al (2012) Analysis of mitochondrial DNA variations in a Chinese family with spinocerebellar ataxia. J Clin Neurosci 19(1):60–64
49. Raposo M, Ramos A, Kazachkova N, Teixeira B, Bettencourt C, Lima M (submitted) Accumulation of mitochondrial DNA common deletion since the preataxic stage of Machado-Joseph disease
50. Kazachkova N, Raposo M, Ramos A, Montiel R, Lima M (2017) Promoter variant alters expression of the Autophagic BECN1 gene: implications for clinical manifestations of Machado-Joseph disease. The Cerebellum
51. Cunha-Santos J, Duarte-Neves J, Carmona V, Guarente L, Pereira de Almeida L, Cavadas C (2016) Caloric restriction blocks neuropathology and motor deficits in Machado-Joseph disease mouse models through SIRT1 pathway. Nat Commun 11(7):11445
52. da Silva Carvalho G, Saute JAM, Haas CB, Torrez VR, Brochier AW, Souza GN, Furtado GV, Gheno T, Russo A, Monte TL, Schumacher-Schuh A, D'Avila R, Donis KC, Castilhos RM, Souza DO, Saraiva-Pereira ML, Torman VL, Camey S, Portela LV, Jardim LB (2016) Cytokines in Machado Joseph disease/Spinocerebellar Ataxia 3. The Cerebellum 15 (4):518–525
53. Saute JAM, da Silva ACF, Muller AP, Hansel G, de Mello AS, Maeda F, Vedolin L, Saraiva-Pereira ML, Souza DO, Arpa J, Torres-Aleman I, Portela LVC, Jardim LB (2011) Serum insulin-like system alterations in patients with Spinocerebellar Ataxia type 3. Mov Disord 26(4):731–735

Chapter 17
Planning Future Clinical Trials for Machado-Joseph Disease

Jonas Alex Morales Saute and Laura Bannach Jardim

Abstract Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD) is an autosomal dominant multiple neurological systems degenerative disorder caused by a CAG repeat expansion at *ATXN3* gene. Only a few treatments were evaluated in randomized clinical trials (RCT) in SCA3/MJD patients, with a lack of evidence for both disease-modifying and symptomatic therapies. The present chapter discuss in detail major methodological issues for planning future RCT for SCA3/MJD. There are several potential therapies for SCA3/MJD with encouraging preclinical results. Route of treatment, dosage titration and potential therapy biomarkers might differ among candidate drugs; however, the core study design and protocol will be mostly the same. RCT against placebo group is the best study design to test a disease-modifying therapy; the same cannot be stated for some symptomatic treatments. Main outcomes for future RCT are clinical scales: the Scale for the Assessment and Rating of ataxia (SARA) is currently the instrument of choice to prove efficacy of disease-modifying or symptomatic treatments against ataxia, the most important disease feature. Ataxia quantitative scales or its composite scores can be used as primary outcomes to provide preliminary evidence of efficacy in phase 2 RCT, due to a greater sensitivity to change. Details regarding eligibility

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criteria, randomization, sample size estimation, duration and type of analysis for both disease modifying and symptomatic treatment trials, were also discussed. Finally, a section anticipates the methodological issues for testing novel drugs when an effective treatment is already available. We conclude emphasizing four points, the first being the need of RCT for a number of different aims in the care of SCA3/MJD. Due to large sample sizes needed to warrant power, RCT for disease-modifying therapies should be multicenter enterprises. There is an urge need for surrogate markers validated for several drug classes. Finally, engagement of at risk or presymptomatic individuals in future trials will enable major advances on treatment research for SCA3/MJD.

Keywords SCA3 · Machado-Joseph disease · Clinical trials · Treatment
Study design

17.1 Context

Most clinical trials for neurodegenerative disorders have been performed for common conditions like Alzheimer's (AD) and Parkinson's disease (PD). In such complex disorders, uniformity or homogeneity of causes are not guaranteed among recruited participants, representing a major issue for both experimental and observational studies. In contrast, polyglutamine diseases (polyQ diseases), monogenic conditions caused by CAG repeat expansions (CAGexp) in coding regions of specific genes, would theoretically present a better scenario for clinical trials relying on more homogeneous samples. In spite of that, quite a few clinical trials have been done in polyQ diseases and the most important reason for that is their rarity, when adequate sample size and statistical power of a given trial are at stake. Rarity of polyQ disorders explains other major reasons for the few number of trials performed so far: lack of knowledge of its natural history for many years, and absence of good clinical biomarkers. This chapter will present suggestions on how to face these challenges when planning future trials in polyQ diseases in general, and in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD), in particular.

As previously reviewed in this book, SCA3/MJD is an autosomal dominant, multiple neurological systems degenerative disorder in which cerebellar ataxia is the core feature [1–3]. SCA3/MJD is caused by a CAGexp at *ATXN3* that codes for ataxin-3 [1, 4, 5]. Age at onset (AO), clinical manifestations, natural history, and survival are very important factors when it comes to build up a clinical trial: they have been described in details in Chap. 10. Currently, there is no proven disease-modifying therapy for this condition and only a few treatments were tested in controlled clinical trials.

Cellular mechanisms have been reviewed in Chap. 10. Complex, multicellular pathways might be targeted by different therapeutic approaches. Interventions acting on distal mechanisms might be safer, but will probably be limited in time and

to symptomatic effects—such as those related to neurotransmission, neuronal firing-rate and general neuroprotection. Interventions acting on proximal disease mechanisms such as gene silencing may be potentially curative, but might be associated with increased risks for major adverse events such as those associated with tumor suppressor genes and invasive procedures to deliver treatment to target tissues.

A brief, historical review on first clinical trials in SCA3/MJD will be presented. After that, methodological issues for future studies on potential therapies, the aim of this chapter, will be discussed in detail.

17.2 First Clinical Trials in SCA3/MJD and the CONSORT Era

The first randomized clinical trials (RCT) in SCA3/MJD were published in 1995, one year after mutation was identified, and reported short-term benefit of sulfamethoxazole-trimethoprim (SMT + TMP) [6, 7]. Later, a trial with longer follow-up discarded SMT + TMP efficacy on ataxic manifestations in SCA3/MJD [8] and this therapy was abandoned. Subsequent trials with other interventions aimed to modify the disease progression or reduce disease symptoms; they resulted negative or not conclusive. These pioneer studies were hampered by methodological problems such as lack of validated clinical scales to assess ataxia and neurological severity, lack of knowledge about disease natural history, and absence of surrogate biomarkers. Many trials were limited by lack of randomization and of a placebo group, multiple primary endpoints, inclusion of different types of cerebellar ataxia, and short periods of follow-up. Trials with physostigmine, fluoxetine, tandospirone, lamotrigine, buspirone and IGF-1, are examples that illustrate the problems researchers face when trying to study novel treatments for rare diseases [3].

Consolidated Standards of Reporting Trials (CONSORT) first appeared in 1996, and intended to recommend a minimum set of evidence-based characteristics for performing and reporting RCT. Being consensual among methodologists, translational specialists and editors, CONSORT 2010 guidelines give us a tool to critically understand if a trial for a given disease was well designed, analyzed, and interpreted [9].

Mostly due to historical reasons, only a few clinical trials performed in SCA were reported according to CONSORT 2010 guidelines [10–14]; even so, methodological limitations were not prevented in some cases. For those interested, we suggest a recent review on the theme [3].

17.3 Designing New Clinical Trials for SCA3/MJD

Evidence on efficacy is lacking for disease-modifying and symptomatic treatments for SCA3/MJD. However, a number of symptomatic therapies already exist for non-ataxia neurological signs of SCA3/MJD, such as spasticity and dystonia. Some of these interventions are already established treatments for neurological signs irrespective of the etiology (Eg: botulinum toxin for spasticity or focal dystonia) and evaluation of their specific efficacy in SCA3/MJD might preclude placebo groups. Therefore, while RCT against placebo group are the best design for a disease modifying therapy, this might not be the best approach to test some symptomatic treatments. Finally, if a given therapy is proved to be effective, in the future, new interventions with the same aim should be tested by superiority or non-inferiority analysis against established standard treatment and not against placebo.

17.3.1 Designing a Clinical Trial for SCA3/MJD When There Is no Intervention Already Established

There are several potential therapies for SCA3/MJD, with encouraging preclinical results. Route of treatment, dosage titration and potential therapy biomarkers might differ among candidate drugs RCT; however, the core study design and protocol will be mostly the same.

17.3.1.1 Disease Modifying Versus Symptomatic Treatment

One of the first questions to be answered before ongoing on a RCT of a candidate drug, is whether researchers expect a symptomatic or a disease modifying effect. Disease-modifying therapies exert their effects on the underlying pathophysiology of the disease rather than simply acting on symptoms. When started after the disease process is established and the clinical picture is already evident, disease-modifying therapies are likely just to halt or slow disease progression. Improvements are not expected, in general. On the other hand, symptomatic therapies aim to improve the target (a neurological function) irrespective of the disease stage—although improvements might not last.

Even when researchers have reasons to believe that an intervention will modify the disease process, there is a great chance that the observed effect is only symptomatic. Misclassification here can have clinical consequences. A disease modifying therapy should be offered to all affected individuals and it should be discontinued only in case of significant adverse event that preclude its use. Even worsening of the clinical picture is not sufficient evidence of failure for a disease-modifying drug. In contrast, loss of effect after a given time is just the usual

prognosis for symptomatic drugs. Moreover, symptomatic treatments are usually tried for a short period in real life; if no benefit is evident or no harm is seen after its discontinuation, the drug can be withdrawn.

Clinical trials with a single period study can hardly determine whether a treatment effect is symptomatic, disease-modifying, or both. The inclusion of a second period, in a withdrawal or a delayed-start design [15], might help to uncover which kind of effect the intervention presents.

Subjects randomized to active treatment followed by placebo (A/P) are compared to subjects randomized to placebo followed by placebo/nothing (P/P) in the withdrawal design (Fig. 17.1a, b). If the active treatment has a disease modifying effect, final average scores from A/P and P/P groups will maintain their differences in time; if it has a symptomatic effect, then final average scores will gradually approach one to each other. Although the alternatives in the progression rate are very clear-cut, this design is prone to non-adherence and losses. In the delayed-start design, subjects randomized to active treatment followed by active treatment (A/A) are compared to those randomized to placebo followed by active treatment (P/A) (Fig. 17.1c, d). Again, if the active treatment has a disease-modifying effect, final scores (or lines) from both groups will not approach one to each other. Considering that a difference between groups favoring active treatment (if effective) is a *sine qua non* condition for applying such designs, the delayed start design is also ethically preferred due to less time exposure to placebo.

Although important, building a two-period trial adequately is a difficult task. Deciding for how long the second period should last is crucial and it is frequently

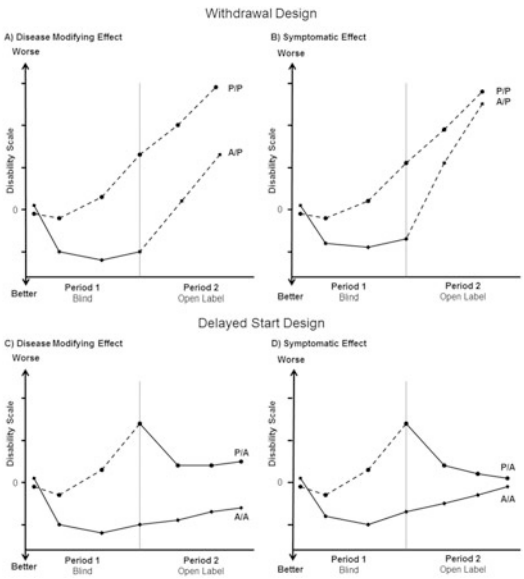

Fig. 17.1 Symptomatic versus disease-modifying effects. In withdrawal paradigm, **a** represents a given treatment with both symptomatic and disease-modifying effects while **b** has only symptomatic effect. In delayed-start paradigm, **c** represents a given treatment with both symptomatic and disease-modifying treatment effect and **d** has only symptomatic effect

unknown. For instance, a too short follow-up could inadequately lead to a parallel slope appearance, indicative of a disease-modifying effect. In addition, demonstration of a disease-modifying effect depends on equivalence of slopes [16]. Studies looking for equivalence between groups usually require a large number of patients, which is a major drawback in the context of rare diseases.

17.3.1.2 Outcomes for Clinical Trials

Knowledge of disease natural history, of sensitivity to change of a given outcome measurement, and of the estimated sample size according to the expected effect are crucial to adequately design a RCT. The most widely used instruments to measure disease progression in SCA3/MJD are clinical scales. Different clinical scales have been widely explored in SCA3/MJD and we have considerable information about its natural history, sensitivity to change and sample size estimations, which are presented below.

As clinical scales were not proven to be sensitive outcomes for disease-modifying RCT of short duration, much is expected from surrogate markers, at least for phase II clinical trials. Biochemical, neurophysiological and neuroimaging studies are arising as candidate biomarkers for SCA3/MJD. Knowledge about them is limited: characteristics such as natural history and sensitivity to change must be determined before their use as main outcomes.

Neurological Scales

Four main type of scales were developed to evaluate SCAs: (1) semi-quantitative ataxia scales as the International Cooperative Ataxia Rating Scale (ICARS) [17], and the Scale for the Assessment and Rating of ataxia (SARA) [18]; (2) semi-quantitative ataxia and non-ataxia scales as the Neurological Examination Score for SCA (NESSCA) [19]; (3) semi-quantitative non-ataxia scales, like the Inventory of Non-ataxia Symptoms (INAS) [20]; and (4) quantitative scales, like Composite Cerebellar Functional Score (CCFS) [21] and SCA Functional Index (SCAFI) [22]. All were validated for SCA3/MJD, and each of them present advantages and disadvantages according to the planned outcome for a given trial [23].

Progression Rate of Clinical Scales: Natural History and Placebo Group Studies

Semi-quantitative Ataxia Scales

Worsening of clinical scales is supposed to mirror disease progression, and have been used to describe the natural history (NH) of SCA. Progression rates have been frequently measured between a baseline and further observations of a given clinical

scale. In few other studies, statistical models considered the relationship between the slope of progression and disease duration (DD) for each patient, generally resulting in slower progression rates than the former modeling. For instance, ICARS (range: 0–100 points) worsened 5.1 points per year in a Brazilian SCA3/MJD cohort, when baseline was compared to follow-ups [24], and 2.25 points/year in a Dutch cohort where DD was adjusted to the model [25]. SARA (range: 0–40 points) progression between observations was more frequently studied and worsened 0.65, 1.56–1.70 and 3.0 points per year among Americans [26], Europeans [27–29] and Taiwanese SCA3/MJD patients [30].

Progression rate of placebo groups would be theoretically more helpful for planning clinical trials than those obtained from NH studies. In two recent RCTs, SARA progression was 1.34 in 31 SCA3/MJD patients [12, 31, 32] and 1.67 points/year in 27 patients with other SCAs and Friedreich ataxia [11]—results very similar to those obtained in NH studies using intervals between observations.

ICARS has interdependency and redundancy of some items, which may under or overestimate sensitivity to change, raising doubts about effect size measures of the scale. SARA, on the other hand, is very reliable, and less time-consuming than ICARS [23]. SARA is currently the most widely used semi-quantitative ataxia scale in SCA3/MJD.

Semi-quantitative Ataxia and Non-ataxia Scales

NESSCA (range: 0–40 points) progression was 1.26 points/year in a NH study of 156 SCA3/MJD patients that considered DD for the model. Markovian chains showed that extra-pyramidal findings presented a slower progression in the first stages of disease [33]. Mean placebo group progression of NESSCA was similar: 1.45 points/year [12]. In the same period and placebo group, cerebellar NESSCA items (range: 0–7) progressed 0.74 points, while the sum of pyramidal, extrapyramidal and peripheral NESSCA items (range: 0–19) progressed 0.23 points/year, only [32], confirming that non-ataxic findings presented slower progression than ataxia in SCA3/MJD.

INAS count (range: 0–16) is the sum of categorical observations among several neurological findings. Probably due to its dichotomized variables (strong, moderate, or mildly positive responses being reduced to a single value), INAS count showed an unsatisfactory responsiveness when compared to other kind of scales [27, 34]. Annual INAS count progression was 0.37 (SE 0.08) points in SCA3/MJD. Furthermore, INAS did not follow a linear progression in a longer follow-up of the same cohort [29], a phenomenon similar to some NESSCA non-ataxia items [33].

Since ataxia is the core disease sign and since non-ataxia signs progress quite slowly, NESSCA and INAS count are not recommended as primary outcomes for future RCT designed to evaluate disease-modifying treatments. However, their use as secondary outcomes in future RCT is warranted. Extra-cerebellar manifestations are an important burden in SCA3/MJD, and the effect of future trials on them should be followed, either for efficacy or for safety reasons.

Quantitative Scales

Quantitative composite scores CCFS and SCAFI were developed to reliably detect small clinical changes over short periods. However, face and content validity of quantitative tasks of composite scores are hard to define since they are timed tasks. For instance, a delay in the 9-hole pegboard test or 8-m walking time (8 MW) performances might be due to upper limb or gait ataxia, but also to bradykinesia, dystonic movements, and other extracerebellar phenomena [23].

NH studies showed that CCFS worsened 0.025 [SE: 0.004] per year in SCA3/MJD [28] and that SCAFI progressed −0.159 [SD: 0.33] per year among patients with different SCAs [34]. In placebo group, progression of CCFS and SCAFI were near 0.015 (almost undetectable) and −0.30, respectively [12].

Sensitivity to change measured with standardized response mean (SRM) of CCFS in two cohorts of polyQ SCAs [34, 35] were similar or even lower than SARA sensitivity to change. However, theoretical greater sensitivity to change of quantitative composite scores were confirmed by recent trials. Significant improvements in CCFS and SCAFI were seen with smaller sample sizes and shorter periods than expected, whereas no effect was detected on SARA or NESSCA [12, 36]. Therefore, relative changes in composite scores to an intervention might be larger and easier to detect than changes in semi-quantitative scales [32].

Quantitative scores have the downside of not being feasible in more severely affected patients: those who are not be able to walk, use the pegboard, perform the click test, or even talk. Ceiling effects are probably unavoidable and should preclude the recruitment of more disabled patients. However, quantitative scores used as primary outcomes might hasten and provide preliminary evidence of efficacy in phase 2 RCT. Data simultaneously obtained with an ataxia scale (SARA, ICARS or other), used as a secondary end-point, from phase 2 studies with positive results on composite scores (as primary outcomes), could then provide sample size estimations for phase 3 trials with the same intervention, when the semi-quantitative ataxia scale should be the primary outcome.

Minimally Important Differences (MID) of Clinical Scales

MID is not the same as sensitivity to change or as true responsiveness (clinically important changes) and has seldom been demonstrated [37], particularly for neurodegenerative disorders [38]. Some authors consider any small significant difference as clinically relevant for ataxias [39]. Ideally, MID should include subjective changes as external criterion, such as psychological well-being and quality of life, whose validation properties may differ among specific disorders.

Schmitz-Hubsch and cols tried to define MID for SCA scales by using Patients' Clinical Global Impression of Change (PGI) questionnaire [40] as an external criterion [34]. The upper limit of the 95% confidence interval (CI) of change in the stable subjects was used as a conservative estimate of MID. The SARA MID obtained for different SCAs was 1.1/40 points [34].

Who Should Be Recruited? Which Factors Should Be Controlled, According to Results Obtained from Clinical Scales?

The question deserves a great attention from researchers. Some authors consider that any effective drug will have a better pharmacodynamics scenario in early disease stages [41]. Such strategy was successful in trials for other neurodegenerative genetic disease, like transthyretin familial amyloid polyneuropathy [42].

On lithium trial subgroup analysis, when analyzing only patients able to perform 8 MW, mean NESSCA progression was significantly slower in lithium group [32]. Therefore, independent walking might be an inclusion criterium in a future trial, at expenses of reducing its external validity. More or less stringent inclusion criteria will have pros and cons that researchers need to take into account.

Recruiting presymptomatic carriers for RCT will probably guarantee treatment start at a better time window. The example of PRECREST study should inspire SCA researchers [43]. Prospective observational studies of clinical scales and candidate biomarkers in preclinical phases must be pursued in SCAs [44]. NH results must be preferentially replicated in other cohorts, before recommending any RCT on pre-clinical phases.

Controlling for modifying factors of disease progression is another interesting issue in a RCT protocol. For instance, CAGexp modified the NH of NESSCA [33] and might impact on treatment responses. Interaction of CAG_{exp} randomization strata with treatment response on NESSCA was found in lithium trial [32], with a significant effect of active treatment only for patients with shorter CAG_{exp}. However, a confounding bias was detected: cerebellar items had a higher impact on total NESSCA in this subgroup than in individuals with larger CAG_{exp}. Due to that, authors concluded that the main factor associated with drug efficacy, or higher sensitivity to change, was the greater proportion of ataxic features, and not the CAG_{exp}.

In summary, evidence obtained so far sustains that simple or block randomization may be enough to control for known and unknown factors such as CAGexp. Stratification or minimization randomization might be used only in too small sample size studies [45].

Sample Size Estimations

Disease Modifying Treatments

These strategies are expected to slow down the rate of disease progression, and not to improve or revert it. Natural history studies of SCA3/MJD depicted that the number of patients needed per study arm to detect a 50% reduction of disease progression in a 2-arm trial for any drug, with the respective outcome measure within 1 year, should be 130 for NESSCA [33], 175–202 for SARA [35, 29]), 275 for SCAFI [34]; SCA1, SCA2, SCA3/MJD and SCA6 analyzed together) and 260 for CCFS [35]. A longer follow-up study calculated that these numbers would be 102 within 2 years and 70 within 3 years of study using SARA as the outcome [29].

Sample size estimations from placebo groups provide more accurate information for designing future clinical trials than natural history studies. Table 17.1 presents sample size estimations obtained from an intention-to-treat placebo group [32] and according to the desired relative change on annual progression with intervention. For instance, sample size needed to show a 50% reduction of disease progression with SARA after 12 months will be between 175 (according to a NH study) and 328 (according to placebo study).

Table 17.1 Sample size estimations

Relative effect	N patients per study arm			
	NESSCA	SARA	SCAFI	CCFS
0.1	3937	8180	4681	31,554
0.2	985	2046	1171	7889
0.3	438	910	521	3507
0.4	247	512	293	1973
0.5	158	328	188	1263
0.6	110	228	131	877
0.7	81	168	96	645
0.8	62	129	74	494
0.9	50	102	59	391
1	40	83	48	316
1.1	34	69	40	262
1.2	28	58	33	220
1.3	24	49	29	188
1.4	21	43	25	162
1.5	19	37	22	141
1.6	16	33	19	124
1.7	15	29	17	110
1.8	13	26	15	98
1.9	12	24	14	88
2	11	21	13	80

Estimated sample size (n per trial arm) for a 2 arm interventional trial that aims to reduce progression rates by 1–100% (100% meaning that progression was interrupted) or ameliorate the baseline scores by 101–200% (200% meaning an improvement in the same magnitude of the expected progression in the placebo group for the period), according to the observed variability of progression rates in the placebo group (power 80%, $\alpha = 0.05$) in the intention to treat population (N = 31 patients) of lithium trials [32] with the formula: [(mean placebo progression—mean intervention progression/mean placebo progression)]. *NESSCA* Neurological Examination Score for the Assessment of Spinocerebellar Ataxia. *SARA* Scale for the Assessment and Rating of Ataxia. *SCAFI* SCA Functional Index. *CCFS* Composite Cerebellar Functional Score

These are huge numbers for a rare disease. Even in geographical areas where SCA3/MJD presents a high relative frequency, these sample sizes will be hardly achievable. For instance, the 42.7:100,000 prevalence of symptomatic individuals in Azorean islands actually represents 105 individual for the overall population of 246,102 [46]. Prevalence in Rio Grande do Sul state, Brazil, is 5.6:100,000—or 625 for the 11.2 million inhabitants [47]. Sample sizes for RCT in SCA3/MJD imply that these studies must be multicenter.

It is worth to remember that sample size estimated from NH or placebo groups give us only clues on how to plan a clinical trial. They can be quite different from the numbers needed to show differences obtained from a specific management. This can be exemplified by the experience obtained from the lithium trial. The scale with the least sample size required in a future trial according to SRM of the placebo group, NESSCA (158 patients), would need the largest number of patients per arm in a future trial with lithium, 684. In contrast, both composite quantitative scores SCAFI and CCFS, that would require larger sample sizes according to SRM, presented statistically significant effects with lithium, with 31 patients per study arm [32]. These numbers show us that the responsiveness of scales to treatments cannot be completely foreseen and question the mathematical property of the effect size as measured by SRM to compare different scales over time [34].

Symptomatic Therapy

Sample sizes required to evaluate symptomatic treatments might be smaller than those for disease-modifying therapies. Improvements are expected in the active drug arm and therefore a more clear-cut detachment from the placebo progression over time. However, this might be not so simple, as data so far obtained might teach us.

Few studies evaluated ataxia symptomatic treatments for SCA3/MJD [13, 36, 48]. Two RCT with methodological issues and inconclusive results, showed large effects on placebo groups on short term: mean improvements on SARA score of 0.86 and 1.37 points after 8 and 12 weeks [13, 48]. That is, testing of a symptomatic candidate drug by a short-term trial will be a hard task.

Although not including a single SCA3/MJD patient, studies that evaluated riluzole efficacy for cerebellar ataxias [10, 11] can be used as a model for planning future symptomatic treatment for SCA3/MJD or other SCA. The numbers of recruited patients were 20 [10] and 30 per study arm [11]. The former study elected 5-points improvement in ICARS as the primary outcome after two months, and the latter 1-point improvement in SARA after 12 months. In both studies, a statistically significant effect was detected favoring active treatment with the reported sample sizes.

Primary outcome (1-point improvement in SARA score) was achieved by 26 and 11% patients on the placebo group after 3 and 12 months [11]. Since the proportion of patients with improvements with riluzole treatment was 50% after both periods, the absolute difference between treatment groups was of 24 and 39% after 3 and 12 months of treatment, probably due to larger placebo effect at the beginning of the study [11]—a phenomenon seen in other RCTs [13, 48]. The sample size

required to detect a 24% difference in the proportion of responders between treatments is 63 patients per study arm; to detect a 39% difference (by the 12 month), 21 patients per study arm would fit (power 80%, $\alpha = 0.05$). Estimations obtained from the placebo group of lithium trial indicated similar sample sizes to confirm a symptomatic effect. Minimally important improvement (MID) for SARA is 1.1: since worsening in placebo group is 1.34 points per year, the relative effect would be 1.82 and the number of patients per study arm that should be recruited would be 26 (power 80%, $\alpha = 0.05$, Table 17.1) [12].

Therefore, it seems that 21–26 patients per study-arm should be an adequate sample size to test symptomatic treatments for SCA3/MJD in a 1-year study and that 63–65 patients per study arm would be necessary to perform short-term symptomatic treatment trials. SARA should be the main outcome, and either a dichotomous (rate of responders with 1-point SARA improvement) or a continuous outcome (difference in mean improvements of SARA) can be chosen.

Study Duration

Disease Modifying

Since SARA mean progression was of 1.34 points per year in a placebo group [12], the achievement of SARA MID—1.1 points—in a 1-year study would require the very large relative reduction of 82% in progression rate. Disease-modifying interventions can hardly achieve this milestone. Longer duration studies would be able to detect smaller effects sizes with similar or even smaller sample sizes [29]. Considering the rarity of SCA3/MJD (and remembering that the same patient should not participate in multiple clinical trials), future studies using SARA as their outcome (such as phase 3 trials) might be planned to last at least 24 months. In contrast, trials with higher responsivity outcomes such as composite scores, that can be chosen for phase 2 trials, can have adequate power to detect differences after 12 months.

Symptomatic Therapy

Short-term (2–3 months, 65 patients per arm) and long-term (1-year, 25 patients per arm) studies can test ataxia symptomatic treatments in SCA3/MJD. A reasonable study could last one year with an interim analysis on 3rd and 6th months of treatment with pre-defined interim results. This plan would allow an early study interruption either for efficacy or futility [31].

Surrogate Markers

Treatments have been approved on the basis of their manifest effects on the clinical signs and/or symptoms of the disease for which they were intended. Clinical trials based on clinical outcomes are a highly empirical approach that does not need a complete explanation of the underlying mechanisms to be accepted [16].

Sometimes the proposed clinical benefit might not be detectable in trials of reasonable duration or size. SCA3/MJD might be one of these cases: its slow progression requires RCT with large sample of patients and/or long duration. In these circumstances, use of surrogate markers as the primary outcome measures is warranted.

A biomarker is a measurement that reflects the activity of a disease process. A surrogate marker, in contrast, is a biomarker proven to be a reliable substitute of a clinically meaningful, generally a hard endpoint, to be used in therapeutic trials [49, 16].

If a surrogate marker with better sensitivity to change than those from clinical scales is identified for SCA3/MJD, then its use as primary outcome could hasten phase 2 RCTs and drug discovery for the disease.

Validating a surrogate marker is a complex process that has many interpretative problems and depends on substantial research. Correlation of a measure with clinical progression is not a sufficient condition for establishing a surrogate marker. For instance, the correlation between biomarker and disease might disappear during treatment. Alternatively, the drug may have the effect desired on the surrogate marker, but no effect on the clinical outcome. Clinical outcomes have indubitable face validity; in contrast, the use of surrogate markers requires deeper knowledge of disease process. There may be many pathophysiologic pathways contributing to disease manifestations, and the surrogate marker may be "in" some, but not in other pathways. Drug mechanisms should also be known enough to decide if the surrogate marker will pick them up and translate into the beneficial clinical outcome [50, 51].

Surrogate markers must be validated during RCT using clinical outcomes with established MID: this research process will take some time. Given that there is a number of candidate therapies for SCA3/MJD, it would be wise to validate surrogate markers across many drugs and strategies, so that therapies can be more easily compared (in non-inferiority or superiority approaches).

The number of candidate biomarkers evaluated so far for SCA3/MJD is not very large, but can be divided into candidate markers of disease progression and candidate modifiers of phenotype. Table 17.2 summarizes the evidence obtained so far. Some of the potential biomarkers were tested prospectively in the context of the lithium trial; results are expected soon (Jardim, personal communication).

17.3.2 Designing a Clinical Trial for SCA3/MJD When Comparison Against Placebo Is not Acceptable or Feasible

The use of a comparator placebo group is sometimes difficult or unfeasible to test efficacy of a given management. This is the case of physical therapy, for instance, when patient and physician cannot be blinded; or of consecrated therapies, when

Table 17.2 Data obtained from studies on candidate biomarkers and surrogate markers for SCA3/MJD

Candidate	Associations tested as a biomarker of SCA3/MJD				
	Marker of disease progression ($p < 0.05$)			Modifier of phenotype ($p < 0.05$)	
	Disease duration	Clinical Scales	Other	Age at onset	CAGexp
Neuroimaging					
Caudate nucleus volume loss	0.49*/year, C	–	–	–	None, C
Putamen volume loss	0.48*/year, C	–	–	–	None, C
Thalamus volume loss	–	SARA R = 0.624, D	–	None, D	None, D
Midbrain volume loss	None, A, B 0.41*/year, C	SARA R = −0.467, A	–	–	None, A, C
Pons volume loss	None A, B, 0.20*/year, C	SARA R = −0.560, A	–	–	None, A, C
Total brainstem volume loss	None, A 0.20*/year, C	SARA R = −0.677, A R = 0.581, D	–	None, D	None, A, C, D
Medulla volume loss	None, A	SARA R = −0.479, A	–	–	None, A
Total cerebellum volume loss	None, A, B	SARA R = −0.451, A	–	–	None, A
Peripheral biomarkers (total blood, plasma, serum, blood cells)					
Serum insulin sensitivity	None, E	None, E	–	R = −0.444, E R = 0.585, F	None, E
Serum IGF-1	None, E	None, E	Medulla oblongata and pons volume R = −0.439, E	None, E	None, E

(continued)

Table 17.2 (continued)

Candidate	Associations tested as a biomarker of SCA3/MJD				
	Marker of disease progression ($p < 0.05$)			Modifier of phenotype ($p < 0.05$)	
	Disease duration	Clinical Scales	Other	Age at onset	CAGexp
Serum IGF binding protein 1	None, E	None, E	–	None, E	R = 0.451, E
Serum Neuron-specific enolase [52, 53]	None, H R = 0.259, I	EDSS R = −0.729,H ICARS R = 0.242, I SARA: R = 0.248, I	–	None, H, I	None, H, I
Serum S100B	R = 0.452, H None, I	Montgomery-Asberg depression rating scale R = 0.461, H None, I	–	None, H; I	None, H; I
Serum glutathione peroxidase	None, G	NESSCA R = −0.309, G	–	None, G	None, G
Serum eotaxin (da Silva Carvalho et al. 2015)	Log 0.3/1.3 pg/ml (or 23%) per year, J	None, J	–	None, J	None, J
Serum miRNA-25 and miRNA-125b	+, qualitative data, K	–	–	–	–
mRNA of *TNFSF14*, *FCGR3B*, *CLC*, and *SLA*	+, qualitative data, L	–	–	None, L	None, L

(continued)

Table 17.2 (continued)

Candidate	Associations tested as a biomarker of SCA3/MJD				
	Marker of disease progression ($p < 0.05$)			Modifier of phenotype ($p < 0.05$)	
	Disease duration	Clinical Scales	Other	Age at onset	CAGexp
Biomarkers on cell lines (fibroblast, iPS)					
DNAJB1 expression in fibroblasts	–	–	–	+, qualitative data, M	–

* % of Total Intracranial Volume, TICV

A Schulz et al. [54]
B Guimarães et al. [55]
C Reetz et al. [56]
D De Rezende et al. [57]
E Saute et al. [58]
F Saute et al. [59]
G de Assis et al. [60]
H Tort et al. [52]
I Zhou et al. [53]
J Da Silva Carvalho et al. [61]
K Shi et al. [62]
L Raposo et al. [63]
M Zijlstra et al. [64]

physicians might declare ethical reasons or conflicts of conscience that prevent prescription of placebo; or of invasive interventions where the risk per se does not allow to not offer the active therapy.

There are a number of alternatives to circumvent the absence of a placebo group, all of them inferior to RCT. It is worth to remember that open label trials can hardly test the study hypothesis correctly due to Hawthorne and placebo effects, while at the same time patients are being exposed to risks.

17.3.2.1 When Patient and Physician Cannot Be Blinded

Surgical and other similar interventions cannot be hided to patients and physicians. The alternative proposed is sham, fake or dummy procedures. They aim to act in a similar way to placebo, by masking the technique used in the active group. Some authors raise ethical doubts about these designs, but they can be justified if there is a relevant clinical question to be answered. Although it was never done in SCA3/MJD, use of a sham group might be methodologically necessary for invasive procedures required to deliver gene silencing therapies, for instance.

Another strategy is to apply a delayed onset group combined with a blind evaluation of efficacy. Physical therapy is a good example for this approach. Considered as the standard treatment for cerebellar ataxia, efficacy studies on physical therapy are rare and best protocol of exercises are not clearly established [3]. An RCT on intensive rehabilitation in ataxias (no SCA3/MJD cases) assigned patients to the immediate or delayed entry control group to rehabilitation [65]. The immediate group showed significantly improvement of 3 points on SARA after 4 weeks compared with the delayed group.

17.3.2.2 Consecrated or Symptomatic Therapies Already in Use

This class encompasses several symptomatic treatments for motor and nonmotor manifestations involved in SCA3/MJD. This is an important field of research. While disease-modifying therapies for neurodegenerative diseases were not identified yet, effective symptomatic treatments such as those for parkinsonian symptoms brought significant improvement of patient's quality of life. Short-term RCTs for most of symptomatic treatments will be warranted for SCA3/MJD. Table 17.3 clearly shows the need for class I, II and III studies to provide better recommendation levels on this ground. Recent reviews on symptomatic therapies of SCA3/MJD are also available [3, 66]

Some physicians might not feel comfortable in not offering the considered consecrated study drug to all study individuals. In this case, single-blind longitudinal observations might be planned. Studies with this design used a clinical scale videotaped and blindly scored, as their outcome [73]. Such design is better than an open-label study, because it overcomes assessment bias of unblinded raters. However, positive results due to placebo and Hawthorne effects are not prevented.

Table 17.3 Therapeutic approaches for SCA3/MJD: conceptual frame, praxis and recommendations

Approach and targets	Intervention studied	Recommendation
Disease modifying; long term follow-up ($\geq$ 1 year)	RCT on lithium carbonate [12] Efficacy not proved	Not recommended for reducing disease progression **(Level C)**. Larger phase 3 RCTs are warranted
	Open trial on stem cell therapy [67] Efficacy not proved	Not recommended for reducing disease progression **(Level U)**
Symptomatic	Intervention studied	Recommendation
	RCT on valproic acid [48] Efficacy not proved	Not recommended **(Level U)**
Ataxia	RCT on varenicline [13] Efficacy not proved	Not recommended **(Level U)**
	2 RCT on Riluzole [10, 11] Effective in ataxias of different etiology and hereditary ataxias; not tested for SCA3/MJD	Probably recommended **(Level A)** RCT in SCA3/MJD are needed
	Open trial on 4-Aminopyridine [36] Efficacy not proved.	Not recommended **(Level U)**
	Open and RCT trials on coordinative training [65, 68, 69] Efficacy proved	Recommended **(Level C)** In-patient intensive rehabilitation programs and $\geq$3 times/week outpatient session or daily homemade exercises are probably more effective than less intensive programs **(Level C)**
Spasticity	Case report on botulinum toxin [70]. Expert opinion on others	Physiotherapy, baclofen, tizanidine and botulinum toxin injections are recommended for lower limb spasticity **(Level U)**
Dystonia	Case reports on botulinum toxin [71, 72]	Botulinum toxin may be utilized for blepharospasm and focal dystonia. Additional caution is advisable for cervical dystonia **(Level U)**
	Case reports and a blinded trial on levodopa [73, 74, 75]	Levodopa/carbidopa or benserazide should be tried dystonia; if no response is seen the treatment should be withdrawn **(Level C)**
Parkinsonism	Case reports on dopaminergic drugs response [76–78]	Levodopa, pramipexole, or ropinirole are recommended **(Level U)**

(continued)

Table 17.3 (continued)

Symptomatic	Intervention studied	Recommendation
Dysarthria and dysphagia	Expert opinion	No drug treatment is recommended Speech and swallowing therapy are recommended **(Level U)** SARA scale should be routinely assessed in SCA3/MJD patients and, when scores are >15, videofluoroscopy of swallowing should be ordered to orientate therapy **(Level U)**
Oculomotor features	Expert opinion	Prismatic lenses and/or orthoptic exercises are recommended for diplopia treatment **(Level U)** Botulinum toxin injections or strabismus surgery should only be indicated by neuro-ophtalmologists in reference centers, with experience on the treatment of ataxic patients **(Level U)** [79]
Depressive symptoms	Improvements in depressive symptoms were obtained in open-labeled trials with Fluoxetine [80] and with occupational therapy [81] Efficacy proved Expert Opinion	Depressive manifestations should be actively searched during clinical assessments, preferentially using validated instruments for its diagnosis and screening when performed by non-specialists **(Level U)** Treatment should follow guidelines for depressive disorders in the general population **(Level U)**
Cramps	Series reports on carbamazepine [82] and mexiletine hydrochloride [83] Suggestion of efficacy	Carbamazepine 200 mg b.i.d or mexiletine 150–300 mg q.i.d are recommended for the treatments of cramps that did not improve with physical therapy management alone **(Level C)**
Pain	Case series on nonsteroidal anti-inflammatory drugs and opiates. Case reports on botulinum toxin injections [84]	Chronic pain should be treated according to its characteristics, similar to the general population **(Level U)**
Fatigue	Observational studies of predisposing factors [85] Expert Opinion	Predisposing factors should be addressed first, especially depression, visual symptoms and sleeping disorders **(Level U)**. After that, amantadine and modafinil can be considered for use **(Level U)**

(continued)

Table 17.3 (continued)

Symptomatic	Intervention studied	Recommendation
Sleep disorders	Expert Opinion	Sleep disorders should be treated as in the general population, following specific guidelines **(Level U)**

Adapted from Saute and Jardim [3]
RCT randomized clinical trial. Treatment recommendation according to the American Academy of Neurology classification of evidence

In this case, an objective surrogate marker evaluated by blinded evaluators (like MRI volumetric analysis), could ensure greater reliability of results.

17.3.2.3 High Risk Procedure

Treatments such as intrathecal infusions or hematopoietic stem cell transplantations are related to high risk of adverse events related to the procedure per se. Morbidity and mortality rates might turn a sham group unacceptable. In this scenario, open label trials have been done for neurodegenerative disorders other than SCA3/MJD. If disease progression is not slowed or interrupted, the approach can be abandoned without considering a RCT. If some positive result was obtained, then comparisons with historical cohorts might be useful. Of course, placebo effects are not avoided with this design.

17.3.3 Designing a Clinical Trial for SCA3/MJD When an Effective Intervention Already Exists

17.3.3.1 When the Effective Intervention Was not Clearly Established for SCA3/MJD

As said before, riluzole showed efficacy against ataxia in two independent RCT for cerebellar ataxias. Although its recommendation is debatable, riluzole's case can be the prototype to exemplify an effective intervention against a symptom in general but still not tested for SCA3/MJD. Short (4–12 weeks) and long-term efficacy (12 months) of riluzole were shown [10, 11]. A wise strategy to get evidence for SCA3/MJD would be to design a short-term RCT, where riluzole effect would be tested after 8–12 weeks. If a positive effect occurs, an open label phase would start, assuming long-term efficacy by analogy with results obtained after 12 months in the double-blind study for other ataxic disorders. The required sample size per study arm in this case would be larger than a longer study (see Sample Size Estimation

section); however it would turn possible to raise convincing evidence of efficacy in a very short period. In addition, an open label phase would be of great utility, considering the potential clarification about symptomatic versus disease-modifying effect of this drug (Fig. 17.1) [86].

17.3.3.2 Once an Effective Intervention Is Established, How New Candidates Will Be Tested for SCA3/MJD

This is a utopic but not so far away possibility for SCA3/MJD. There is considerable hope that in a medium run, evidence on efficacy will be obtained for some/any of the candidate managements proposed from bench studies. With this hope in mind, we will briefly summarize the recommendations once this state of art is achieved for SCA3/MJD.

Because SCA3/MJD is an orphan disease, a therapy must be considered as effective after a single Class I, phase 3 trial (Fig. 17.2), or even Class II trial accompanied by registry data in order to get conditional or definite recommendations by regulatory agencies (Level A recommendation) [87]. After this point

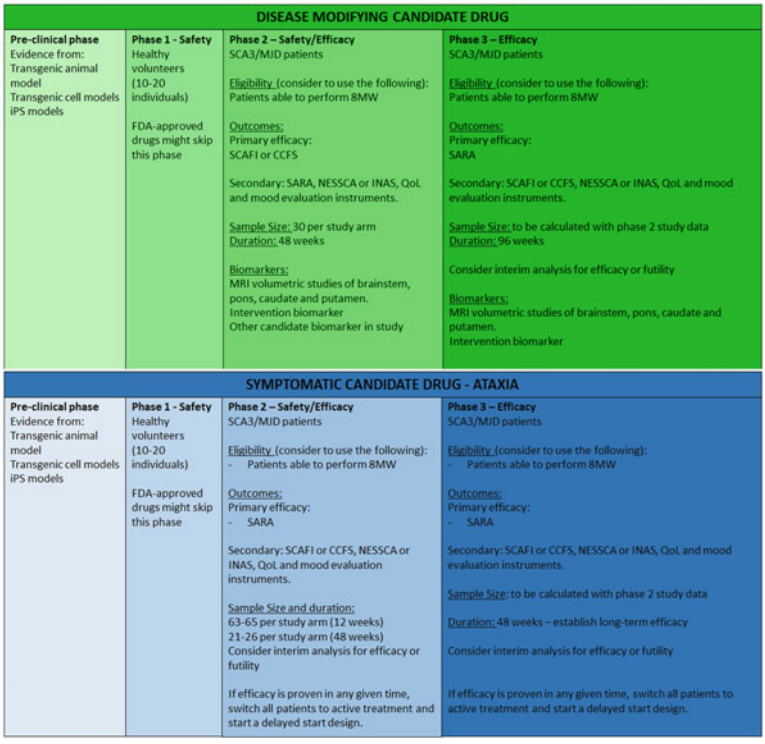

Fig. 17.2 Basic trial designs models

achievement, further RCTs might aim to determine if a new therapy is superior, equivalent or non-inferior to this standard treatment. Comparisons against placebo will not be accepted anymore.

Researchers might propose that the new treatment is similar or even superior to the established therapy in terms of safety and efficacy. If a superiority RCT fails to show a difference between a new treatment and the older one, this result does not mean they are equivalent. A new trial will be needed, with additional costs, and such a failure might improperly shut down a drug pipeline. In order to avoid it, equivalence trials have been proposed: they aim to determine if one intervention is therapeutically similar to another. Investigators then define equivalent results as being those obtained into a pre-select Δ. At the end of the trial, a CI is computed around the difference between two test statistics. If the CI lies strictly within $[-\Delta, +\Delta]$ the two treatments are considered equivalent [88, 89].

Noninferiority trials comprise a third category of RCT against active controls. They seek to determine if a new treatment is not worse than a reference treatment [90]. Although the CI around the difference in the effect size between the new and existing treatments is calculated as the former one, the question of interest is now asymmetric. The new treatment will be non-inferior to existing treatment if the lower bound of CI does not extend beyond the predefined window. In other words, only lower bound for this non-inferiority comparison is under study (one-tailed test). Although non-inferiority trials seem to have statistical advantages when compared to equivalence trials, they should not be used indiscriminately. New treatments tested by non-inferiority trials must have some other advantage, such as greater availability, reduced cost, less invasiveness, fewer adverse effects, or greater ease of administration [88, 89].

17.4 Final Remarks

In the last years, we witnessed a series of discoveries on bench research in SCA3/MJD. Evidence obtained from preclinical studies on transgenic animal and cell models, and on neurons derived from iPS cells will allow new phase 1 and 2 trials in a near future. Observational and RCT raised robust evidences about how to measure efficacy in future trials, and provided very useful clues to future designs. Our final remarks stress four lines of conclusion.

First, there is no therapy established as effective, in SCA3/MJD, and RCT are needed for a number of aims (Table 17.3). Lithium carbonate might be at best judged as possibly effective and the phase 2 study results must be confirmed or refuted in a future phase 3 trial prior to its use. Several other therapies proposed as disease-modifying or symptomatic might enter phase 2 trials for safety and efficacy. RCT designed for symptomatic candidate treatments will be adequately powered by observing around 65 or 25 patients per arm during 3 or 12 months, respectively, whereas trials designed for disease-modifying therapies will need substantially

larger sample sizes. For each specific scenario, sizes and study duration are estimated and proposed in Table 17.1 and Fig. 17.2.

Second, SCA3/MJD is a rare disease: RCT for disease-modifying therapies will only recruit the sample size needed in multicenter enterprises. Costs are very high, adding difficulty to academic initiatives. Since clinical trials sponsored by industry test new drugs and devices, academic researchers interested in repurposing already approved drugs for other conditions must find new forms of funding.

Third, RCT to come need to describe treatment effects on candidate biomarkers. There is an urge need for surrogate markers validated for several drug classes and also surrogate biomarkers that can be used as evidence of efficacy in presymptomatic individuals. They might help defining the best managements in a medium future. And, while there is no proven effective treatment for SCA3/MJD, an effort should be done to collect all placebo groups data in public or shared databases, trying to provide even more accurate and valid data for designing the trials to come.

Finally, some words must be addressed to at-risk individuals. Drug discovery for disease-modifying and potentially curative treatment is a very hard and long process that might take several years before the observation of any positive results. Therefore, genetic counseling that includes information and availability of the many different reproductive options, will remain the central core in SCA3/MJD care in the years to come. Besides that, at-risk or presymptomatic individuals will soon be the subjects of clinical studies, and their participation will be paramount for the future of research in SCA3/MJD.

References

1. Costa MC, Paulson HL (2012) Toward understanding Machado-Joseph disease. Prog Neurobiol 97:239–257
2. Jardim LB, Pereira ML, Silveira I et al (2001) Neurologic findings in Machado-Joseph disease: relation with disease duration, subtypes, and (CAG)n. Arch Neurol 58:899–904
3. Saute JA, Jardim LB (2015) Machado Joseph disease: clinical and genetic aspects, and current treatment. Expert Opin Orphan Drugs 3:517–535
4. Kawaguchi Y, Okamoto T, Taniwaki M et al (1994) CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. Nat Genet 8:221–228
5. Maciel P, Gaspar C, DeStefano AL et al (1995) Correlation between CAG repeat length and clinical features in Machado-Joseph disease. Am J Hum Genet 57:54–61
6. Correia M, Coutinho P, Silva MC et al (1995) Evaluation of the effect of sulphametoxazole and trimethoprim in patients with Machado-Joseph disease. Rev Neurol 23:632–634
7. Sakai T, Matsuishi T, Yamada S et al (1995) Sulfamethoxazole-trimethoprim doubleblind, placebo-controlled, crossover trial in Machado-Joseph disease: sulfamethoxazole-trimethoprim increases cerebrospinal fluid level of biopterin. J Neural Transm Gen Sect 102:159–172
8. Schulte T, Mattern R, Berger K et al (2001) Double-blind crossover trial of trimethoprim-sulfamethoxazole in spinocerebellar ataxia type 3/Machado-Joseph disease. Arch Neurol 58:1451–1457
9. Schulz KF, Altman DG, Moher D (2010) For the CONSORT group. Ann Intern Med 152

10. Ristori G, Romano S, Visconti A, Cannoni S, Spadaro M, Frontali M, Pontieri FE, Vanacore N, Salvetti M (2010) Riluzole in cerebellar ataxia: a randomized, double-blind, placebo-controlled pilot trial. Neurology 74:839–845
11. Romano S, Coarelli G, Marcotulli C, Leonardi L, Piccolo F, Spadaro M, Frontali M, Ferraldeschi M, Vulpiani MC, Ponzelli F, Salvetti M, Orzi F, Petrucci A, Vanacore N, Casali C, Ristori G (2015) Riluzole in patients with hereditary cerebellar ataxia: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 14:985–991
12. Saute JA, de Castilhos RM, Monte TL et al (2014) A randomized, phase 2 clinical trial of lithium carbonate in Machado-Joseph disease. Mov Disord 29:568–573
13. Zesiewicz TA, Greenstein PE, Sullivan KL, Wecker L, Miller A, Jahan I, Chen R, Perlman SL (2012) A randomized trial of varenicline (Chantix) for the treatment of spinocerebellar ataxia type 3. Neurology 78:545–550
14. Saccà F, Puorro G, Brunetti A, Capasso G, Cervo A, Cocozza S, de Leva M, Marsili A, Pane C, Quarantelli M, Russo CV, Trepiccione F, De Michele G, Filla A, Brescia Morra V (2015) A randomized controlled pilot trial of lithium in spinocerebellar ataxia type 2. J Neurol 262(1):149–153
15. Olanow CW, Rascol O, Hauser R, Feigin PD, Jankovic J, Lang A, Langston W, Melamed E, Poewe W, Stocchi F, Tolosa E (2009) ADAGIO study investigators. A double-blind, delayed-start trial of rasagiline in Parkinson's disease. N Engl J Med 361(13):1268–1278
16. Katz R (2004) Biomarkers and surrogate markers: an FDA perspective. NeuroRx 1(2): 189–195
17. Trouillas P, Takayanagi T, Hallett M et al (1997) International cooperative ataxia rating scale for pharmacological assessment of the cerebellar syndrome. The ataxia neuropharmacology committee of the World federation of neurology. J Neurol Sci 145:205–211
18. Schmitz-Hübsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, Giunti P, Globas C, Infante J, Kang JS, Kremer B, Mariotti C, Melegh B, Pandolfo M, Rakowicz M, Ribai P, Rola R, Schöls L, Szymanski S, van de Warrenburg BP, Dürr A, Klockgether T, Fancellu R (2006) Scale for the assessment and rating of ataxia: development of a new clinical scale. Neurology 66(11):1717–1720
19. Kieling C, Rieder CRM, Silva ACF et al (2008) A neurological examination score for the assessment of spinocerebellar ataxia 3 (SCA3). Eur J Neurol 15:371–376
20. Schmitz-Hübsch T, Coudert M, Bauer P, Giunti P, Globas C, Baliko L, Filla A, Mariotti C, Rakowicz M, Charles P, Ribai P, Szymanski S, Infante J, van de Warrenburg BP, Dürr A, Timmann D, Boesch S, Fancellu R, Rola R, Depondt C, Schöls L, Zdienicka E, Kang JS, Döhlinger S, Kremer B, Stephenson DA, Melegh B, Pandolfo M, di Donato S, du Montcel ST, Klockgether T (2008) Spinocerebellar ataxia types 1, 2, 3, and 6: disease severity and nonataxia symptoms. Neurology 71:982–989
21. du Montcel ST, Charles P, Ribai P et al (2008) Composite cerebellar functional severity score: validation of a quantitative score of cerebellar impairment. Brain 131:1352–1361
22. Schmitz-Hübsch T, Giunti P, Stephenson DA et al (2008) SCA Functional Index: a useful compound performance measure for spinocerebellar ataxia. Neurology 71:486–492
23. Saute JA, Donis KC, Serrano-Munuera C et al (2012) Ataxia rating scales–psychometric profiles, natural history and their application in clinical trials. Cerebellum 11:488–504
24. França MC, D'Abreu A, Nucci A et al (2009) Progression of ataxia in patients with Machado-Joseph disease. Mov Disord 24:1387–1390
25. Torman VL, de Vries J, Verbeek D, Brunt E, Kampinga H, Jardim LB (2016) Interrelation between size of CAG-expansion and progression of ICARS scores in a Dutch Cohort of SCA3/MJD patients. International Meeting on Spastic Paraparesis and Ataxias 2016, Abstract 55, page 55, booklet. https://spatax.files.wordpress.com/2016/06/booklet-20161.pdf
26. Tetsuo Ashizawa, Karla P Figueroa, Susan L Perlman, Christopher M Gomez, George R Wilmot, Jeremy D Schmahmann, Sarah H Ying, Theresa A Zesiewicz, Henry L Paulson, Vikram G Shakkottai, Khalaf O Bushara, Sheng-Han Kuo, Michael D Geschwind, Guangbin

Xia, Pietro Mazzoni, Jeffrey P Krischer, David Cuthbertson, Amy Holbert, John H Ferguson, Stefan M Pulst, SH Subramony (2013) Clinical characteristics of patients with spinocerebellar ataxias 1, 2, 3 and 6 in the US; a prospective observational study. Orphanet J Rare Dis 8(1):177
27. Jacobi H, Bauer P, Giunti P et al (2011) The natural history of spinocerebellar ataxia type 1, 2, 3, and 6: a 2-year follow-up study. Neurology 77:1035–1041
28. Tezenas du Montcel S, Charles P, Goizet C, Marelli C, Ribai P, Vincitorio C, Anheim M, Guyant-Maréchal L, Le Bayon A, Vandenberghe N, Tchikviladzé M, Devos D, Le Ber I, N'Guyen K, Cazeneuve C, Tallaksen C, Brice A, Durr A (2012) Factors influencing disease progression in autosomal dominant cerebellar ataxia and spastic paraplegia. Arch Neurol 69 (4):500–508
29. Jacobi H, du Montcel ST, Bauer P, Giunti P, Cook A, Labrum R, Parkinson MH, Durr A, Brice A, Charles P, Marelli C, Mariotti C, Nanetti L, Panzeri M, Rakowicz M, Sulek A, Sobanska A, Schmitz-Hübsch T, Schöls L, Hengel H, Baliko L, Melegh B, Filla A, Antenora A, Infante J, Berciano J, van de Warrenburg BP, Timmann D, Szymanski S, Boesch S, Kang JS, Pandolfo M, Schulz JB, Molho S, Diallo A, Klockgether T (2015) Long-term disease progression in spinocerebellar ataxia types 1, 2, 3, and 6: a longitudinal cohort study. Lancet Neurol 14(11):1101–1108
30. Yi-chung Lee, Yi-chu Liao, Po-shan Wang, I-Hui Lee, Kon-ping Lin, Bing-wen Soong (2011) Comparison of cerebellar ataxias: A three-year prospective longitudinal assessment. Mov Disord 26(11):2081–2087
31. Aggarwal SP, Zinman L, Simpson E, McKinley J, Jackson KE, Pinto H, Kaufman P, Conwit RA, Schoenfeld D, Shefner J, Cudkowicz M (2010) Northeast and Canadian amyotrophic lateral sclerosis consortia. Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 9:481–488
32. Saute JA, Rieder CR, Castilhos RM, Monte TL, Schumacher-Schuh AF, Donis KC, D'Ávila R, Souza GN, Russo AD, Furtado GV, Gheno TC, Souza DO, Saraiva-Pereira ML, Portela LV, Camey S, Torman VB, Jardim LB (2015) Planning future clinical trials in Machado Joseph disease: lessons from a phase 2 trial. J Neurol Sci 358(1–2):72–76
33. Jardim LB, Hauser L, Kieling C et al (2010) Progression rate of neurological deficits in a 10-year cohort of SCA3 patients. Cerebellum 9:419–428
34. Schmitz- Hübsch T, Fimmers R, Rakowicz M et al (2010) Responsiveness of different rating instruments in spinocerebellar ataxia patients. Neurology 74:678–684
35. Chan E, Charles P, Ribai P et al (2011) Quantitative assessment of the evolution of cerebellar signs in spinocerebellar ataxias. Mov Disord 26:534–538
36. Giordano I, Bogdanow M, Jacobi H et al (2013) Experience in a short-term trial with 4-aminopyridine in cerebellar ataxia. J Neurol 260:2175–2176
37. Streiner DL, Norman GR (2008) Health measurement scales: a practical guide to their development and use. Oxford University Press, Oxford
38. Molnar FJ, Man-Son-Hing M, Fergusson D (2009) Systematic review of measures of clinical significance employed in randomized controlled trials of drugs for dementia. J Am Geriatr Soc 57(3):536–546
39. Fahey MC, Corben L, Collins V, Churchyard AJ, Delatycki MB (2007) How is disease progress in Friedreich's ataxia best measured? A study of four rating scales. J Neurol Neurosurg Psychiatry 78:411
40. Guy W (1976) National Institute of Mental Health. ECDEU Assessment manual for psychopharmacology, revised edition. National Institute of Mental Health, Rockville, MD
41. Matilla-Dueñas A, Ashizawa T, Brice A et al (2014) Consensus paper: pathological mechanisms underlying neurodegeneration in spinocerebellar ataxias. Cerebellum 13:269–302
42. Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Planté-Bordeneuve V, Lozeron P, Suhr OB, Campistol JM, Conceição IM, Schmidt HH, Trigo P, Kelly JW,

Labaudinière R, Chan J, Packman J, Wilson A, Grogan DR (2012) Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology 79(8):785–792
43. Rosas HD, Doros G, Gevorkian S, Malarick K, Reuter M, Coutu JP, Triggs TD, Wilkens PJ, Matson W, Salat DH, Hersch SM (2014) PRECREST: a phase II prevention and biomarker trial of creatine in at-risk Huntington disease. Neurology 82:850–857
44. Jacobi H, Reetz K, du Montcel ST, Bauer P, Mariotti C, Nanetti L, Rakowicz M, Sulek A, Durr A, Charles P, Filla A, Antenora A, Schöls L, Schicks J, Infante J, Kang JS, Timmann D, Di Fabio R, Masciullo M, Baliko L, Melegh B, Boesch S, Bürk K, Peltz A, Schulz JB, Dufaure-Garé I, Klockgether T (2013) Biological and clinical characteristics of individuals at risk for spinocerebellar ataxia types 1, 2, 3, and 6 in the longitudinal RISCA study: analysis of baseline data. Lancet Neurol 12:650–658
45. Suresh K (2011) An overview of randomization techniques: an unbiased assessment of outcome in clinical research. J Hum Reprod Sci 4(1):8–11
46. Coutinho P, Silva MC, Gonçalves AF et al (1994) Epidemiologia da doença de Machado-Joseph em Portugal. Rev Port Neurol 3:69–76
47. G.N. Souza, N. Kersting, A.C. Krum-Santos, A.S.P. Santos, G.V. Furtado, D. Pacheco, T.A. Gonçalves, J.A. Saute, L. Schuler-Faccini, E.P. Mattos, M.L. Saraiva-Pereira, L.B. Jardim (2016) Spinocerebellar ataxia type 3/Machado-Joseph disease: segregation patterns and factors influencing instability of expanded CAG transmissions. Clin Genet 90(2):134–140
48. Lei LF, Yang GP, Wang JL, Chuang DM, Song WH, Tang BS, Jiang H (2016) Safety and efficacy of valproic acid treatment in SCA3/MJD patients. Parkinsonism Relat Disord 26: 55–61
49. Temple R (1999) Are surrogate markers adequate to assess cardiovascular disease drugs? JAMA 282:790–795
50. Fleming TR, DeMets DL (1996) Surrogate endpoints in clinical trials: are we being misled? Ann Intern Med 125:605–613
51. Fleming TR, Powers JH (2012) Biomarkers and surrogate endpoints in clinical trials. Stat Med 31(25):2973–2984
52. Tort ABL, Portela LV, Rockenbach IC et al (2005) S100B and NSE serum concentrations in Machado Joseph disease. Clin Chim Acta 351:143–148
53. Zhou J, Lei L, Shi Y et al (2011) Serum concentrations of NSE and S100B in spinocerebellar ataxia type 3/Machado-Joseph disease. Zhong Nan Da Xue Xue Bao Yi Xue Ban 36:504–510
54. Schulz JB, Borkert J, Wolf S et al (2010) Visualization, quantification and correlation of brain atrophy with clinical symptoms in spinocerebellar ataxia types 1, 3 and 6. Neuroimage 49:158–168
55. Guimarães RP, D'Abreu A, Yasuda CL et al (2013) A multimodal evaluation of microstructural white matter damage in spinocerebellar ataxia type 3. Mov Disord 28:1125–1132
56. Reetz K, Costa AS, Mirzazade S et al (2013) Genotype-specific patterns of atrophy progression are more sensitive than clinical decline in SCA1, SCA3 and SCA6. Brain 136:905–917
57. de Rezende TJ, D'Abreu A, Guimarães RP, Lopes TM, Lopes-Cendes I, Cendes F, Castellano G, França MC Jr (2015) Cerebral cortex involvement in Machado-Joseph disease. Eur J Neurol 22(2):277–283, e23–e24
58. Saute JA, da Silva ACF, Muller AP et al (2011) Serum insulin-like system alterations in patients with spinocerebellar ataxia type 3. Mov Disord 26:731–735
59. Saute JA, Souza GN, Haas CB et al (2016) Peripheral insulin sensitivity and body composition alterations in early stage Machado Joseph disease [abstract]. Mov Disord 31 (suppl 2)
60. de Assis AM, Saute JAM, Longoni A, Haas CB, Torrez VR, Brochier AW, Souza GN, Furtado GV, Gheno TC, Russo A, Monte TL, Castilhos RM, Schumacher-Schuh A, D'Avila R, Donis KC, de Mello Rieder CR, Souza DO, Camey S, Leotti VB, Jardim LB, Portela LV (2017) Peripheral Oxidative Stress Biomarkers in Spinocerebellar Ataxia Type 3/Machado-Joseph Disease. Front Neurol 8:485

61. da Silva Carvalho G, Saute JA, Haas CB, Torrez VR, Brochier AW, Souza GN, Furtado GV, Gheno T, Russo A, Monte TL, Schumacher-Schuh A, D'Avila R, Donis KC, Castilhos RM, Souza DO, Saraiva-Pereira ML, Torman VL, Camey S, Portela LV, Jardim LB (2016) Cytokines in Machado Joseph disease/spinocerebellar ataxia 3. Cerebellum 15:518–525
62. Shi Y, Huang F, Tang B et al (2014) MicroRNA profiling in the serums of SCA3/MJD patients. Int J Neurosci 124:97–101
63. Raposo M, Bettencourt C, Maciel P et al (2015) Novel candidate blood-based transcriptional biomarkers of Machado-Joseph disease. Mov Disord 30:968–975
64. Zijlstra MP, Rujano MA, Van Waarde MA et al (2010) Levels of DNAJB family members (HSP40) correlate with disease onset in patients with spinocerebellar ataxia type 3. Eur J Neurosci 32:760–770
65. Miyai I, Ito M, Hattori N et al (2012) Cerebellar ataxia rehabilitation trial in degenerative cerebellar diseases. Neurorehabil Neural Repair 26:515–522
66. Pedroso JL, França MC, Braga-Neto P et al (2013) Nonmotor and extracerebellar features in Machado-Joseph disease: a review. Mov Disord 28:1200–1208
67. Jin J-L, Liu Z, Lu Z-J et al (2013) Safety and efficacy of umbilical cord mesenchymal stem cell therapy in hereditary spinocerebellar ataxia. Curr Neurovasc Res 10:11–20
68. Ilg W, Synofzik M, Br€otz D et al (2009) Intensive coordinative training improves motor performance in degenerative cerebellar disease. Neurology 73:1823–1830
69. Ella M.R. Fonteyn, Anita Heeren, Jasper-Jan C. Engels, Jasper J. Den Boer, Bart P.C. van de Warrenburg, Vivian Weerdesteyn (2014) Gait adaptability training improves obstacle avoidance and dynamic stability in patients with cerebellar degeneration. Gait Posture 40(1):247–251
70. Freeman W, Wszolek Z (2005) Botulinum toxin type A for treatment of spasticity in spinocerebellar ataxia type 3 (Machado-Joseph disease). Mov Disord 20:644
71. Tuite PJ, Lang AE (1996) Severe and prolonged dysphagia complicating botulinum toxin A injections for dystonia in Machado-Joseph disease. Neurology 46:846
72. Cardoso F, de Oliveira JT, Puccioni-Sohler M et al (2000) Eyelid dystonia in Machad Joseph disease. Mov Disord 15:1028–1030
73. Nunes MB, Martinez AR, Rezende TJ, Friedman JH, Lopes-Cendes I, D'Abreu A, França MC Jr (2015) Dystonia in Machado-Joseph disease: clinical profile, therapy and anatomical basis. Parkinsonism Relat Disord 21:1441–1447
74. Nandagopal R, Moorthy SGK (2004) Dramatic levodopa responsiveness of dystonia in a sporadic case of spinocerebellar ataxia type 3. Postgrad Med J 80:363–365
75. Wilder-Smith E, Tan EK, Law HY et al (2003) Spinocerebellar ataxia type 3 presenting as an L-DOPA responsive dystonia phenotype in a Chinese family. J Neurol Sci 213:25–28
76. Buhmann C, Bussopulos A, Oechsner M (2003) Dopaminergic response in parkinsonian phenotype of Machado-Joseph disease. Mov Disord 18:219–221
77. Lu CS, Chang HC, Kuo PC et al (2004) The parkinsonian phenotype of spinocerebellar ataxia type 3 in a Taiwanese family. Parkinsonism Relat Disord 10:369–373
78. Siebert M, Donis KC, Socal M et al (2012) Glucocerebrosidase gene variants in parkinsonian patients with Machado Joseph/spinocerebellar ataxia 3. Parkinsonism Related Disord 18: 185–190
79. Russo AD, Reckziegel ER, Krum-Santos AC et al (2015) clinical scales predict significant videofluoroscopic dysphagia in Machado Joseph disease patients. Mov Disord Clin Pract 2:260–266
80. Monte TL, Rieder CRM, Tort AB et al (2003) Use of fluoxetine for treatment of Machado-Joseph disease: an open-label study. Acta Neurol Scand 107:207–210
81. Silva RCR, Saute JA, Silva ACF et al (2010) Occupational therapy in spinocerebellar ataxia type 3: an open-label trial. Brazilian J Med Biol Res 43:537–542
82. França MC, D'Abreu A, Nucci A, Lopes-Cendes I (2008) Muscle excitability abnormalities in Machado-Joseph disease. Arch Neurol 65:525–529
83. Kanai K, Kuwabara S, Arai K et al (2003) Muscle cramp in Machado-Joseph disease: altered motor axonal excitability properties and mexiletine treatment. Brain 126:965–973

84. França MC, D'Abreu A, Friedman JH et al (2007) Chronic pain in Machado-Joseph disease: a frequent and disabling symptom. Arch Neurol 64:1767–1770
85. Brusse E, Brusse-Keizer MGJ, Duivenvoorden HJ, van Swieten JC (2011) Fatigue in spinocerebellar ataxia: patient self-assessment of an early and disabling symptom. Neurology 76:953–959
86. Jonas Alex Morales Saute, Laura Bannach Jardim (2016) Riluzole in patients with hereditary cerebellar ataxia. Lancet Neurol 15(8):788–789
87. Haffner ME (2006) Adopting orphan drugs–two dozen years of treating rare diseases. N Engl J Med 354:445–447
88. Jennifer Schumi, Janet T Wittes, (2011) Through the looking glass: understanding non-inferiority. Trials 12 (1)
89. Gilda Piaggio, Diana R. Elbourne, Stuart J. Pocock, Stephen J. W. Evans, Douglas G. Altman, for the CONSORT Group (2012) Reporting of Noninferiority and Equivalence Randomized Trials. JAMA 308(24):2594–2604
90. Steven M Snapinn, Current Controlled Trials in Cardiovascular Medicine 1(1):19–21
91. Alves S, Nascimento-Ferreira I, Auregan G et al (2008) Allele-specific RNA silencing of mutant ataxin-3 mediates neuroprotection in a rat model of Machado-Joseph disease. PLoS One 3:e3341

Chapter 18
Molecular Mechanisms and Cellular Pathways Implicated in Machado-Joseph Disease Pathogenesis

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Abstract Machado-Joseph disease (MJD) is a dominantly inherited disorder originally described in people of Portuguese descent, and associated with the expansion of a CAG tract in the coding region of the causative gene *MJD1/ATX3*. The CAG repeats range from 10 to 51 in the normal population and from 55 to 87 in SCA3/MJD patients. *MJD1* encodes ataxin-3, a protein whose physiological function has been linked to ubiquitin-mediated proteolysis. Despite the identification of the causative mutation, the pathogenic process leading to the neurodegeneration observed in the disease is not yet completely understood. In the past years, several studies identified different molecular mechanisms and cellular pathways as being impaired or deregulated in MJD. Autophagy, proteolysis or post-translational modifications, among other processes, were implicated in MJD pathogenesis. From these studies it was possible to identify new targets for therapeutic intervention, which in some cases proved successful in models of disease.

Keywords Pathogenesis · Molecular mechanisms · Machado-Joseph disease

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In this chapter we review the current knowledge on the different molecular mechanisms and cellular pathways that are implicated in MJD pathogenesis.

The genetic basis of Machado-Joseph disease (MJD) is well defined and understood, nevertheless only now we start to understand the complexity of the molecular mechanisms implicated in the pathogenesis. In this chapter we describe several molecular mechanisms and cellular pathways, which were identified as being implicated in the MJD pathogenesis (Fig. 18.1).

18.1 Toxicity of the Ataxin-3 Polyglutamine Stretch

A common feature of Polyglutamine (polyQ) diseases is the deposition of insoluble intracellular ubiquitinated inclusions containing the misfolded disease protein within the neurons in the affected regions.

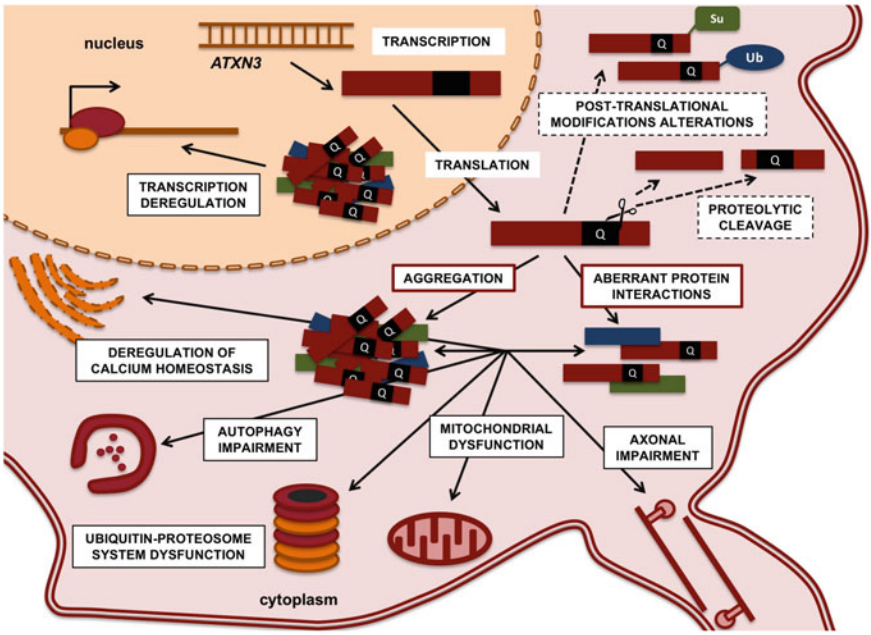

Fig. 18.1 Molecular mechanisms and cellular pathways implicated in MJD pathogenesis. The polyQ expansion in mutant ataxin-3 proteins leads to a cascade of events, including the impairment and dysfunction of different pathways that culminate in the neuronal death

Ataxin-3 is preferentially a cytoplasmic protein expressed throughout the brain [1, 2]. However, the expanded polyQ stretch leads to conformational alterations, which disable the proper folding of the protein [3, 4]. These changes cause the aggregation of the mutated protein and its nuclear accumulation in specific neuronal regions that are undergoing degeneration [5]. In fact, the presence of the mutant ataxin-3 intranuclear inclusions in MJD-affected brain regions is considered a hallmark of the disease. Moreover, in vitro and in vivo studies suggest that aggregation is required to the establishment of the disease-associated dysfunction and degeneration [6, 7]. However, there is no clear correlation between the presence of aggregates and neurodegeneration. Indeed, evidence seem to point that aggregation is not needed or sufficient to cause cell degeneration [8–11].

Although ataxin-3 functions have not been fully elucidated, it seems to be involved in the regulation of protein degradation, in the cellular response to heat stress, in transcriptional regulation, and it is also important to cytoskeletal organization (reviewed in [12–14]). Considering these cellular functions and the fact that homozygous MJD patients have an earlier onset of disease and a more severe phenotype than heterozygous patients [15], it was speculated that the loss of normal ataxin-3 contributes to MJD pathology. However, animals that do not express ataxin-3 are mostly normal [16, 17]. Thus, it is generally accepted that MJD pathology is mainly due to a toxic gain of function of the expanded ataxin-3. Indeed, mutant ataxin-3 inclusions recruit many other proteins, including chaperones [18] and transcriptional co-activators [19, 20], affecting several cellular pathways and mechanisms and thus causing cellular toxicity. Along with the polyQ tract toxicity, recent evidence suggest that the expanded CAG repetition can also lead to the formation of an elongated CAG repeat mRNA, which can by itself cause toxicity (reviewed in [14]).

18.2 Proteolytic Cleavage of Mutant Ataxin-3

It has become increasingly evident that proteolytic cleavage of polyQ proteins is a biologically relevant phenomenon in the context of the disease pathogenesis.

In MJD, previous work using cell and animal models demonstrated that expression of polyQ expanded ataxin-3 fragments resulted in increased aggregation and toxicity as compared to the full-length protein [1, 11, 21, 22], leading to the idea referred to as the toxic fragment hypothesis.

In fact, an ataxin-3 fragment was detected in Q71-MJD transgenic mice and *post mortem* brain tissue of MJD patients, whose levels increased with disease severity, supporting a relation between ataxin-3 proteolysis and disease progression [23]. Additionally, Ikeda and collaborators [21] found no pathology upon expression of full-length ataxin-3, while truncated protein produced a strong phenotype [21]. Furthermore, not only the ataxin-3 C-terminal fragment is cytotoxic [5, 21, 23], but the non-polyQ N-terminal fragment may also contribute to pathology through an impaired unfolded protein response [24]. Overall, proteolysis of the host protein to

liberate a toxic fragment would be required for pathology and would be the trigger of the aggregation process, a hallmark of the disease [25–27]. However, while the N-terminal fragment promotes the formation of cytoplasmic aggregates [24], the C-terminal fragment bearing the polyQ stretch leads to the formation of intranuclear inclusions [25, 27], being the nucleus considered a more important subcellular localization site than the cytoplasm for polyQ pathogenesis [7]. Therefore, despite the toxicity of the cytoplasmatic N-terminal ataxin-3, a more pronounced phenotype is observed upon ataxin-3 nuclear localization [6], suggesting a determinant contribution of the nuclear-localized C-terminal fragment of mutant ataxin-3.

In this sense, identifying protease(s) responsible for human mutant ataxin-3 processing and its cleavage site(s) could contribute to understand the mechanism of proteolysis involved and reveal potential candidates for therapy. Accordingly, mutant ataxin-3 has been shown to be a substrate for caspases [25, 28, 29, 30] and calpains [31–35], though without consensual results.

On one hand, either caspase-3 or caspase-1 generated ataxin-3 cleavage products in vitro. Interestingly, neither the reversible competitive tetrapeptide aldeyde inhibitor Ac-DEVD-CHO nor Ac-YVAD-CHO, which inhibit the effector and interleukin-1-β converting enzyme (ICE/caspase-1)-like caspases, respectively, were able to inhibit the fragments formation, suggesting that other enzymes could generate those products [30]. However, in cells undergoing apoptosis, proteolysis was mediated predominantly by caspase-1. Site-directed mutagenesis experiments narrowed the major cleavage event to a cluster of aspartate residues within the UIM2 near the polyQ tract [25, 29]. In a MJD *Drosophila* model, results suggested that ataxin-3 cleavage is conserved in the fly and that the process may also be caspase-dependent. Nevertheless, even though neuronal loss was aggravated, no significant modifications in nuclear inclusion formation were observed [28].

On the other hand, the role of calpains in ataxin-3 proteolysis was first reported by Haacke and collaborators in 2007 in mouse neuroblastoma N2a cells [31] and then further confirmed in L-glutamate-induced excitation of patient-specific induced pluripotent stem cell (iPSC)-derived neurons [33]. In both models, calcium-dependent proteolysis of ataxin-3 was followed by the formation of SDS-insoluble aggregates, a process abolished by calpain inhibition [31, 33]. Simões and collaborators [34] demonstrated for the first time in vivo, without external protease activation, that calpain-mediated proteolysis has a determinant role not only in ataxin-3 toxic fragments formation, but also in ataxin-3 translocation to the nucleus and MJD pathogenesis [34]. Furthermore, calpain inhibition mediated by its endogenous specific inhibitor calpastatin and by the synthetic compound BDA-410, through either in situ viral transduction in the brain or oral administration, respectively, decreased neurotoxicity and neurodegeneration in vivo. Calpain activation could come about by: (a) deranged calcium signalling reported in MJD transgenic mice [36] and (b) calpastatin depletion, observed in the *post mortem* dentate nucleus of MJD patients and in MJD mouse models [34]. Accordingly, knocking out calpastatin in a MJD transgenic mouse model leads to an aggravated neurological phenotype with an increased number of nuclear aggregates and accelerated neurodegeneration in the cerebellum [32].

The above divergent results concerning the proteases involved in ataxin-3 proteolysis might be explained by variations in experimental design and methods, which lead to different protein cellular contexts. Additionally, one cannot exclude that ataxin-3 cleavage may be a multi-step process, similarly to what has been reported for huntingtin [37].

In summary, mutant ataxin-3 has been proposed to have proteolytic sites at amino acids 145, 171, 225, 228 [30]; 241, 244, 248 [25, 29]; 286 [22]; 257 [27]; within the N-terminus of amino acid 190 [23, 38]; 60, 200 [31]; and 260 [24, 31, 35] (Fig. 18.2). Nevertheless, independently of the protease involved or the specific cleavage site, proteolysis of mutant ataxin-3 between the nuclear export signal (NES) sites and the nuclear localization signal (NLS) site would promote the C-terminus translocation to the nucleus and consequent aggregation and neurotoxicity [39].

18.3 Altered Protein Interactions

Several studies have revealed the importance of protein-protein interactions in understanding the normal function of the polyQ disease-causing protein [40–44].

As mentioned above, the toxicity of the expanded ataxin-3 or even its fragments does not fully explain the selective neuronal degeneration observed. One idea is that the normal function of ataxin-3 or its interactions with other proteins in each neuronal subpopulation might contribute to explain its selective toxicity [45]. Interestingly, normal ataxin-3 is found in nuclear inclusions of SCA2 patients, whereas normal ataxin-2 is found in the inclusions of MJD patients [46]. It was suggested that mutant ataxin-3 drives a reduction of ataxin-2 levels (and its subcellular localization), which contributes decisively to the disease pathogenesis [47]. Other studies showed that ataxin-3 interacts with several proteins linked to ubiquitination, sumoylation, phosphorylation, or calcium homeostasis, which might turn relevant in a context of disease (see next sections in this chapter for these topics).

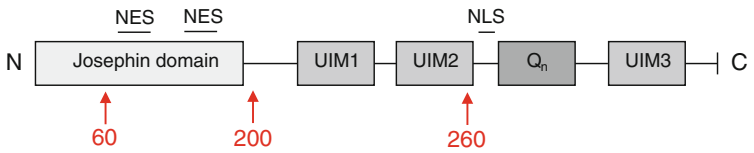

Fig. 18.2 Proteolytic cleavage of mutant ataxin-3. Mutant ataxin-3 might be cleaved by calpains at amino acids 60, 200 [31] and 260 [24, 31, 35]

18.4 Ubiquitin-Proteasome System Dysfunction

The ubiquitin-proteasome system (UPS) is the main regulated pathway for the clearance of damaged and misfolded intracellular proteins, with a central role in maintenance of cellular homeostasis [48–51].

First evidence pointing out a dysfunction of UPS in neurodegenerative diseases is from 1987, when Mori et al. described the presence of ubiquitin in inclusion bodies of Alzheimer's disease brain patients [52]. The presence of ubiquitin, chaperones and others UPS components in neuronal inclusions was later described for polyQ disorders, including MJD [5, 18, 53, 54, 55, 56, 57].

Ataxin-3 is a deubiquitinating enzyme directly involved in UPS. Ataxin-3 has an ubiquitin hydrolase activity within the N-terminal of the Josephin-domain [53, 58, 59, 60], it binds to ubiquitin, polyUb chains and ubiquitylated proteins through the ubiquitin-interacting motifs (UIM) located at the C-terminal region [53, 58, 59, 60, 61, 62, 63] and directly interacts with proteasome subunits and other proteasome components, regulating proteosomal degradation of protein substrates [59, 60, 61, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76]. The alteration of normal ataxin-3 function by the presence of the polyQ tract may compromise UPS activity, resulting in deficient degradation of misfolded proteins. Though mutant ataxin-3 has similar DUB activity to normal ataxin-3 [58, 61], it can differently interact with components of UPS system and interfere with the degradation of proteolytic substrates [61, 63, 64, 66, 67, 70, 72, 74, 77]. Accumulation of misfolded proteins can directly inhibit proteasome activity [78] and may overload an already impaired UPS system, exacerbating the toxic effects and resulting into cellular dysfunction and death.

Moreover, ataxin-3 has been demonstrated to have affinity to Lys63-linked chains [63]. Lys63-linked chains can regulate endocytosis, DNA repair, translational control and protein kinase activation, which suggests that the deubiquitinating activity of ataxin-3 might play a role in a variety of cellular processes [79]. In agreement, loss of ataxin-3 DUB activity in neuronal cells showed to affect differentiation and cytoskeleton organization [80], which may contribute to neurodegeneration.

18.5 Autophagy Impairment

In polyQ disorders there are strong evidences that mutant proteins might be degraded by macroautophagy, a mechanism with a crucial role in degradation of insoluble aggregate-prone proteins and essential for neuronal survival [81–83].

In MJD, mutant ataxin-3, similarly to other polyQ diseases proteins have shown to be cleared by autophagy [84, 85]. Furthermore, autophagy activation mediated by temsirolimus in mouse brains led to reduced number of aggregates, as well as, reduced levels of soluble mutant-ataxin-3 in the cytoplasm [86]. No alterations were reported in the levels of endogenous wild-type ataxin-3 in nucleus and cytoplasm, suggesting that mutant ataxin-3 is more prone to autophagic degradation than

wild-type ataxin-3 [86]. Reduction of *Atg5* autophagy gene in a *Drosophila* model of degeneration-dependent on polyQ ataxin-3 enhanced retinal toxicity; protein aggregation and protein accumulation in the cytoplasm of photoreceptor axons, thus indicating that autophagy can have a protective role in MJD [87]. A comparative analysis of MJD patients' putamen and controls revealed a pronounced immunoreactivity for autophagic markers such as p62, Atg16L and LC3 in MJD patients [88]. In a late stage MJD transgenic mice-expressing ataxin-3-71Q, an increase in the amount of p62-positive aggregates and LC3-II was also reported. Moreover, in a rat lentiviral model of MJD expressing human full-length mutant-ataxin-3 with 72 glutamines, p62 started to co-localize with ubiquitinated mutant-ataxin-3 aggregates in the nucleus at an early stage of the disease and autophagosomes accumulated in the cytoplasm at late stages of disease progression [88]. Assessment of endogenous beclin-1 levels revealed significant decreases in both fibroblasts from MJD patients and in the striatum of MJD transgenic mice [88]. Beclin-1 was also shown to co-localize with mutant-ataxin-3 aggregates in the nucleus in a more advanced stage of disease progression in rat lentiviral model [88]. Very recently, it was also shown that wild-type ataxin-3 deubiquitinates beclin-1, protecting it from proteasome-mediated degradation and thereby enabling autophagy. Such interaction involves the normal length polyQ tract of ataxin-3 but is abrogated in the presence of a competing longer polyQ mutation in a disease protein, such as mutant ataxin-3 or mutant huntingtin [89].

18.6 Mitochondrial Impairments and Oxidative Stress

Mitochondrial dysfunction has been associated with aging and age-related diseases, particularly with neurodegenerative diseases [90]. In polyQ diseases, mitochondrial dysfunction was first described in Huntington's disease (HD; [91–94], but it has also been reported in spinal and bulbar muscular atrophy (SBMA; [95], dentatorubral-pallidoluysian atrophy (DRPLA; [96], spinocerebellar ataxia type 1 (SCA1; [97]) and in MJD.

In 1996, Matsuishi and colleagues described a metabolic disruption in the cerebrospinal fluid of MJD patients, suggesting that mitochondrial function could be affected [98]. In fact, later on, in vitro studies, using cellular models of MJD, showed that the expression of mutant ataxin-3 promotes mitochondrial-mediated cell death, increases oxidative stress and reduces mitochondrial DNA copy number, suggesting an alteration on mitochondrial function [99–101]. Moreover, wild-type and mutant ataxin-3 were found in mitochondria, but fragments of ataxin-3 resulting from proteolysis were found in mitochondria only for mutant ataxin-3, suggesting a potential mitochondrial damage role for these fragments [102]. More recently, mitochondria from a MJD mouse model were found dysfunctional, particularly on complex II of the respiratory chain [76]. Lately, it was also reported that mtDNA is damaged in blood and brain samples from a transgenic MJD mouse model further suggesting a compromised of mitochondrial function [103, 104].

18.7 Impairment of Axonal Transport

As it was already mentioned, the presence of intranuclear aggregates of mutant ataxin-3 in the neurons of the affected brain regions is a hallmark of MJD. Interestingly, ataxin-3 was also found in the axons of neurons [2]. This data might be important in the light that the function and survival of neurons demands continuous axonal transport of mRNA and proteins. Moreover, several studies suggest that axonal transport disturbance is an attractive hypothesis that could explain the vulnerability of neurons [105–107]. In 2010, Seidel and collaborators reported the presence of mutant ataxin-3 aggregates in axons of several brain regions of MJD patients affected by neurodegeneration. The authors hypothesized that the presence of these aggregates in axons might be detrimental to axonal transport mechanisms and thereby contribute to neuronal degeneration in MJD. This finding is in line with the description that MJD patients revealed some phenotype symptoms linked to axonal neuropathy [108]. Also, some metabolic abnormalities observed in the deep white matter in MJD patients suggest extensive neuronal and axonal dysfunction [109]. Despite all these evidence suggesting that aggregates may impair axonal transport the consequences for the pathogenesis remain to be elucidated.

18.8 Deregulation of Intracellular Ca^{2+} Homeostasis

Calcium is a signaling ion involved in many cellular processes. In neurons, calcium homeostasis is essential to key processes such as neurite outgrowth, neurotransmitter release and synaptic plasticity. Abnormal neuronal calcium signaling may disrupt key cellular pathways contributing to neurodegeneration [110–113]. In the context of MJD, and in opposition to wild type, mutant ataxin-3 specifically binds to InsP3R1 intracellular calcium channel present in endoplasmatic reticulum, increasing its sensibility to activation by InsP3 and potentiating Ca^{2+} release from ER storages [36]. In cerebellar granule cells, ataxin-3 oligomers showed to interact with the lipid raft ganglioside GM1, glutamatergic receptors and voltage dependent calcium channels, inducing an influx of free intracellular Ca^{2+} [114]. Intracellular calcium overload can cause cell death by activation of several cytotoxic mechanisms, such as mitochondria permabilization, oxidative stress, cytoskeletal organization disruption and activation of calcium dependent proteins such as the previously mentioned calpains [113]. Administration of Dantrolene, an inhibitor of Ca^{2+} release, significantly improved motor coordination and gait deficits and prevented neuronal death in YAC84Q MJD transgenic mice [36], suggesting that Ca^{2+} signaling has indeed a role in the pathology of MJD.

18.9 Transcriptional Deregulation

Expanded polyQ proteins tend to accumulate in the nucleus, where the high protein concentration creates favourable conditions for interaction with transcriptional factors or cofactors [115, 116].

In MJD, mutant ataxin-3 has been shown to be involved in transcriptional deregulation, contributing to the disease pathogenesis [20, 117, 118]. Ataxin-3 can act by recruiting specific transcription factors to polyQ rich nuclear inclusions, as TATA-binding protein (TBP) and cAMP response element-binding protein-binding protein (CBP), suggesting an altered transcription mediated by these transcription factors that may contribute to the neurotoxicity [19, 118].

Furthermore, the normal function of ataxin-3 in the regulation of transcription can be modified in pathological conditions [119, 120]. Both normal and expanded ataxin-3 are able to interact with the cAMP response-element binding protein (CREB)-binding protein, p300, and p300/CREB-binding protein-associated factor (PCAF), leading to inhibition of transcription. However, the C-terminus containing the pathological polyQ stretch is able to bind more efficiently the coactivators than the normal polyQ, suggesting a possible pathological function of ataxin-3 [120]. In addition, ataxin-3 represses transcription by binding specific regions of MMP-2 gene promoter and interacting with histone deacetylase 3 (HDAC3) and nuclear receptor co-repressor (NCor). When mutated, ataxin-3 displays altered DNA and chromatin binding which reduces its histone deacetylase activity and repressor function [119]. Furthermore, when compared with normal ataxin-3, mutant ataxin-3 exhibited a reduced capability to activate the FOXO4-mediated SOD2 expression during the oxidative stress, promoting cytotoxicity [121].

Therefore, considering the abnormal interaction of the expanded ataxin-3 with the transcription regulators and the defect on the transcription regulation activity of normal ataxin-3, a deregulation of some biological processes is expectable. In fact, genes involved in inflammation, cell-surface associated processes and cell signalling have been shown to exhibit an altered expression in MJD models [122–124]. In a MJD transgenic model, the cerebellar dysfunction and ataxia were correlated with the disruption of the normal pattern of transcription. The contribution of the transcription deregulation for the initiation of the pathogenesis is supported by the observation of downregulated mRNA levels of genes involved in glutamatergic signalling and signal transduction in animals without neurological phenotype [122].

Furthermore, evidence of downregulation of the miRNA biogenesis and of miRNA machinery has recently been shown in different MJD models. Given that 3'UTR of the human ATXN3 mRNA is targeted by miRNAs, which mediate post-transcriptional negative regulation of ATXN3 itself, this further reinforces the relevance of transcriptional dysregulation contribution to MJD disease pathogenesis [125].

18.10 Post-translational Modifications Alterations

Different post-translational modifications of ataxin-3 have been identified as key steps in MJD pathogenic cascade. The importance of ataxin-3 phosphorylation to MJD pathogenesis was first shown by Fei et al. [126]. This study showed that conversion of serine 256 to alanine in ataxin-3 prevented phosphorylation mediated by glycogen synthase kinase 3 and resulted in protein aggregation of expanded ataxin-3 [126]. In another study, casein kinase 2 (CK2) -dependent phosphorylation of serines 340 and 352 in ataxin-3 was shown to modulate ataxin-3 stability and determine a nuclear distribution of both normal and expanded ataxin-3 [127]. On the contrary, mutation of these sites, pharmacological inhibition of CK2 and the association of CK2α with ataxin-3 decreased nuclear localization of ataxin-3 and nuclear inclusions formation [127]. More recently, a novel phosphorylation site at serine 12 (S12) was described for ataxin-3 [128]. Simulating constitutive phosphorylation on this residue reverted the dendrite and synapse loss caused by expanded ataxin-3 in cortical neurons. Furthermore, in vivo modification of ataxin-3 S12 reduced aggregation, neuronal degeneration and synapse loss in the striatum of an MJD lentiviral rat model [128]. Ataxin-3, being a deubiquitinating enzyme (DUb), can also be modified by ubiquitination [129]. Ubiquitination of ataxin-3 was shown to directly enhance ataxin-3 enzymatic capacity [129]. In vitro, both expanded and wild-type ataxin-3 were similarly activated by ubiquitination [129]. In a mouse model of MJD, an increased ubiquitination of expanded ataxin-3 was obtained in the brain lysates soluble fraction compared to normal ataxin-3 [129]. Ubiquitination at lysine 117 residue was identified as the main ubiquitination site in ataxin-3 and was shown to improve ataxin-3 functions comprising the increase in aggresome formation and reduction of ubiquitinated aggregates in cultured mammalian cells [130]. In a *Drosophila* model, ubiquitin-dependent activation of ataxin-3 improved its protective capacity at later stages and more severe forms of polyQ-dependent degeneration [131]. In this case, ubiquitinatable ataxin-3 was able to suppress eye degeneration by reducing protein aggregation, contrary to what was observed in cultured cells, and without changing the overall levels of the toxic protein [131]. Ataxin-3 can also undergo SUMOylation [132, 133]. SUMO-1 modification of ataxin-3 at lysine 166 led to an increase of mutant ataxin-3 stability and cytotoxicity in HEK293T cells [133]. Subcellular distribution, ubiquitination and formation of aggregates were not altered upon SUMOylation [133]. In another study, covalent bonding of SUMO at lysine 356 in the ataxin-3 isoform predominantly expressed in the brain induced differences in ataxin-3 self-assembly, while thermal stability, enzymatic activity and overall secondary structure of ataxin-3 did not suffer alterations [132]. Furthermore, it was shown that SUMOylation of ataxin-3 resulted in a decreased percentage of cortical neurons exhibiting ataxin-3 inclusions and increased the affinity of ataxin-3 for its interacting partner p97 [132].

18.11 Aging-Related Dysfunction

Aging can be defined as a natural time-dependent process, characterized by the progressive decline of biological functions, after the organism has achieved its maximal reproductive capability, towards mortality [134]. During this process, changes derived from the formation of subsidiary products of metabolism and the accumulation of cellular damage, can lead to disease formation or induce a faster progression of already established diseases. These changes can be defined as hallmarks of aging. Until now at least nine cellular and molecular hallmarks of aging were proposed, meaning that they manifest during normal aging, its experimental aggravation should accelerate aging, and its experimental amelioration should retard the normal aging process and therefore increase healthy lifespan (Reviewed in [135]).

In MJD and during aging, quality-control pathways, namely, ubiquitin-proteosome system and autophagy, are dysfunctional [136–138] and thus, aging can contribute to the decline of the cell's ability to remove misfolded proteins accumulated with the disease. Moreover, as previously referred in this chapter, one of the pathogenic mechanisms behind MJD is the mitochondrial dysfunction being also a hallmark of aging (reviewed in [139]). On the other hand, aging is associated with an increase in transcriptional noise. Microarray-based studies comparing young and old tissues from numerous species have identified changes in genes encoding key components of mitochondrial, inflammatory, and lysosomal degradation components, as well as apoptosis, cell cycle and cellular senescence biomarkers (Reviewed in [135]). Some strong evidence also relate neuronal death and transcriptional abnormalities seen in neurodegenerative disorders [122, 140, 141, 142] as well as in MJD as described in this chapter.

The evolutionary theories of aging predict as well that many genes potentially influence-ageing rate, among them are the ones included in the Sirtuin family of protein deacetylases [143, 144]. Sirtuins are highly sensitive nutrient-sensors and are intrinsically related with aging. Since the beginning of the millennium it is known that the increase in the expression or activity of sirtuin 1 leads to an increase in longevity, improving some pathways that usually are disrupted with aging [145]. Interestingly the same was observed in a transgenic mouse model of MJD. The molecular or pharmacological increase of sirtuin 1 levels and activity led to a significant amelioration of the disease phenotype [146].

18.12 Concluding Remarks

MJD belongs to the group of Polyglutamine disorders sharing a common cause, which is an abnormal repetition of a CAG motif within the ORF of the causative genes. In MJD, this mutation is then translated in the ataxin-3 protein leading to the neurodegenerative process observed in the disease. Despite this known cause,

it is controversial whether the disease pathogenesis results from a protein gain of toxic function or rather results from a loss of the normal protein function. On the other hand, it is more consensual that several molecular mechanisms account for the disease pathogenesis, and in the past years several cellular pathways were shown to be impaired or deregulated in the disease. In this chapter we review the molecular mechanisms implicated in MJD pathogenesis (Fig. 18.1), which might be tested as targets for therapeutic interventions. In fact, several studies showed that targeting some of these deregulated pathways are beneficial in the mitigation of MJD-related abnormalities (see Chaps. 20 and 21 on advanced therapies for polyQ disorders).

References

1. Paulson HL, Das SS, Crino PB, Perez MK, Patel SC, Gotsdiner D, Fischbeck KH, Pittman RN (1997) Machado-Joseph disease gene product is a cytoplasmic protein widely expressed in brain. Ann Neurol 41:453–462
2. Schmidt T et al (1998) An isoform of ataxin-3 accumulates in the nucleus of neuronal cells in affected brain regions of SCA3 patients. Brain Pathol 8:669–679
3. Masino L, Musi V, Menon RP, Fusi P, Kelly G, Frenkiel TA, Trottier Y, Pastore A (2003) Domain architecture of the polyglutamine protein ataxin-3: a globular domain followed by a flexible tail. FEBS Lett 549:21–25
4. Nagai Y, Inui T, Popiel HA, Fujikake N, Hasegawa K, Urade Y, Goto Y, Naiki H, Toda T (2007) A toxic monomeric conformer of the polyglutamine protein. Nat Struct Mol Biol 14:332–340
5. Paulson HL, Perez MK, Trottier Y, Trojanowski JQ, Subramony SH, Das SS, Vig P, Mandel JL, Fischbeck KH, Pittman RN (1997) Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. Neuron 19:333–344
6. Bichelmeier U et al (2007) Nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3: in vivo evidence. J Neurosci 27:7418–7428
7. Yang W, Dunlap JR, Andrews RB, Wetzel R (2002) Aggregated polyglutamine peptides delivered to nuclei are toxic to mammalian cells. Hum Mol Genet 11:2905–2917
8. Boy J et al (2010) A transgenic mouse model of spinocerebellar ataxia type 3 resembling late disease onset and gender-specific instability of CAG repeats. Neurobiol Dis 37:284–293
9. Silva-Fernandes A et al (2010) Motor uncoordination and neuropathology in a transgenic mouse model of Machado-Joseph disease lacking intranuclear inclusions and ataxin-3 cleavage products. Neurobiol Dis 40:163–176
10. Trottier Y, Cancel G, An-Gourfinkel I, Lutz Y, Weber C, Brice A, Hirsch E, Mandel JL (1998) Heterogeneous intracellular localization and expression of ataxin-3. Neurobiol Dis 5:335–347
11. Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, Pittman RN, Bonini NM (1998) Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in Drosophila. Cell 93:939–949
12. Matos CA, de Macedo-Ribeiro S, Carvalho AL (2011) Polyglutamine diseases: the special case of ataxin-3 and Machado-Joseph disease. Prog Neurobiol 95:26–48
13. Costa Mdo C, Paulson HL (2012) Toward understanding Machado-Joseph disease. Prog Neurobiol 97:239–257
14. Evers MM, Toonen LJ, van Roon-Mom WM (2014) Ataxin-3 protein and RNA toxicity in spinocerebellar ataxia type 3: current insights and emerging therapeutic strategies. Mol Neurobiol 49:1513–1531

15. Carvalho DR, La Rocque-Ferreira A, Rizzo IM, Imamura EU, Speck-Martins CE (2008) Homozygosity enhances severity in spinocerebellar ataxia type 3. Pediatr Neurol 38:296–299
16. Rodrigues AJ, Coppola G, Santos C, Costa Mdo C, Ailion M, Sequeiros J, Geschwind DH, Maciel P (2007) Functional genomics and biochemical characterization of the C. elegans orthologue of the Machado-Joseph disease protein ataxin-3. FASEB J 21:1126–1136
17. Schmitt I, Linden M, Khazneh H, Evert BO, Breuer P, Klockgether T, Wuellner U (2007) Inactivation of the mouse Atxn3 (ataxin-3) gene increases protein ubiquitination. Biochem Biophys Res Commun 362:734–739
18. Chai Y, Koppenhafer SL, Bonini NM, Paulson HL (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. J Neurosci 19:10338–10347
19. Chai Y, Wu L, Griffin JD, Paulson HL (2001) The role of protein composition in specifying nuclear inclusion formation in polyglutamine disease. J Biol Chem 276:44889–44897
20. McCampbell A et al (2000) CREB-binding protein sequestration by expanded polyglutamine. Hum Mol Genet 9:2197–2202
21. Ikeda H, Yamaguchi M, Sugai S, Aze Y, Narumiya S, Kakizuka A (1996) Expanded polyglutamine in the Machado-Joseph disease protein induces cell death in vitro and in vivo. Nat Genet 13:196–202
22. Yoshizawa T, Yamagishi Y, Koseki N, Goto J, Yoshida H, Shibasaki F, Shoji S, Kanazawa I (2000) Cell cycle arrest enhances the in vitro cellular toxicity of the truncated Machado-Joseph disease gene product with an expanded polyglutamine stretch. Hum Mol Genet 9:69–78
23. Goti D et al (2004) A mutant ataxin-3 putative-cleavage fragment in brains of Machado-Joseph disease patients and transgenic mice is cytotoxic above a critical concentration. J Neurosci 24:10266–10279
24. Hubener J et al (2011) N-terminal ataxin-3 causes neurological symptoms with inclusions, endoplasmic reticulum stress and ribosomal dislocation. Brain 134:1925–1942
25. Berke SJ, Schmied FA, Brunt ER, Ellerby LM, Paulson HL (2004) Caspase-mediated proteolysis of the polyglutamine disease protein ataxin-3. J Neurochem 89:908–918
26. Breuer P, Haacke A, Evert BO, Wullner U (2010) Nuclear aggregation of polyglutamine-expanded ataxin-3: fragments escape the cytoplasmic quality control. J Biol Chem 285:6532–6537
27. Haacke A, Broadley SA, Boteva R, Tzvetkov N, Hartl FU, Breuer P (2006) Proteolytic cleavage of polyglutamine-expanded ataxin-3 is critical for aggregation and sequestration of non-expanded ataxin-3. Hum Mol Genet 15:555–568
28. Jung J, Xu K, Lessing D, Bonini NM (2009) Preventing Ataxin-3 protein cleavage mitigates degeneration in a Drosophila model of SCA3. Hum Mol Genet 18:4843–4852
29. Liman J, Deeg S, Voigt A, Vossfeldt H, Dohm CP, Karch A, Weishaupt J, Schulz JB, Bahr M, Kermer P (2014) CDK5 protects from caspase-induced Ataxin-3 cleavage and neurodegeneration. J Neurochem 129:1013–1023
30. Wellington CL et al (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. J Biol Chem 273:9158–9167
31. Haacke A, Hartl FU, Breuer P (2007) Calpain inhibition is sufficient to suppress aggregation of polyglutamine-expanded ataxin-3. J Biol Chem 282:18851–18856
32. Hubener J et al (2013) Calpain-mediated ataxin-3 cleavage in the molecular pathogenesis of spinocerebellar ataxia type 3 (SCA3). Hum Mol Genet 22:508–518
33. Koch P et al (2011) Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. Nature 480:543–546
34. Simoes AT, Goncalves N, Koeppen A, Deglon N, Kugler S, Duarte CB, Pereira de Almeida L (2012) Calpastatin-mediated inhibition of calpains in the mouse brain prevents mutant ataxin 3 proteolysis, nuclear localization and aggregation, relieving Machado-Joseph disease. Brain 135:2428–2439

35. Simoes AT, Goncalves N, Nobre RJ, Duarte CB, Pereira de Almeida L (2014) Calpain inhibition reduces ataxin-3 cleavage alleviating neuropathology and motor impairments in mouse models of Machado-Joseph disease. Hum Mol Genet 23:4932–4944
36. Chen X, Tang TS, Tu H, Nelson O, Pook M, Hammer R, Nukina N, Bezprozvanny I (2008) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. J Neurosci 28:12713–12724
37. Kim YJ, Yi Y, Sapp E, Wang Y, Cuiffo B, Kegel KB, Qin ZH, Aronin N, DiFiglia M (2001) Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. Proc Natl Acad Sci U S A 98:12784–12789
38. Colomer Gould VF, Goti D, Pearce D, Gonzalez GA, Gao H, Bermudez de Leon M, Jenkins NA, Copeland NG, Ross CA, Brown DR (2007) A mutant ataxin-3 fragment results from processing at a site N-terminal to amino acid 190 in brain of Machado-Joseph disease-like transgenic mice. Neurobiol Dis 27:362–369
39. Antony PM, Mantele S, Mollenkopf P, Boy J, Kehlenbach RH, Riess O, Schmidt T (2009) Identification and functional dissection of localization signals within ataxin-3. Neurobiol Dis 36:280–292
40. Chen S, Peng GH, Wang X, Smith AC, Grote SK, Sopher BL, La Spada AR (2004) Interference of Crx-dependent transcription by ataxin-7 involves interaction between the glutamine regions and requires the ataxin-7 carboxy-terminal region for nuclear localization. Hum Mol Genet 13:53–67
41. Goehler H et al (2004) A protein interaction network links GIT1, an enhancer of huntingtin aggregation, to Huntington's disease. Mol Cell 15:853–865
42. Lim J et al (2006) A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. Cell 125:801–814
43. Steffan JS, Kazantsev A, Spasic-Boskovic O, Greenwald M, Zhu YZ, Gohler H, Wanker EE, Bates GP, Housman DE, Thompson LM (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc Natl Acad Sci U S A 97:6763–6768
44. Tsuda H et al (2005) The AXH domain of Ataxin-1 mediates neurodegeneration through its interaction with Gfi-1/senseless proteins. Cell 122:633–644
45. Takahashi T, Katada S, Onodera O (2010) Polyglutamine diseases: where does toxicity come from? what is toxicity? where are we going? J Mol Cell Biol 2:180–191
46. Uchihara T, Fujigasaki H, Koyano S, Nakamura A, Yagishita S, Iwabuchi K (2001) Non-expanded polyglutamine proteins in intranuclear inclusions of hereditary ataxias–triple-labeling immunofluorescence study. Acta Neuropathol 102:149–152
47. Nobrega C, Carmo-Silva S, Albuquerque D, Vasconcelos-Ferreira A, Vijayakumar UG, Mendonca L, Hirai H, de Almeida LP (2015) Re-establishing ataxin-2 downregulates translation of mutant ataxin-3 and alleviates Machado-Joseph disease. Brain 138:3537–3554
48. Clague MJ, Coulson JM, Urbe S (2012) Cellular functions of the DUBs. J Cell Sci 125:277–286
49. Kleiger G, Mayor T (2014) Perilous journey: a tour of the ubiquitin-proteasome system. Trends Cell Biol 24:352–359
50. Reyes-Turcu FE, Ventii KH, Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. Annu Rev Biochem 78:363–397
51. Suresh B, Lee J, Kim H, Ramakrishna S (2016) Regulation of pluripotency and differentiation by deubiquitinating enzymes. Cell Death Differ 23:1257–1264
52. Mori H, Kondo J, Ihara Y (1987) Ubiquitin is a component of paired helical filaments in Alzheimer's disease. Science 235:1641–1644
53. Chow MK, Mackay JP, Whisstock JC, Scanlon MJ, Bottomley SP (2004) Structural and functional analysis of the Josephin domain of the polyglutamine protein ataxin-3. Biochem Biophys Res Commun 322:387–394

54. Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. Nat Genet 19:148–154
55. Schmidt T, Lindenberg KS, Krebs A, Schols L, Laccone F, Herms J, Rechsteiner M, Riess O, Landwehrmeyer GB (2002) Protein surveillance machinery in brains with spinocerebellar ataxia type 3: redistribution and differential recruitment of 26S proteasome subunits and chaperones to neuronal intranuclear inclusions. Ann Neurol 51:302–310
56. Zander C, Takahashi J, El Hachimi KH, Fujigasaki H, Albanese V, Lebre AS, Stevanin G, Duyckaerts C, Brice A (2001) Similarities between spinocerebellar ataxia type 7 (SCA7) cell models and human brain: proteins recruited in inclusions and activation of caspase-3. Hum Mol Genet 10:2569–2579
57. Seidel K, den Dunnen WF, Schultz C, Paulson H, Frank S, de Vos RA, Brunt ER, Deller T, Kampinga HH, Rub U (2010) Axonal inclusions in spinocerebellar ataxia type 3. Acta Neuropathol 120:449–460
58. Berke SJ, Chai Y, Marrs GL, Wen H, Paulson HL (2005) Defining the role of ubiquitin-interacting motifs in the polyglutamine disease protein, ataxin-3. J Biol Chem 280:32026–32034
59. Mao Y, Senic-Matuglia F, Di Fiore PP, Polo S, Hodsdon ME, De Camilli P (2005) Deubiquitinating function of ataxin-3: insights from the solution structure of the Josephin domain. Proc Natl Acad Sci U S A 102:12700–12705
60. Nicastro G, Menon RP, Masino L, Knowles PP, McDonald NQ, Pastore A (2005) The solution structure of the Josephin domain of ataxin-3: structural determinants for molecular recognition. Proc Natl Acad Sci U S A 102:10493–10498
61. Burnett B, Li F, Pittman RN (2003) The polyglutamine neurodegenerative protein ataxin-3 binds polyubiquitylated proteins and has ubiquitin protease activity. Hum Mol Genet 12:3195–3205
62. Chai Y, Berke SS, Cohen RE, Paulson HL (2004) Poly-ubiquitin binding by the polyglutamine disease protein ataxin-3 links its normal function to protein surveillance pathways. J Biol Chem 279:3605–3611
63. Winborn BJ, Travis SM, Todi SV, Scaglione KM, Xu P, Williams AJ, Cohen RE, Peng J, Paulson HL (2008) The deubiquitinating enzyme ataxin-3, a polyglutamine disease protein, edits Lys63 linkages in mixed linkage ubiquitin chains. J Biol Chem 283:26436–26443
64. Boeddrich A et al (2006) An arginine/lysine-rich motif is crucial for VCP/p97-mediated modulation of ataxin-3 fibrillogenesis. EMBO J 25:1547–1558
65. Donaldson KM, Li W, Ching KA, Batalov S, Tsai CC, Joazeiro CA (2003) Ubiquitin-mediated sequestration of normal cellular proteins into polyglutamine aggregates. Proc Natl Acad Sci U S A 100:8892–8897
66. Doss-Pepe EW, Stenroos ES, Johnson WG, Madura K (2003) Ataxin-3 interactions with rad23 and valosin-containing protein and its associations with ubiquitin chains and the proteasome are consistent with a role in ubiquitin-mediated proteolysis. Mol Cell Biol 23:6469–6483
67. Durcan TM, Kontogiannea M, Thorarinsdottir T, Fallon L, Williams AJ, Djarmati A, Fantaneanu T, Paulson HL, Fon EA (2011) The Machado-Joseph disease-associated mutant form of ataxin-3 regulates parkin ubiquitination and stability. Hum Mol Genet 20:141–154
68. Jana NR, Dikshit P, Goswami A, Kotliarova S, Murata S, Tanaka K, Nukina N (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. J Biol Chem 280:11635–11640
69. Matsumoto M, Yada M, Hatakeyama S, Ishimoto H, Tanimura T, Tsuji S, Kakizuka A, Kitagawa M, Nakayama KI (2004) Molecular clearance of ataxin-3 is regulated by a mammalian E4. EMBO J 23:659–669
70. Scaglione KM et al (2011) Ube2w and ataxin-3 coordinately regulate the ubiquitin ligase CHIP. Mol Cell 43:599–612

71. Tsai YC, Fishman PS, Thakor NV, Oyler GA (2003) Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. J Biol Chem 278:22044–22055
72. Wang Q, Li L, Ye Y (2006) Regulation of retrotranslocation by p97-associated deubiquitinating enzyme ataxin-3. J Cell Biol 174:963–971
73. Ying Z, Wang H, Fan H, Zhu X, Zhou J, Fei E, Wang G (2009) Gp78, an ER associated E3, promotes SOD1 and ataxin-3 degradation. Hum Mol Genet 18:4268–4281
74. Zhong X, Pittman RN (2006) Ataxin-3 binds VCP/p97 and regulates retrotranslocation of ERAD substrates. Hum Mol Genet 15:2409–2420
75. Durcan TM, Fon EA (2013) Ataxin-3 and its e3 partners: implications for machado-joseph disease. Front Neurol 4:46
76. Laco MN, Cortes L, Travis SM, Paulson HL, Rego AC (2012) Valosin-containing protein (VCP/p97) is an activator of wild-type ataxin-3. PLoS ONE 7:e43563
77. Laco MN, Oliveira CR, Paulson HL, Rego AC (2012) Compromised mitochondrial complex II in models of Machado-Joseph disease. Biochim Biophys Acta 1822:139–149
78. Bence NF, Sampat RM, Kopito RR (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. Science 292:1552–1555
79. Chen ZJ, Sun LJ (2009) Nonproteolytic functions of ubiquitin in cell signaling. Mol Cell 33:275–286
80. Neves-Carvalho A et al (2015) Dominant negative effect of polyglutamine expansion perturbs normal function of ataxin-3 in neuronal cells. Hum Mol Genet 24:100–117
81. Cuervo AM (2004) Autophagy: in sickness and in health. Trends Cell Biol 14:70–77
82. Cuervo AM (2004) Autophagy: many paths to the same end. Mol Cell Biochem 263:55–72
83. Williams A, Jahreiss L, Sarkar S, Saiki S, Menzies FM, Ravikumar B, Rubinsztein DC (2006) Aggregate-prone proteins are cleared from the cytosol by autophagy: therapeutic implications. Curr Top Dev Biol 76:89–101
84. Berger Z et al (2006) Rapamycin alleviates toxicity of different aggregate-prone proteins. Hum Mol Genet 15:433–442
85. Ravikumar B, Duden R, Rubinsztein DC (2002) Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum Mol Genet 11:1107–1117
86. Menzies FM, Huebener J, Renna M, Bonin M, Riess O, Rubinsztein DC (2010) Autophagy induction reduces mutant ataxin-3 levels and toxicity in a mouse model of spinocerebellar ataxia type 3. Brain 133:93–104
87. Bilen J, Bonini NM (2007) Genome-wide screen for modifiers of ataxin-3 neurodegeneration in Drosophila. PLoS Genet 3:1950–1964
88. Nascimento-Ferreira I et al (2011) Overexpression of the autophagic beclin-1 protein clears mutant ataxin-3 and alleviates Machado-Joseph disease. Brain 134:1400–1415
89. Ashkenazi A, Bento CF, Ricketts T, Vicinanza M, Siddiqi F, Pavel M, Squitieri F, Hardenberg MC, Imarisio S, Menzies FM, Rubinsztein DC (2017) Polyglutamine tracts regulate beclin 1-dependent autophagy. Nature 545:108–111
90. Knott AB, Perkins G, Schwarzenbacher R, Bossy-Wetzel E (2008) Mitochondrial fragmentation in neurodegeneration. Nat Rev Neurosci 9:505–518
91. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, Bird ED, Beal MF (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol 41:646–653
92. Lou S, Lepak VC, Eberly LE, Roth B, Cui W, Zhu XH, Oz G, Dubinsky JM (2016) Oxygen consumption deficit in Huntington disease mouse brain under metabolic stress. Hum Mol Genet
93. Orr AL, Li S, Wang CE, Li H, Wang J, Rong J, Xu X, Mastroberardino PG, Greenamyre JT, Li XJ (2008) N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. J Neurosci 28:2783–2792

94. Panov AV, Gutekunst CA, Leavitt BR, Hayden MR, Burke JR, Strittmatter WJ, Greenamyre JT (2002) Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. Nat Neurosci 5:731–736
95. Beauchemin AM, Gottlieb B, Beitel LK, Elhaji YA, Pinsky L, Trifiro MA (2001) Cytochrome c oxidase subunit Vb interacts with human androgen receptor: a potential mechanism for neurotoxicity in spinobulbar muscular atrophy. Brain Res Bull 56:285–297
96. Lodi R, Schapira AH, Manners D, Styles P, Wood NW, Taylor DJ, Warner TT (2000) Abnormal in vivo skeletal muscle energy metabolism in Huntington's disease and dentatorubropallidoluysian atrophy. Ann Neurol 48:72–76
97. Kish SJ, Mastrogiacomo F, Guttman M, Furukawa Y, Taanman JW, Dozic S, Pandolfo M, Lamarche J, DiStefano L, Chang LJ (1999) Decreased brain protein levels of cytochrome oxidase subunits in Alzheimer's disease and in hereditary spinocerebellar ataxia disorders: a nonspecific change? J Neurochem 72:700–707
98. Matsuishi T, Sakai T, Naito E, Nagamitsu S, Kuroda Y, Iwashita H, Kato H (1996) Elevated cerebrospinal fluid lactate/pyruvate ratio in Machado-Joseph disease. Acta Neurol Scand 93:72–75
99. Chou AH, Yeh TH, Kuo YL, Kao YC, Jou MJ, Hsu CY, Tsai SR, Kakizuka A, Wang HL (2006) Polyglutamine-expanded ataxin-3 activates mitochondrial apoptotic pathway by upregulating Bax and downregulating Bcl-xL. Neurobiol Dis 21:333–345
100. Tsai HF, Tsai HJ, Hsieh M (2004) Full-length expanded ataxin-3 enhances mitochondrial-mediated cell death and decreases Bcl-2 expression in human neuroblastoma cells. Biochem Biophys Res Commun 324:1274–1282
101. Yu YC, Kuo CL, Cheng WL, Liu CS, Hsieh M (2009) Decreased antioxidant enzyme activity and increased mitochondrial DNA damage in cellular models of Machado-Joseph disease. J Neurosci Res 87:1884–1891
102. Pozzi C, Valtorta M, Tedeschi G, Galbusera E, Pastori V, Bigi A, Nonnis S, Grassi E, Fusi P (2008) Study of subcellular localization and proteolysis of ataxin-3. Neurobiol Dis 30:190–200
103. Kazachkova N, Raposo M, Montiel R, Cymbron T, Bettencourt C, Silva-Fernandes A, Silva S, Maciel P, Lima M (2013) Patterns of mitochondrial DNA damage in blood and brain tissues of a transgenic mouse model of Machado-Joseph disease. Neurodegener Dis 11:206–214
104. Ramos A, Kazachkova N, Silva F, Maciel P, Silva-Fernandes A, Duarte-Silva S, Santos C, Lima M (2015) Differential mtDNA damage patterns in a transgenic mouse model of Machado-Joseph disease (MJD/SCA3). J Mol Neurosci 55:449–453
105. Caviston JP, Ross JL, Antony SM, Tokito M, Holzbaur EL (2007) Huntingtin facilitates dynein/dynactin-mediated vesicle transport. Proc Natl Acad Sci U S A 104:10045–10050
106. Gunawardena S, Her LS, Brusch RG, Laymon RA, Niesman IR, Gordesky-Gold B, Sintasath L, Bonini NM, Goldstein LS (2003) Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. Neuron 40:25–40
107. Szebenyi G et al (2003) Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. Neuron 40:41–52
108. Klockgether T et al (1999) Age related axonal neuropathy in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). J Neurol Neurosurg Psychiatry 66:222–224
109. D'Abreu A, Franca MC Jr, Paulson HL, Lopes-Cendes I (2010) Caring for Machado-Joseph disease: current understanding and how to help patients. Parkinsonism Relat Disord 16:2–7
110. Bading H (2013) Nuclear calcium signalling in the regulation of brain function. Nat Rev Neurosci 14:593–608
111. Brini M, Cali T, Ottolini D, Carafoli E (2014) Neuronal calcium signaling: function and dysfunction. Cell Mol Life Sci 71:2787–2814
112. Clapham DE (2007) Calcium signaling. Cell 131:1047–1058
113. Zhivotovsky B, Orrenius S (2011) Calcium and cell death mechanisms: a perspective from the cell death community. Cell Calcium 50:211–221

114. Pellistri F et al (2013) Different ataxin-3 amyloid aggregates induce intracellular Ca(2+) deregulation by different mechanisms in cerebellar granule cells. Biochim Biophys Acta 1833:3155–3165
115. Lim J, Crespo-Barreto J, Jafar-Nejad P, Bowman AB, Richman R, Hill DE, Orr HT, Zoghbi HY (2008) Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature 452:713–718
116. Yamada M, Tsuji S, Takahashi H (2000) Pathology of CAG repeat diseases. Neuropathology 20:319–325
117. Kazantsev A, Preisinger E, Dranovsky A, Goldgaber D, Housman D (1999) Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells. Proc Natl Acad Sci U S A 96:11404–11409
118. Perez MK, Paulson HL, Pendse SJ, Saionz SJ, Bonini NM, Pittman RN (1998) Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. J Cell Biol 143:1457–1470
119. Evert BO, Araujo J, Vieira-Saecker AM, de Vos RA, Harendza S, Klockgether T, Wullner U (2006) Ataxin-3 represses transcription via chromatin binding, interaction with histone deacetylase 3, and histone deacetylation. J Neurosci 26:11474–11486
120. Li F, Macfarlan T, Pittman RN, Chakravarti D (2002) Ataxin-3 is a histone-binding protein with two independent transcriptional corepressor activities. J Biol Chem 277:45004–45012
121. Araujo J, Breuer P, Dieringer S, Krauss S, Dorn S, Zimmermann K, Pfeifer A, Klockgether T, Wuellner U, Evert BO (2011) FOXO4-dependent upregulation of superoxide dismutase-2 in response to oxidative stress is impaired in spinocerebellar ataxia type 3. Hum Mol Genet 20:2928–2941
122. Chou AH, Yeh TH, Ouyang P, Chen YL, Chen SY, Wang HL (2008) Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. Neurobiol Dis 31:89–101
123. Evert BO, Vogt IR, Kindermann C, Ozimek L, de Vos RA, Brunt ER, Schmitt I, Klockgether T, Wullner U (2001) Inflammatory genes are upregulated in expanded ataxin-3-expressing cell lines and spinocerebellar ataxia type 3 brains. J Neurosci 21:5389–5396
124. Evert BO, Vogt IR, Vieira-Saecker AM, Ozimek L, de Vos RA, Brunt ER, Klockgether T, Wullner U (2003) Gene expression profiling in ataxin-3 expressing cell lines reveals distinct effects of normal and mutant ataxin-3. J Neuropathol Exp Neurol 62:1006–1018
125. Carmona V, Cunha-Santos J, Onofre I, Simões AT, Vijayakumar U, Davidson BL, de Almeida Pereira (2017) Unravelling endogenous microRNA system dysfunction as a new pathophysiological mechanism in Mahcado-Joseph disease. Mol Ther 25:1028–1055
126. Fei E, Jia N, Zhang T, Ma X, Wang H, Liu C, Zhang W, Ding L, Nukina N, Wang G (2007) Phosphorylation of ataxin-3 by glycogen synthase kinase 3 beta at serine 256 regulates the aggregation of ataxin-3. Biochem Biophys Res Commun 357:487–492
127. Mueller T, Breuer P, Schmitt I, Walter J, Evert BO, Wullner U (2009) CK2-dependent phosphorylation determines cellular localization and stability of ataxin-3. Hum Mol Genet 18:3334–3343
128. Matos CA, Nóbrega C, Louros SR, Almeida B, Ferreiro E, Valero J, Pereira de Almeida L, Macedo-Ribeiro S, Carvalho AL (2016) Ataxin-3 phosphorylation decreases neuronal defects in spinocerebellar ataxia type 3 models. J Cell Biol 212(4):465–480
129. Todi SV, Winborn BJ, Scaglione KM, Blount JR, Travis SM, Paulson HL (2009) Ubiquitination directly enhances activity of the deubiquitinating enzyme ataxin-3. EMBO J 28:372–382
130. Todi SV, Scaglione KM, Blount JR, Basrur V, Conlon KP, Pastore A, Elenitoba-Johnson K, Paulson HL (2010) Activity and cellular functions of the deubiquitinating enzyme and polyglutamine disease protein ataxin-3 are regulated by ubiquitination at lysine 117. J Biol Chem 285:39303–39313

131. Tsou WL, Burr AA, Ouyang M, Blount JR, Scaglione KM, Todi SV (2013) Ubiquitination regulates the neuroprotective function of the deubiquitinase ataxin-3 in vivo. J Biol Chem 288:34460–34469
132. Almeida B, Abreu IA, Matos CA, Fraga JS, Fernandes S, Macedo MG, Gutierrez-Gallego R, Pereira PJ, Carvalho AL, Macedo-Ribeiro S (2015) SUMOylation of the brain-predominant Ataxin-3 isoform modulates its interaction with p97. Biochim Biophys Acta 1852:1950–1959
133. Zhou YF et al (2013) SUMO-1 modification on K166 of polyQ-expanded ataxin-3 strengthens its stability and increases its cytotoxicity. PLoS ONE 8:e54214
134. Farooqui T, Farooqui AA (2009) Aging: an important factor for the pathogenesis of neurodegenerative diseases. Mech Ageing Dev 130:203–215
135. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153:1194–1217
136. Carrard G, Bulteau AL, Petropoulos I, Friguet B (2002) Impairment of proteasome structure and function in aging. Int J Biochem Cell Biol 34:1461–1474
137. Ferrington DA, Husom AD, Thompson LV (2005) Altered proteasome structure, function, and oxidation in aged muscle. FASEB J 19:644–646
138. Keller JN, Dimayuga E, Chen Q, Thorpe J, Gee J, Ding Q (2004) Autophagy, proteasomes, lipofuscin, and oxidative stress in the aging brain. Int J Biochem Cell Biol 36:2376–2391
139. Boveris A, Navarro A (2008) Brain mitochondrial dysfunction in aging. IUBMB Life 60:308–314
140. Chang KH et al (2011) Nuclear envelope dispersion triggered by deregulated Cdk5 precedes neuronal death. Mol Biol Cell 22:1452–1462
141. Lee KY, Clark AW, Rosales JL, Chapman K, Fung T, Johnston RN (1999) Elevated neuronal Cdc2-like kinase activity in the Alzheimer disease brain. Neurosci Res 34:21–29
142. Shelton SB, Johnson GV (2004) Cyclin-dependent kinase-5 in neurodegeneration. J Neurochem 88:1313–1326
143. Bishop NA, Lu T, Yankner BA (2010) Neural mechanisms of ageing and cognitive decline. Nature 464:529–535
144. Kirkwood TB (2005) Understanding the odd science of aging. Cell 120:437–447
145. Cohen HY, Miller C, Bitterman KJ, Wall NR, Hekking B, Kessler B, Howitz KT, Gorospe M, de Cabo R, Sinclair DA (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 305:390–392
146. Cunha-Santos J, Duarte-Neves J, Carmona V, Guarente L, Pereira de Almeida L, Cavadas C (2016) Caloric restriction blocks neuropathology and motor deficits in Machado-Joseph disease mouse models through SIRT1 pathway. Nat Commun 7:11445

Chapter 19
Pharmacological Therapies for Machado-Joseph Disease

Sara Duarte-Silva and Patrícia Maciel

Abstract Machado-Joseph disease (MJD), also known as Spinocerebellar Ataxia type 3 (SCA3), is the most common autosomal dominant ataxia worldwide. MJD integrates a large group of disorders known as polyglutamine diseases (polyQ). To date, no effective treatment exists for MJD and other polyQ diseases. Nevertheless, researchers are making efforts to find treatment possibilities that modify the disease course or alleviate disease symptoms. Since neuroimaging studies in mutation carrying individuals suggest that in nervous system dysfunction begins many years before the onset of any detectable symptoms, the development of therapeutic interventions becomes of great importance, not only to slow progression of manifest disease but also to delay, or ideally prevent, its onset. Potential therapeutic targets for MJD and polyQ diseases can be divided into (i) those that are aimed at the polyQ proteins themselves, namely gene silencing, attempts to enhance mutant protein degradation or inhibition/prevention of aggregation; and (ii) those that intercept the toxic downstream effects of the polyQ proteins, such as mitochondrial dysfunction and oxidative stress, transcriptional abnormalities, UPS impairment, excitotoxicity, or activation of cell death. The existence of relevant animal models and the recent contributions towards the identification of putative molecular mechanisms underlying MJD are impacting on the development of new drugs. To date only a few preclinical trials were conducted, nevertheless some had very promising results and some candidate drugs are close to being tested in humans. Clinical trials for MJD are also very few to date and their results not very promising, mostly due to trial design constraints. Here, we provide an overview of the pharmacological therapeutic strategies for MJD studied in animal models and patients, and of their possible translation into the clinical practice.

Keywords PolyQ diseases · Machado-Joseph disease · Pharmacologic therapy

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19.1 Machado-Joseph Disease or Spinocerebellar Ataxia Type 3

Machado-Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3), is known to exist worldwide [1], representing the most common dominantly inherited ataxia (Reviewed in [1–3]) and the second most common polyQ disease [4]. In the last years a large effort has been put forward towards the understanding of the pathologic mechanism(s) underlying polyQ diseases, however, and unfortunately, the therapeutic approaches and drug development did not reach the desirable outcomes yet. Despite the increasing number of therapeutic strategies assessed in mouse models of polyQ diseases (around 250 preclinical therapeutic trials have already been described) [5], there are no effective treatments for these disorders, including MJD, and currently available therapeutic approaches are only able to provide limited symptomatic relief (Reviewed in [6, 7]).

The core clinical feature in MJD is a slowly progressive ataxia starting in adulthood, being the average age at onset of 40 years and the mean survival time of 21 years [8]. Numerous other clinical symptoms, including weight loss, dystonia, dysarthria, spasticity, rigidity, fasciculations, postural instability, proprioceptive loss, dysphagia, amyotrophy, corticospinal and autonomic nervous system dysfunctions and neuropathy, are also frequently observed in MJD patients [9–11]. Non-motor symptoms are also present, such as cramps, fatigue, sleep disturbances, mild cognitive affection and mood-related diseases [12–16]. Neuropathologically, MJD is characterized by neuronal loss in the cerebellum, *substantia nigra*, striatum, thalamus, pontine nuclei, spinal cord and cranial nerves, precerebellar brainstem nuclei, cholinergic and dopaminergic midbrain, as well as visual, auditory, vestibular, somatosensory, and ingestion and urination-related systems (Reviewed in [11]). Retained integrity of the cortical and subcortical regions of the limbic system and mild degeneration of cerebral and cerebellar cortices, white matter of cerebellum, inferior olive and Purkinje cells, are also characteristic of MJD [11]. The ataxin-3 protein (the MJD disease protein) is expressed ubiquitously and when it bears the expanded allele it tends to aggregate forming neuronal nuclear inclusion bodies (NNIs) in the brain [17, 18]. These NNIs are present in functionally affected and non-affected brain regions, indicating that there is no direct correlation between the occurrence of these protein aggregates and neuronal dysfunction [11, 19, 20]. Axonal aggregates have also been found in human patients and, as the intranuclear aggregates, they were immunopositive for ubiquitin and p62; one can hypothesize that axonal inclusions might be detrimental to axonal transport mechanisms, contributing to degeneration of nerve cells in MJD [21].

The clinical presentation of MJD is highly pleomorphic and led to the definition of four clinical sub-phenotypes: **type I**, characterised by the predominance of pyramidal and extrapyramidal anomalies, in addition to ataxia and other signs, with an early age-at-onset and fast progression; **type II**, with typical cerebellar ataxia, progressive external ophthalmoplegia and pyramidal signs appearing at an intermediate age; **type III**, with late onset and slow progression of peripheral signs,

such as loss of proprioception and muscle atrophies; and **type IV**, the rarest, characterised by the presence of Parkinsonic signs, associated to the core clinical features [10, 22, 23].

Here, we provide an overview of the current situation concerning small molecule therapeutics for MJD, including a brief description of the symptomatic therapies used in the clinics to improve patients' daily life, followed by a section on the recent drug discovery and development efforts, outlining the disease-modifying therapies tested so far in animal models of this disorder. In the end, we also provide a summary of the clinical trials performed to date in MJD patients.

19.2 Symptomatic Therapies for Machado-Joseph Disease

Despite the lack of efficacious disease-modifying therapies for MJD to date, several treatments, including specific drugs and multi-professional supportive approaches, are used to ameliorate neurological symptoms and increase the quality of life of the patients (Reviewed in [24], *updated in 2015*).

Non-pharmacological therapies include genetic counselling [25, 26], speech therapy, exercise/physiotherapy [27, 28], and occupational therapy [29]. The occupational therapy combined with antidepressants is thought to be helpful to fight the depression symptoms reported in MJD [30].

The pharmacological therapies prescribed by the physicians are mainly based on the knowledge of other related diseases or based on the patient's needs. Yet, the efficacy of those therapies has not been proven scientifically in MJD patients. Importantly, none of the clinical trials performed to date in MJD patients were based on data obtained in animal models of the disease. Nowadays, and with available animal models that closely mimic the human condition, the connection between preclinical and clinical studies should be strengthened. Pharmacological therapy includes levodopa or dopamine agonists for the restless leg syndrome as well as for the parkinsonism-like symptoms [31]. Adverse events may occur with levodopa treatment, namely worsening of the motor symptoms as shown for Parkinson's disease patients [32]. Modafinil, a psychostimulant, can be used to improve daytime fatigue, which is very frequent in MJD, and mexiletine or carbamazepine for cramps [33]. Together, these examples show that symptomatic MJD patients may benefit from available pharmacological approaches, which provide an important combination for the quality of life and the patients' feeling of independence.

19.3 Disease-Modifying Therapies for Machado-Joseph Disease: Lessons from Preclinical Trials

Despite the existence of a variety of MJD rodent models ([34] reviewed in [35]) and their potentialities, only a few preclinical trials have been performed until now using these models (see Table 19.1), and even less have then been translated to

Table 19.1 Preclinical trials performed in MJD mouse models using pharmacological approaches

Therapeutic molecule	Dosage	REF	Target/action	Treatment onset	Treatment duration (weeks)	Route of administration	Control groups	Outcome		Model
								Phenotype	Pathology	
Dantrolene	5 mg/kg	Chen et al. [85]	Stabilizer of intracellular Ca^{2+} signaling	Post-symptomatic	40	Food supplementation	Wild-type animals (treated and vehicle); MJD mice vehicle	Improvement in the beam walk test; improved gait deficits;	Restored brain weight; restored neuronal loss in PN; SN-TH neuronal cell loss is improved	Cemal et al. [83]
CCI-779	20 mg/kg	Menzies et al. [48]	Autophagy inducer	Post-symptomatic	8	i.p injection (3x/week)	Wild-type animals (treated and vehicle)-data not shown; MJD mice vehicle	Improvement in Rotarod (no phenotype was detected in basal conditions)	Reduced aggregate number in the motor cortex; reduction in soluble ataxin-3	Bichelmeier et al. [57]
Sodium butyrate	400 and 800 mg/kg	Chou et al. [78]	HDAC inhibitor	Pre-symptomatic	36	i.p injection (daily)	MJD mice vehicle	Prevention of weight loss; improvement in the rotarod; improved ataxic symptoms; improved hypoactivity; prolonged survival	Ameliorates mutant ataxin-3-induced degeneration of Purkinje neurons; restored hypoacetylation status in cerebellum	Chou et al. [62]
H1152	10 mg/kg	Wang et al. [63]	Rho-kinase (ROCK) inhibitor	Pre-symptomatic	12	i.p injection (daily)	Wild-type animals (treated); MJD mice vehicle	Partial improvement in the rotarod; increase in locomotor activity deficit	Reduction of ataxin-3 levels in the cerebellum, cerebral cortex, pontine nuclei or spinal cord; prevention of neuronal loss in the pontine nuclei	Chou et al. [62]

(continued)

Table 19.1 (continued)

Therapeutic molecule	Dosage	REF	Target/action	Treatment onset	Treatment duration (weeks)	Route of administration	Control groups	Outcome		Model
								Phenotype	Pathology	
Caffeine	1 g/L	Goncalves et al. [89]	Non-selective adenosine receptor antagonist	Pre-symptomatically	12	Drinking water	C57Bl6 animals vehicle (expressing mutant and wild-type ataxin-3 in the striatum)	ND	Ameliorates mutant ataxin-3 induced neurodegeneration; reduction in inclusions in the basal ganglia; reactive gliosis was reduced	Goncalves et al. [89]
17-DMAG	25 mg/kg	Silva-Fernandes et al. [42]	Hsp90 inhibitor	Pre-symptomatic	25	i.p injection (3x/week)	Wild-type animals (treated and vehicle); MJD mice vehicle	Delayed and improved motor deficits onset. Improved swimming performance, rotarod deficits and balance problems	Reduced aggregate number in the pontine nuclei and soluble ataxin-3 protein levels; decreased the number of pyknotic cells in the pontine nuclei	Silva-Fernandes et al. [42]
Lithium chloride	10.4 mg/kg	Duarte-Silva et al. [59]	Autophagy inducer	Pre-symptomatic	19	i.p injection (3x/week)	Wild-type animals (treated and vehicle); MJD mice vehicle	No overall effect; reduction of the tremors at endstage	No effect on mutant ataxin-3 levels	Silva-Fernandes et al. [42]
Citalopram	8 and 13 mg/kg	Teixeira-Castro et al. [101]	Selective serotonin reuptake inhibitor	Pre-symptomatic	29	Drinking water	Wild-type animals (treated and vehicle); MJD mice vehicle	Improved body weight, gait and motor deficits (footprinting, beam walk and motor swimming tests)	Reduced ataxin-3-posivite aggregates in several affected brain regions; reduced astrogliosis; increased number of ChAT + cells in the spinal cord and in the 7 N; increased Calbindin staining in Purkinje cells	Silva-Fernandes et al. [42]

(continued)

Table 19.1 (continued)

Therapeutic molecule	Dosage	REF	Target/action	Treatment onset	Treatment duration (weeks)	Route of administration	Control groups	Outcome		Model
								Phenotype	Pathology	
Valproic acid	200 mg/kg	Esteves et al. [79]	HDCA inhibitor	Pre-symptomatic	25	i.p (5 consecutive days/week)	Wild-type animals (treated and vehicle); MJD mice vehicle	Minor effects on body weight, balance problems, exploratory activity, swimming deficits and motor uncoordination in the rotarod	No effect on mutant ataxin-3-positive neuronal aggregates	Silva-Fernandes et al. [42]
Lithium chloride + CCI-779	10.4 mg/kg + 20 mg/kg	Duarte-Silva et al. [61]	Autophagy inducers	Pre-symptomatic	19	i.p injection (3x/week)	Wild-type animals (treated and vehicle); MJD mice vehicle	No overall effect in several behavior paradigms; combined therapy showed to be toxic to transgenic and wild-type mice	Reduction of soluble mutant ataxin-3 and the number of neuronal aggregates in the pontine nuclei	Silva-Fernandes et al. [42]
Riluzole	10 mg/kg	Schmidt et al. [110]	Glutamate antagonist	Post-symptomatic	40	Drinking water	Single transgenic for the MJD responder (treated and vehicle)	No improvement on motor deficits measured by rotarod, on home cage activity or body weight	Reduction of the soluble ataxin-3 level and an increase in ataxin-3 positive accumulations; reduction of calbindin expression in Purkinje cells in riluzole treated mice	Boy et al. [109]
Resveratrol	10 mg/kg	Cunha-Santos et al. [94]	Sirtuin 1 inducer	Post-symptomatic	8	i.p injection (daily)	MJD mice vehicle	Improved motor deficits and balance	Restored SIRT1 mRNA levels. Neuropathology was not evaluated	Torashima et al. [99]

clinical trials. Those studies were performed considering different approaches: (i) more directly targeting mutant ataxin-3 synthesis, folding and degradation and (ii) reducing the downstream deleterious effects of mutant ataxin-3 accumulation. The hypothesized pathogenic mechanism(s) involved in MJD and discussed throughout this chapter are represented in Fig. 19.1, as well as the possible therapeutic targets.

19.3.1 Mutant Ataxin-3 Refolding and Degradation: Autophagy and Proteasome Inducers

Restoration of global protein homeostasis, or proteostasis, is a promising approach to reduce the toxicity of mutant ATXN3 in MJD. Several studies in rodent models demonstrated the efficacy of activating the cellular machinery involved in maintaining adequate conformation and solubility of proteins or, in case this fails, send them for degradation, such as molecular chaperones, the ubiquitin-proteasome system (UPS) and autophagy, which will be discussed hereafter.

For instance, Hsp90 inhibitors are known to possess the unique pharmacological effect of inducing a heat stress response and, in addition to their use as anticancer agents, have also been developed as pharmacological HSP inducers for application in protein folding disorders [36, 37]. Several studies demonstrated the positive effects of 17-AAG and its analogues (including 17-DMAG, which is less toxic) as Hsp90 inhibitors in models of polyQ diseases [38–41]. The efficacy of 17-DMAG in improving the behavioral deficits was tested in the CMVMJD135 mice [42]. In this study it was shown that the behavioral deficits were transiently improved by 17-DMAG administration and neuropathologic features were ameliorated. Surprisingly, 17-DMAG did not induce the HSR in the brain of CMVMJD135 animals as expected. However, the protein levels of mutant ataxin-3 as well as the aggregate load were diminished after 17-DMAG treatment suggesting that other mechanism(s) would be occurring in the cells. Indeed, it was proposed that 17-DMAG was inducing autophagy and therefore probably the degradation of mutant ataxin-3 through this mechanism (not excluding others, as the UPS). In spite of the promising results in mouse models, establishing proof of concept, 17-DMAG is known to exert several important adverse effects in humans [43], which must be taken in consideration given the expected need for chronic treatment of MJD patients. Chemical modifications should be conducted in 17-DMAG to decrease its toxicity while keeping its beneficial effects; only after that should such an approach be considered for clinical trials in MJD.

Autophagy induction seems to be a promising target to modulate protein aggregation in polyQ diseases and, in addition to the abovementioned results, there is an extensive body of literature demonstrating its beneficial effects in polyQ diseases [43–56]. In order to verify the therapeutic efficacy of autophagy induction in MJD, Menzies and colleagues used the mouse model generated by Bichelmeier et al. [57]

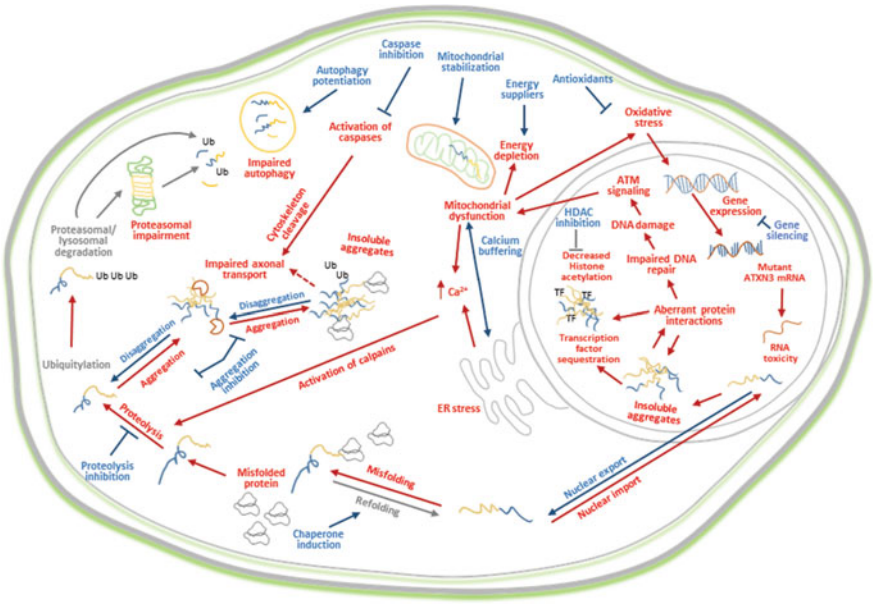

Fig. 19.1 Schematic representation of the potential pathogenic mechanisms underlying MJD and possible therapeutic targets. Intracellular candidate pathogenesis pathways in MJD are represented in red. These include the formation of cytoplasmic and nuclear aggregates/inclusions, transcriptional deregulation, mitochondrial dysfunction, impairment of degradation mechanisms (autophagy/proteasome) and activation of caspases/calpains. Possible intracellular therapeutic targets are represented in blue

which they chronically treated with an autophagy inducer—temsirolimus (codenamed CCI-779), a rapamycin analog. Although the authors were not able to reproduce the phenotype previously described for this model [57], at the end of a two months preclinical trial they report that treated-MJD animals performed better in the accelerating rod when compared to placebo-treated mice, and that this compound had no effect in wild-type (WT) animals in the rotarod. Also, temsirolimus was able to reduce mutant ataxin-3 aggregates in the motor cortex and the soluble cytoplasmic, but not nuclear, mutant ataxin-3 in total brain extracts. Finally, the authors performed a microarray study at basal conditions and after temsirolimus treatment. Overall, the transcriptional alterations found were very small, probably correlating to the absence of a clear phenotype in this cohort of MJD mice. Yet, it was possible to identify genes with decreased expression in MJD-vehicle mice, which was increased after temsirolimus treatment; the opposite effect was not found [48]. The potential beneficial effects of autophagy induction were further reinforced in studies using beclin-1 overexpression in rodent models of MJD [58]. Thus, and also considering the beneficial effects of 17-DMAG, other autophagy inducers were tested in the CMVMJD135 mice: lithium chloride and CCI-779. Unexpectedly, the use of lithium chloride had no overall effect on the behavioral deficits of CMVMJD135 mice, in spite of activating autophagy as expected [59]. Accordingly,

a human clinical trial using lithium carbonate was performed in the same year, demonstrating that albeit well tolerated, lithium had no major impact on disease progression in MJD patients [60] (see Sect. 19.4 in the present chapter). In another attempt to increase autophagy, a combination of two autophagy inducers acting independently and dependently of mTOR—lithium and CCI-779, respectively—was tested in the CMVMJD135 mouse model. This combinatory therapy showed no beneficial effects and even proved to be deleterious to both transgenic and wild-type mice, affecting neurological function and general health [61], at doses shown to be safe in mice when administered alone [48, 59]. These results suggest that overactivation of autophagy could also be dangerous, however, other effects of the drug combination cannot be excluded.

Using the mouse model developed by their team [62], Wang and colleagues developed a preclinical trial using H1152, a Rho-kinase (ROCK) inhibitor [63]. ROCK is a kinase and acts as the downstream effector of small GTP-binding proteins of the Rho subfamily, and its abnormal activation has been implicated in several neurodegenerative diseases [64]. Also, ROCK inhibitors were shown to decrease the levels of mutant huntingtin in brain as well as improve motor function in a mouse model of Huntington's disease (HD) [65]. This study confirmed that H1152 could also decrease the brain level of pathogenic ataxin-3 and exert a therapeutic effect on the MJD mouse model. The authors tested several ROCK inhibitors in vitro and showed that H1152 was the most potent in reducing ataxin-3 protein levels, and that acted by increasing proteasome activity. Daily intraperitoneal injections of H1152 in the MJD mice improved motor coordination and locomotor activity deficits. H1152 administration significantly decreased mutant ataxin-3 levels in the cerebellum, cerebral cortex, pontine nuclei and spinal cord and decreased the cell death (reduction in NeuN positive cells) observed in the pontine nuclei of vehicle-treated transgenic animals [63]. Fasudil, a first-generation ROCK inhibitor, has been studied widely in clinical trials for the treatment of pulmonary arterial hypertension as well as for subarachnoid hemorrhage [66], constituting a safe drug in humans. A phase II clinical trial is ongoing for the study of its safety and efficacy in amyotrophic lateral sclerosis patients (NCT01935518). Indeed, its protective effects were recently shown in a model of HD [67]. In this sense, the inhibition of ROCK can be regarded as a promising avenue for therapeutic intervention in various neurological disorders, including MJD and other polyQ diseases.

19.3.2 Therapies Targeting Downstream Molecular Events

19.3.2.1 Transcriptional Regulation

Transcriptional deregulation is a unifying feature of polyQ disorders [68–73]; however, the relationship between polyQ-induced deregulation of gene expression and the ongoing degenerative processes remains unclear.

More than 20 nuclear proteins relevant for transcription are known to interact with polyQ disease associated-proteins [69, 74]. Mutant ataxin-3 has been shown to interact abnormally with several proteins involved in the transcription machinery, namely CREB-binding protein (CBP) and p300/CREBBP associated factor (PCAF), suppressing their histone acetyltransferase activity [72, 75]. Overexpression of some of these transcription regulators was shown to overcome polyQ toxicity, both in cellular models for MJD, Spinal and Bulbar Muscular Atrophy (SBMA), and HD [70, 76] as well as in vivo, in a polyQ model in Drosophila [71]. This suggests that expanded polyQ proteins may contribute for the depletion of key transcriptional regulators with toxic effects to the cell and reinforces the idea of an important role for transcription deregulation in polyQ pathogenesis. Acetylation of histones relaxes the DNA structure, promoting transcription, whereas hypoacetylation represses gene activity [77]. The equilibrium of histone acetylation/deacetylation is controlled by histone acetyltransferases (HATs) and deacetylases (HDACs).

Previously, based on expression data, Chou and collaborators suggested that a global transcriptional deregulation was occurring in the cerebellum of a MJD transgenic model [62]. More specifically, they have shown a generalized hypoacetylation of H3 and H4. In order to modulate these alterations in the transcriptome, the same authors treated their mouse model with sodium butyrate (SB), an HDAC inhibitor. They observed that daily administration of SB was able to revert histone hypoacetylation as well as the transcription downregulation in the cerebellum. Importantly, SB treatment improved motor performance of transgenic animals in the rotarod, an effect that was less evident in later stages. The gait-related symptoms, quantified through the footprint pattern, were also ameliorated with SB, as well as the spontaneous locomotor activity, body weight loss and survival [78].

In contrast, Esteves S and colleagues, demonstrated that chronic treatment of the CMVMJD135 mice with valproic acid (VPA), also known to act as an HDAC inhibitor led to limited effects concerning the improvement of motor deficits and had no effect on mutant ataxin-3 aggregation in the brain. Nevertheless, VPA treatment increased the levels of GRP78, an endoplasmic reticulum chaperone involved in the folding of newly synthetized proteins and in the translocation of aberrant proteins for degradation by the proteasome, which might explain the small improvement in motor coordination seen after a long treatment duration [79]. These results contrast with the findings of a study in human patients, in which a beneficial effect was observed (see Sect. 19.4 in the present chapter).

19.3.2.2 Calcium Signaling Stabilizers

Calcium signaling is thought to play an important role in polyQ pathogenesis. This hypothesis is based on previous studies demonstrating that mutant huntingtin can bind and activate specifically type 1 inositol 1,4,5-triphosphate receptors (InsP3R1, an intracellular calcium release channel), influencing calcium signaling [80]. Deranged calcium signaling was also observed in neuronal primary cultures from the YAC128 HD mouse model [81, 82]. Later on, mutant ATXN3 was also proven

to bind to InsP3R1 and to perturb calcium signaling. Taking advantage of the YAC transgenic model of MJD generated by Cemal et al. in [83], Chen and collaborators performed a chronic treatment to these mice, using food supplemented with dantrolene. This compound is a ryanodine antagonist and a clinically relevant Ca^{2+} signaling stabilizer, being commonly used as a skeletal muscle relaxant to treat hyperthermia and muscle spasticity [84]. Dantrolene-treated MJD mice showed an improved performance in the balance beam test (taking less time to traverse the different beams, with a number of foot slips identical to WT), reduction of the crawling behavior seen in the MJD-vehicle group, and a significant improvement in the footprinting pattern. To evaluate the neuroprotective effect of dantrolene, the brains of the four groups used were weighed, however there was no improvement in this parameter. Dantrolene food supplementation did, nevertheless, diminish the loss of NeuN positive cells in the pontine nuclei and of TH-positive cells in the *substantia nigra* of MJD mice [85]. In spite of its beneficial effects, no further studies with this compound were performed in MJD patients. The known side effects of dantrolene originate in the central nervous system, and include drowsiness, lightheadedness, headaches, anorexia, diarrhea, nausea, and vomiting [86]. To our knowledge, no clinical trials with dantrolene have been performed in neurodegenerative diseases, suggesting that this compound might not be a good candidate for MJD treatment.

19.3.2.3 Neuroprotection

Neuronal dysfunction and synaptotoxicity are thought to play a major role in polyQ disease pathogenesis. Indeed, it was previously suggested that neuronal dysfunction may precede neurodegeneration and clinical symptoms in HD [87, 88]. In MJD, loss of synaptic markers was proposed to be an early feature in a lentiviral-based disease model, suggesting a putative role for ataxin-3 in the control of synapse function [89]. Furthermore, Silva-Fernandes and colleagues have shown the presence of a clear motor phenotype in the CMVMJD135 mouse model of MJD, without major early neuronal loss, suggesting once again, that neuronal dysfunction may precede neurodegeneration [42]. These hypotheses were not deeply explored, so far, in MJD; nevertheless, some compounds known to have neuroprotective effects have been tested in MJD models.

Treatment with caffeine (a non-selective adenosine receptor antagonist) as well as with selective blockers of the adenosine A_2A receptor ($A_{2A}R$) have been shown to be neuroprotective in several brain diseases, including HD [90–92]. In a study by Gonçalves et al., caffeine was administered to a lentiviral model of MJD (overexpression of human wild-type—atx3-27Q—or mutant ataxin-3-atx3-72Q) in the drinking water for 3 months (maximum), in a 1 g/L dose, corresponding to a human daily consumption of 5 cups of coffee. Chronic caffeine treatment rescued the striatal shrinkage observed in the mutant ATXN3 transduced animals and slightly reduced the number of pycnotic cells. Also, caffeine was able to avoid the loss of NeuN positive cells observed in the atx3-72Q animals. These data suggest

that chronic caffeine treatment is neuroprotective towards ataxin-3 overexpression in the striatum. Furthermore, loss of DARPP-32 staining volume, astrogliosis and putative microgliosis were improved in the treated group. Nevertheless, the beneficial effects of caffeine were shown to be transient. Finally, and intriguingly, caffeine-treated mice showed an increase in the number of nuclear inclusions when compared to water-drinking animals. These observations might indicate that the final stages of aggregation, visible neuronal inclusions, are protective rather than toxic [89], but this was not explored further. Several studies support the use of caffeine for different neurodegenerative diseases (reviewed in [93]). The neuroprotective effects of caffeine observed in the lentiviral-mediated model of MJD, and considering the well-define and side-effect profile, being in general well tolerated comparing to other drugs, support the use of antagonists of adenosine receptors as potential therapeutic tools to treat MJD and other polyQ diseases. Further studies in MJD patients should be performed to prove the clinical utility of this approach.

Recently, Cunha-Santos and colleagues tested the potential of resveratrol, a Sirtuin-1 (SIRT1) activator, as potential therapeutic strategy for MJD [94]. SIRT1 belongs to the group of the histone deacetylase enzymes being a NAD^+-dependent histone and protein deacetylase that plays an important role in several cellular and physiological processes, including an important involvement in neurodegeneration [95]. Indeed, induction of SIRT1 was shown to have a protective role in HD and SBMA models [96–98]. Resveratrol treatment in the MJD mouse model [99] was shown to improve motor and balance deficits after disease onset. This study pointed SIRT1 activation as a potential therapeutic target for MJD 94]. Resveratrol, being a multitarget compound with several neuroprotective roles, represents an interesting candidate for the treatment of MJD. Nevertheless, it is important to remember resveratrol solubility and bioavailability limitations [100], which can be solved by appropriate chemical modifications. Resveratrol was already tested in a phase 2 clinical trial in Alzheimer's disease patients and showed to be safe and well tolerated; nevertheless, the small number of participants did not allow researchers to determine whether resveratrol may be beneficial or not.

It was also stated that "More potent and bioavailable SIRT1 activators are also in development" (see *Study Results* of the NCT01504854 clinical trial), which could be useful for this and other neurodegenerative diseases.

19.3.2.4 Modulators of the Serotonergic and Glutamatergic Systems

Recently, and departing from an unbiased screening of FDA-approved small molecules, Teixeira-Castro and collaborators identified Citalopram (Selective Serotonin Reuptake Inhibitor—SSRI) as a hit compound able to modify the neurotoxic effect of mutant ATXN3 in the nematode *C. elegans*, but also its aggregation. The effect required early treatment initiation and a minimum duration. The compound was further tested in a mouse model of the disease (CMVMJD135) and shown to delay disease progression, decrease mutant ATXN3 aggregation and neuropathology. This work also demonstrated, using pharmacogenetic approaches,

that activation of the serotonergic signaling was beneficial in both animal models of MJD [101]. Intriguingly, improvement in the mouse model happened in spite of normal neurotransmitter levels at the basal state. This intriguing link between serotonin signaling and protein homeostasis has been recognized by the work of Prahlad and colleagues [102], and may imply a new perspective for usage of these established compounds in neurodegenerative diseases, including other polyQ-associated SCAs.

Although evidence for excitotoxicity is not as strong as for HD, perturbed glutamate transmission has also been proposed to play a role in MJD [62, 103, 104], namely through very intriguing links to mutant protein cleavage and aggregation. Interestingly, clinical trials using the antiglutamatergic drug riluzole demonstrated a beneficial effect in patients with different ataxias [105, 106]. Unfortunately, MJD patients were not included in these clinical trials. Considering this, and also the fact that riluzole was shown to have protective effects in cellular models of HD [107, 108], Schmidt and colleagues have studied the potential beneficial effects of riluzole in a conditional MJD mouse model [109]. Post-symptomatic chronic treatment with riluzole had no effect on motor deficits of the mouse despite the observed reduction of soluble mutant ataxin-3 protein levels. Furthermore, riluzole increased the levels of ataxin-3 aggregation. Also, and very importantly, the authors showed that treatment with riluzole decreased the Calbindin expression in Purkinje cells of the cerebellum, suggestive of possible toxicity, which might indicate that this compound might not be commendable to test in humans with MJD, or, at least, that it should be tested with caution [110].

19.4 Clinical Trials in MJD Patients

Currently, no disease modifying treatment exists for MJD. Yet, some symptomatic treatment is available, including genetic counseling, physical therapy programs, and speech and swallowing training as discussed above. The translation of findings from model systems to human patients is an important and urgent issue. Considering the lack of information on the key aspects of the pathogenic mechanism(s), the clinical and molecular heterogeneity of MJD patients and the scarcity of human biological tissues available for research, the development of translational approaches is very difficult. Still, some clinical trials have been performed for MJD (see Table 19.2). The detection of undesired side effects is also of major importance in clinical trials and must be taken in consideration. Most of the MJD clinical trials to date were performed using very few patients (less than 10) and only short-term effects were investigated, thus their outcome assessment might be compromised.

The combination of sulphamethoxazole and trimethoprim (Bactrim, a broad-spectrum antibiotic used in ear and urinary infections) was suggested to reduce disease symptoms in a small double-blind clinical trial using 8 MJD patients. The authors observed mild improvements in some of the parameters evaluated, such as hyperreflexia of knee jerks and rigospasticity of the legs in the patients treated

Table 19.2 Clinical trials performed to date in MJD patients

Therapeutic molecule	REF	Target	Design	Treatment duration (weeks)	Number of patients	Mean age (years)	Mean repeat length	Dosage	Known collateral effects	Outcome
Buspirone	Friedman et al. [121]	Serotonin 5-HT1A receptor partial agonist	Case-study	15	1	NA	NA	12.5 mg/day	Dizziness, drowsiness and headache, nausea, diarrhea, increase in appetitte	**Mild effect**; improved gait and balance; clinical rating scale for ataxia was used
Tandospirone	Takei et al. [122]	Serotonin 5-HT1A receptor partial agonist	Case-study	8	1	51	NA	30 mg/day	Dizziness, drowsiness, headache, dry mouth, insomnia	**Strong effect**; ataxia, depression, insomnia, anorexia, and leg pain were improved; ICARS and SDS scales were used
Tandospirone	Takei et al. [123]	Serotonin 5-HT1A receptor partial agonist	Open-labeled	7	10	50.6 ± 12	NA	30 mg/day 15 mg/day	Dizziness, drowsiness, headache, dry mouth, insomnia	**Strong effect**; ataxia, depression, insomnia, anorexia, and leg pain were improved; ICARS and SDS scales were used
Lamotrigine	Liu et al. [127]	Sodium channel blocking agent	Open-labeled	9	6	27	78 ± 2	25 mg twice a day	Blurred vision, changes in vision,clumsiness or unsteadiness, double vision, poor coordination, skin rash	**Positive effect**; OLST and TGI tests were performed and improved
Varenicline (Chantix)	Zesiewicz et al. [128]	Agonist of α4β2 sub-type of the nicotinic receptor	Doubled-blinded	8	20	50.6 ± 11	NA	1 mg twice a day	Abnormal dreams, change in taste, dry mouth, flatulence, headache, lack or loss of strength, nausea, sleeplessness, stomach pain, trouble sleeping, unusual tiredness or weakness	**Positive effect**; SARA scale, a timed 25-foot walk and 9-hole peg test, measurements of mood and anxiety, and adverse events

(continued)

Table 19.2 (continued)

Therapeutic molecule	REF	Target	Design	Treatment duration (weeks)	Number of patients	Mean age (years)	Mean repeat length	Dosage	Known collateral effects	Outcome
Lithium carbonate	Saute et al. [140]	Mood stabilizer (mode of action is still unknow)	Doubled-blinded	48	62	40 ± 9	75 ± 3	Weekly lithium doses were given until a target of 0.5–0.8 milliequivalents per liter (mEq/L)	Confusion, poor memory, or lack of awareness, fainting fast or slow heartbeat, frequent urination, increased thirst, irregular pulse, stiffness of the arms or legs, troubled breathing (especially during hard work or exercise), unusual tiredness or weakness, weight gain, intentional tremor	**No overall effect**; NESSCA (6) and SARA scale, 9-hole peg test, 8 m Walking Time, Click Test and PATA-rate, Composite Cerebellar Functional Score, Quality-of-Life Questionnaire, Beck Depression Inventory, Clinical Global Impression of Change
Valproic acid	Lei et al. [142]	Histone deacetylase inhibitor	Double-blinded	12	36	37 ± 6	76 ± 3	12 patients: 800 mg/day; 12 patients: 1200 mg/day	Infection, congenital anomalies, alopecia, thrombocytopenia, nausea, vomiting, abdominal pain, weakness, drowsiness, tremor, flu-like symptoms, dizziness, diarrhea, and anorexia	**Positive effect;** improvement in locomotor function given by the decrease in global SARA score which was more evident in the 1200 mg/day cohort

with Bactrim. It was also shown that the levels of biopterins and homovanillic acid were reduced in the cerebrospinal fluid (CSF) of MJD patients when compared with controls with other neurodegenerative diseases, and that the short-term treatment with Bactrim increased these levels [111]. In the same year, another double-blind clinical trial was performed using Bactrim in 8 additional patients. In this study, three parameters were evaluated: subjective performance, neurological examination and timed tests. The treatment with Bactrim again demonstrated an improvement on gait and coordination. The authors suggested that further clinical trials using Bactrim should be performed due to the promising results obtained with this small number of patients [112]. Indeed, in 2001, a third double-blind clinical trial using Bactrim was performed in 22 MJD patients. In this trial, and in contrast to previous observations, chronic treatment with Bactrim had no effect in the parameters evaluated, such as ataxia ranking scale, self-assessment score, posturography and computer assisted motor performance test of Schoppe. The visual system function and mental health were also evaluated, but no effect was observed with Bactrim treatment [113].

The progression of MJD usually confines the patients to a wheelchair and ultimately the patients will be bedridden. In this condition, and in contrast to cognitive preservation, the patients might suffer depressive symptoms. Furthermore, the serotoninergic system in the cerebellum seems to play a role in motor output, such as locomotion. Serotonergic system impairment in the cerebellum was demonstrated to induce cerebellar ataxia [114]. The selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, are commonly used in the treatment of depression and present few side-effects [115]. In fact, as discussed above, citalopram (a commonly used antidepressant) proved to ameliorate the phenotype and neuropathology of the CMVMJD135 mouse model of MJD, suggesting that serotonergic system modulation might have an important role in MJD counteracting pathogenesis. Indeed, and long before this preclinical evidence emerged, some clinical trials using antidepressants have been performed in MJD patients, however the trial design was often less than optimal for detection of an effect. Monte et al. performed an open-label trial in 13 molecularly confirmed MJD patients, and saw that after 6 weeks of treatment, fluoxetine had no overall effect on motor abilities measured by functional scales and had no beneficial effect on the other neuropsychological tests [116]. Again, the outcome of the study may have been compromised by the small number of patients and particularly by the short duration of the study.

The use of 5-HT1A agonists has been controversial for the treatment of cerebellar ataxia, but several reports have suggested the efficacy of these agonists for the treatment of MJD [117–120]. Indeed, Friedman and collaborators have shown mild effects of buspirone in one MJD patient [121]. Later, Takei et al., reported the positive effects of tandospirone, another 5-HT1A agonist, in one MJD patient, that showed improvement in ataxia, depression, insomnia, anorexia and leg pain [122]. These positive effects led the authors to pursue a larger clinical trial using 10 MJD patients. In this trial, the patients started tandospirone treatment at an initial dose of 15 mg/kg (as the previous case study) that was further increased to 30 mg/kg for

7 weeks. The patients were examined using the international cooperative ataxia ranking scale (ICARS), the total length traveled (TLT) by stabolimetry test and the self-rating depression scale (SDS). All these parameters were alleviated with tandospirone treatment. Interestingly, all the symptoms were aggravated after a transient stop of tandospirone, and improved when the therapy was restarted [123]. These results suggested that 5-HT1A agonists could be effective in MJD, although more studies need to be performed to confirm these assumptions. Interestingly, it was suggested that the effects of 5-HT1A agonists might be potentiated by the concomitant use of SSRI's (e.g. citalopram) and *vice versa* [124, 125], which could be an interesting approach considering the results of these human trials and the promising data resultant of the study showing the beneficial effects of citalopram (but also of 5-HT receptor agonists) in MJD animal models [101]

The involvement of excessive *N-methyl-d-aspartate* (NMDA)-mediated signaling in the mechanism of neuronal inclusion formation has been proposed [126]. It was recently shown that L-glutamate-induced excitation of iPSC cells of MJD patients leads to Ca^{2+}-dependent proteolysis of ATXN3 followed by the formation of insoluble aggregates. The formation of those aggregates was also dependent on Na^{+} and K^{+} channels as well as on voltage-gated Ca^{2+} channels [103]. These very intriguing observations could provide a link between excitotoxicity and ATXN3 aggregation. A pilot study was performed in 6 MJD patients using Lamotrigine (25 mg twice a day during 9 weeks), a commonly used antiepileptic drug acting as a sodium channel-blocking agent that might be related to the reduction of NMDA-induced toxicity. In this trial, the patients were evaluated in the one leg standing test (OLST) and tandem gait index (TGI). Both OLST and TGI were improved during Lamotrigine treatment, comparing the values obtained with the normal values for Chinese population. Furthermore, and given these positive results, the authors cultured lymphoblastoid cells of one MJD patient and treated those cells with Lamotrigine. Mutant, but not normal ataxin-3, was reduced with Lamotrigine at concentrations within the therapeutic range in humans. The mechanism underlying the reduction in mutant ataxin-3 levels was not investigated in this work and this effect was not confirmed in the trial subjects [127].

Recently, Zesiewicz and collaborators carried out a short-term clinical trial in 20 MJD patients using Varenicline (Chantix, 1 mg twice a day for 9 weeks) [128]. Chantix (partial agonist of the $\alpha 4\beta 2$ neuronal nicotinic acetylcholine receptors) is used for smoking cessation. The rationale for this study was the fact that, although the major components of the cholinergic system seem to be spared in MJD, which may be reflected by the absence of dementia in MJD patients, the midbrain cholinergic pars compacta of the pedunculopontine nucleus suffers cell loss during disease progression [11], contributing for example to REM sleep disturbances, hence targeting the cholinergic neurotransmission could be a good approach. Chantix was also shown to be beneficial in SCA patients in previous case studies [129, 130]. In this trial, patients were evaluated at baseline and at the end of the treatment (after 8 weeks) primarily using the Scale for the Assessment and Rating of Ataxia (SARA scale). Secondary measurements consisted of a timed 25-foot walk, a 9-hole peg test, Beck depression inventory (BDI), Beck anxiety inventory

(BAI), clinical global impression (CGI), patient global impression (PGI) and the Short-Form 36 (SF36) to evaluate daily living. Chantix was able to significantly improve some subscores of the SARA scale, such as gait and rapid alternating movements. Also, the timed 25-foot walk was ameliorated by Chantix treatment, as well as the BDI score. The BDI score improved in both groups (Chantix and placebo) probably because the patients that were enrolled in the trial became hopeful regarding new treatment possibilities. A problem concerning this study was a high rate of dropout in the placebo group (4 out of 10 patients), interpreted as probably reflecting the difficulty of patients to reach the academic center. Regarding adverse events, it is possible to observe that Chantix caused, to a higher extent, gastrointestinal effects when compared to placebo. The mechanism by which Chantix improves ataxic symptoms was not evaluated in this study or elsewhere [131]. No follow up studies with larger groups of patients have been undertaken after this first promising result.

More recently, Saute and colleagues conducted a phase II clinical trial in 62 MJD patients using Lithium Carbonate. Lithium is commonly used to treat bipolar disorder, and is also used adjunctively with mood stabilizers and antidepressants to enhance, prolong and facilitate treatment response and remission of mood disorders [132, 133]. Lithium treatment was shown to have beneficial effects in several models of different neurodegenerative diseases [134–138], by the inhibition of glycogen synthase kinase-3β (GSK-3β) and autophagy activation. Importantly, however, irreversible cerebellar toxicity, leading to ataxia, nystagmus and dysarthria has also been observed due to lithium intoxication (reviewed in [139]). In this long-term clinical trial, Lithium (at therapeutic dosages of 0.5–0.8 mEq/L) was well tolerated by patients. After 48 weeks of follow-up, patients treated with Lithium did not show significant differences in disease progression, given by the results by Neurological Examination Score for the Assessment of Spinocerebellar Ataxia (NESSCA) and SARA scale. Nevertheless, the authors were able to observe that Lithium-treated MJD patients had a slower progression concerning the PATA test (word speed) and the Click test (finger-pointing coordination) as well as in the SCAFI (spinocerebellar ataxia functional index) and CCFS (composite cerebellar functional score), when compared to patients receiving placebo [133]. They suggested that larger clinical trials should be performed in order to understand the value of Lithium in the treatment of MJD.

The vast literature regarding transcription deregulation involvement in polyQ pathogenesis, led some researchers to conduct a clinical trial in MJD patients using Valproic Acid (VPA). VPA is commonly used as an anticonvulsant drug in the treatment of bipolar disorder. It has several known functions, including the increase in GABA neurotransmission, inhibition of voltage-gated sodium channels, T-type sodium channels and HDAC. In the preclinical trial field, the literature is controversial, since it was shown to be neuroprotective in a *Drosophila* MJD model [141], but showing limited therapeutic effects in a transgenic mouse model of the disease [79], as discussed above. Nevertheless, a clinical trial was recently performed in

MJD patients using VPA. In this study, Lei and collaborators used two different study designs. In the first, a randomized, open-label, dose-escalation study was performed to evaluate safety of VPA administration. In this first part of the study, it was possible to observe that VPA was safe in all the dosages tested (400, 600 and 800 mg/twice a day). In the second approach, 36 MJD patients were enrolled and randomly allocated to placebo, 800 and 1200 mg/day VPA dosing. After 12 weeks of treatment, the patients were evaluated using the SARA scale, and it was possible to observe a decrease in the total SARA score in both VPA dosages, indicating a significant improvement of the patients' motor coordination [142].

There are many concerns regarding the clinical trials performed to date in MJD: (i) the small cohorts of patients, which might be difficult to overcome due to the fact that this is a rare disorder and also the collaboration of patients might represent a problem; (ii) the clinical heterogeneity of the patients; (iii) the short-term observation of the patients, that contrasts with the slow progression of the disease (except for the Lithium Carbonate trial, which had a duration of 48 weeks); (iv) the outcomes used for ataxia measurement, which might be difficult to analyze due to the multisystem involvement in this disease; (v) the design of the studies, as randomized double-blinded trials with quantifiable ataxia scales and non-ataxia measurements should be used, which was not often the case, and (vi) the lack of useful biomarkers. Despite the existence of several scales to measure ataxia (reviewed in [143]), other non-ataxia scores should be applied to MJD patients since these patients also present non-ataxia symptoms, such as pyramidal and extrapyramidal signs, as well as peripheral findings [144].

19.5 Concluding Remarks

The search for disease-modifying therapeutic approaches for most neurodegenerative diseases has not been very productive to date; in the specific case of MJD, an important link between preclinical and clinical studies is still lacking. It is important to pursue well-designed clinical trials based on robust preclinical studies. Certainly, efforts are being made to perform good preclinical trials, and the scientific community is nowadays conducting better clinical studies with promising results for MJD. Other, non-pharmacological, disease-modifying therapeutic strategies may also be very promising.

Despite being rare diseases, MJD and other SCAs affect a large number of people worldwide. Given our current efficacy measures, large clinical trials, involving multiple centers and of long duration, are necessary which, in turn, implies high costs. Pharmaceutical companies are increasingly aware of the relevance of studying diseases of well-defined etiology, such as MJD, and their contribution could help to speed this process in a significant manner.

References

1. Paulson H (2012) Machado-Joseph disease/spinocerebellar ataxia type 3. Handb Clin Neurol 103:437–449
2. Bettencourt C, Lima M (2011) Machado-Joseph disease: from first descriptions to new perspectives. Orphanet J Rare Dis 6:35
3. Matos CA, de Macedo-Ribeiro S, Carvalho AL (2011) Polyglutamine diseases: the special case of ataxin-3 and Machado-Joseph disease. Prog Neurobiol 95(1):26–48
4. Schols L, Bauer P, Schmidt T, Schulte T, Riess O (2004) Autosomal dominant cerebellar ataxias: clinical features, genetics, and pathogenesis. Lancet Neurol 3(5):291–304
5. Switonski PM, Szlachcic WJ, Gabka A, Krzyzosiak WJ, Figiel M (2012) Mouse models of polyglutamine diseases in therapeutic approaches: review and data table. Part II. Mol Neurobiol 46(2):430–466
6. Bauer PO, Nukina N (2009) The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. J Neurochem 110(6):1737–1765
7. Saute JAM, Jardim LB (2015) Machado Joseph disease: clinical and genetic aspects, and current treatment. Expert Opin Orphan Drugs 3(5):517–535
8. Coutinho P, Sequeiros J (1981) Clinical, genetic and pathological aspects of Machado-Joseph disease. J Genet Hum 29(3):203–209
9. Riess O, Rub U, Pastore A, Bauer P, Schols L (2008) SCA3: neurological features, pathogenesis and animal models. Cerebellum 7(2):125–137
10. Rosenberg RN (1992) Machado-Joseph disease: an autosomal dominant motor system degeneration. Mov Disord 7(3):193–203
11. Rub U, Brunt ER, Deller T (2008) New insights into the pathoanatomy of spinocerebellar ataxia type 3 (Machado-Joseph disease). Curr Opin Neurol 21(2):111–116
12. Franca MC Jr, D'Abreu A, Nucci A, Lopes-Cendes I (2008) Muscle excitability abnormalities in Machado-Joseph disease. Arch Neurol 65(4):525–529
13. Friedman JH, Amick MM (2008) Fatigue and daytime somnolence in Machado Joseph disease (spinocerebellar ataxia type 3). Mov Disord 23(9):1323–1324
14. Schols L, Haan J, Riess O, Amoiridis G, Przuntek H (1998) Sleep disturbance in spinocerebellar ataxias: is the SCA3 mutation a cause of restless legs syndrome? Neurology 51(6):1603–1607
15. Kawai Y, Takeda A, Abe Y, Washimi Y, Tanaka F, Sobue G (2004) Cognitive impairments in Machado-Joseph disease. Arch Neurol 61(11):1757–1760
16. Silva UC, Marques W Jr, Lourenco CM, Hallak JE, Osorio FL (2015) Psychiatric disorders, spinocerebellar ataxia type 3 and CAG expansion. J Neurol 262(7):1777–1779
17. Paulson HL, Perez MK, Trottier Y, et al (1997) Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. Neuron 19(2):333–344
18. Schmidt T, Landwehrmeyer GB, Schmitt I, et al (1998) An isoform of ataxin-3 accumulates in the nucleus of neuronal cells in affected brain regions of SCA3 patients. Brain Pathol 8 (4):669–679
19. Rub U, Seidel K, Ozerden I, et al (2007) Consistent affection of the central somatosensory system in spinocerebellar ataxia type 2 and type 3 and its significance for clinical symptoms and rehabilitative therapy. Brain Res Rev 53(2):235–249
20. Yamada M, Tan CF, Inenaga C, Tsuji S, Takahashi H (2004) Sharing of polyglutamine localization by the neuronal nucleus and cytoplasm in CAG-repeat diseases. Neuropathol Appl Neurobiol 30(6):665–675
21. Seidel K, den Dunnen WF, Schultz C, et al (2010) Axonal inclusions in spinocerebellar ataxia type 3. Acta Neuropathol 120(4):449–460
22. Coutinho P, Andrade C (1978) Autosomal dominant system degeneration in Portuguese families of the Azores Islands. A new genetic disorder involving cerebellar, pyramidal, extrapyramidal and spinal cord motor functions. Neurology 28(7):703–709

23. Lima L, Coutinho P (1980) Clinical criteria for diagnosis of Machado-Joseph disease: report of a non-Azoren Portuguese family. Neurology 30(3):319–322
24. Paulson H (1993). Spinocerebellar ataxia type 3. In: Pagon RA, Adam MP, Ardinger HH et al (eds) GeneReviews(R). Seattle, WA
25. Maciel P, Costa MC, Ferro A, et al (2001) Improvement in the molecular diagnosis of Machado-Joseph disease. Arch Neurol 58(11):1821–1827
26. Rodrigues CS, de Oliveira VZ, Camargo G, et al (2012) Presymptomatic testing for neurogenetic diseases in Brazil: assessing who seeks and who follows through with testing. J genet couns 21(1):101–112
27. Miyai I, Ito M, Hattori N, et al (2012) Cerebellar ataxia rehabilitation trial in degenerative cerebellar diseases. Neurorehabil Neural Repair 26(5):515–522
28. Svensson M, Lexell J, Deierborg T (2015) Effects of physical exercise on neuroinflammation, neuroplasticity, neurodegeneration, and behavior: what we can learn from animal models in clinical settings. Neurorehabil Neural Repair 29(6):577–589
29. Silva RC, Saute JA, Silva AC, Coutinho AC, Saraiva-Pereira ML, Jardim LB (2010) Occupational therapy in spinocerebellar ataxia type 3: an open-label trial. Braz J Med Biol Res = Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica [et al] 43(6):537–542
30. Cecchin CR, Pires AP, Rieder CR, et al (2007) Depressive symptoms in Machado-Joseph disease (SCA3) patients and their relatives. Commun Genet 10(1):19–26
31. Nandagopal R, Moorthy SG (2004) Dramatic levodopa responsiveness of dystonia in a sporadic case of spinocerebellar ataxia type 3. Postgrad Med J 80(944):363–365
32. Thanvi B, Lo N, Robinson T (2007) Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J 83(980):384–388
33. D'Abreu A, Franca MC Jr, Paulson HL, Lopes-Cendes I (2010) Caring for Machado-Joseph disease: current understanding and how to help patients. Parkinsonism Relat Disord 16(1): 2–7
34. Szlachcic WJ, Switonski PM, Kurkowiak M, Wiatr K, Figiel M (2015) Mouse polyQ database: a new online resource for research using mouse models of neurodegenerative diseases. Mol Brain 8(1):69
35. Colomer Gould VF (2012) Mouse models of spinocerebellar ataxia type 3 (Machado-Joseph disease). Neurotherapeutics 9(2):285–296
36. Whitesell L, Bagatell R, Falsey R (2003) The stress response: implications for the clinical development of hsp90 inhibitors. Curr Cancer Drug Targets 3(5):349–358
37. Bagatell R, Paine-Murrieta GD, Taylor CW, et al (2000) Induction of a heat shock factor 1-dependent stress response alters the cytotoxic activity of hsp90-binding agents. Clin Cancer Res 6(8):3312–3318
38. Fujikake N, Nagai Y, Popiel HA, Okamoto Y, Yamaguchi M, Toda T (2008) Heat shock transcription factor 1-activating compounds suppress polyglutamine-induced neurodegeneration through induction of multiple molecular chaperones. J Biol Chem 283(38):26188–26197
39. Tokui K, Adachi H, Waza M, et al (2009) 17-DMAG ameliorates polyglutamine-mediated motor neuron degeneration through well-preserved proteasome function in an SBMA model mouse. Hum Mol Genet 18(5):898–910
40. Waza M, Adachi H, Katsuno M, et al (2005) 17-AAG, an Hsp90 inhibitor, ameliorates polyglutamine-mediated motor neuron degeneration. Nat Med 11(10):1088–1095
41. Thomas M, Harrell JM, Morishima Y, Peng HM, Pratt WB, Lieberman AP (2006) Pharmacologic and genetic inhibition of hsp90-dependent trafficking reduces aggregation and promotes degradation of the expanded glutamine androgen receptor without stress protein induction. Hum Mol Genet 15(11):1876–1883
42. Silva-Fernandes A, Duarte-Silva S, Neves-Carvalho A, et al (2014) Chronic treatment with 17-DMAG improves balance and coordination in a new mouse model of Machado-Joseph disease. Neurother: j Am Soc Exp NeuroTher 11(2):433–449

43. Jhaveri K, Taldone T, Modi S, Chiosis G (2012) Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers. Biochim Biophys Acta 1823(3): 742–755
44. del Cano-Espinel M, Acebes JR, Sanchez D, Ganfornina MD (2015) Lazarillo-related Lipocalins confer long-term protection against type I Spinocerebellar ataxia degeneration contributing to optimize selective autophagy. Mol Neurodegeneration 10:11
45. Menzies FM, Garcia-Arencibia M, Imarisio S, et al (2015) Calpain inhibition mediates autophagy-dependent protection against polyglutamine toxicity. Cell Death Differ 22(3): 433–444
46. Tsunemi T, Ashe TD, Morrison BE, et al (2012) PGC-1alpha rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB function. Sci Transl Med 4(142):142–197
47. Roscic A, Baldo B, Crochemore C, Marcellin D, Paganetti P (2011) Induction of autophagy with catalytic mTOR inhibitors reduces huntingtin aggregates in a neuronal cell model. J Neurochem 119(2):398–407
48. Menzies FM, Huebener J, Renna M, Bonin M, Riess O, Rubinsztein DC (2010) Autophagy induction reduces mutant ataxin-3 levels and toxicity in a mouse model of spinocerebellar ataxia type 3. Brain : J neurol 133(Pt 1):93–104
49. Sarkar S, Krishna G, Imarisio S, Saiki S, O'Kane CJ, Rubinsztein DC (2008) A rational mechanism for combination treatment of Huntington's disease using lithium and rapamycin. Hum Mol Genet 17(2):170–178
50. Williams A, Jahreiss L, Sarkar S, et al (2006) Aggregate-prone proteins are cleared from the cytosol by autophagy: therapeutic implications. Curr Top Dev Biol 76:89–101
51. Ravikumar B, Duden R, Rubinsztein DC (2002) Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum Mol Genet 11(9): 1107–1117
52. Ravikumar B, Vacher C, Berger Z, et al (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet 36(6):585–595
53. Xilouri M, Stefanis L (2015) Chaperone mediated autophagy to the rescue: a new-fangled target for the treatment of neurodegenerative diseases. Mol Cell Neurosci 66(Pt A):29–36
54. Frake RA, Ricketts T, Menzies FM, Rubinsztein DC (2015) Autophagy and neurodegeneration. J Clin Invest 125(1):65–74
55. Harris H, Rubinsztein DC (2012) Control of autophagy as a therapy for neurodegenerative disease. Nat Rev Neurol 8(2):108–117
56. Cortes CJ, La Spada AR (2015) Autophagy in polyglutamine disease: imposing order on disorder or contributing to the chaos? Mol Cell Neurosci 66(Pt A): 53–61
57. Bichelmeier U, Schmidt T, Hubener J, et al (2007) Nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3: in vivo evidence. J Neurosci: Official J Soc Neurosci 27(28):7418–7428
58. Nascimento-Ferreira I, Santos-Ferreira T, Sousa-Ferreira L, et al (2011) Overexpression of the autophagic beclin-1 protein clears mutant ataxin-3 and alleviates Machado-Joseph disease. Brain: J neurol 134(Pt 5):1400–1415
59. Duarte-Silva S, Neves-Carvalho A, Soares-Cunha C, et al (2014) Lithium chloride therapy fails to improve motor function in a transgenic mouse model of Machado-Joseph disease. Cerebellum 13(6):713–727
60. Saute JA, de Castilhos RM, Monte TL, et al (2014) A randomized, phase 2 clinical trial of lithium carbonate in Machado-Joseph disease. Mov Disord 29(4):568–573
61. Duarte-Silva S, Silva-Fernandes A, Neves-Carvalho A, Soares-Cunha C, Teixeira-Castro A, Maciel P (2016) Combined therapy with m-TOR-dependent and -independent autophagy inducers causes neurotoxicity in a mouse model of Machado-Joseph disease. Neuroscience 313:162–173

62. Chou AH, Yeh TH, Ouyang P, Chen YL, Chen SY, Wang HL (2008) Polyglutamine-expanded ataxin-3 causes cerebellar dysfunction of SCA3 transgenic mice by inducing transcriptional dysregulation. Neurobiol Dis 31(1):89–101
63. Wang HL, Hu SH, Chou AH, Wang SS, Weng YH, Yeh TH (2013) H1152 promotes the degradation of polyglutamine-expanded ataxin-3 or ataxin-7 independently of its ROCK-inhibiting effect and ameliorates mutant ataxin-3-induced neurodegeneration in the SCA3 transgenic mouse. Neuropharmacology 70:1–11
64. Mueller BK, Mack H, Teusch N (2005) Rho kinase, a promising drug target for neurological disorders. Nat Rev Drug Discov 4(5):387–398
65. Li M, Huang Y, Ma AA, Lin E, Diamond MI (2009) Y-27632 improves rotarod performance and reduces huntingtin levels in R6/2 mice. Neurobiol Dis 36(3):413–420
66. Zhao J, Zhou D, Guo J, et al (2006) Effect of fasudil hydrochloride, a protein kinase inhibitor, on cerebral vasospasm and delayed cerebral ischemic symptoms after aneurysmal subarachnoid hemorrhage. Neurol Med Chir 46(9):421–428
67. Ahmed LA, Darwish HA, Abdelsalam RM, Amin HA (2015) Role of Rho kinase inhibition in the protective effect of Fasudil and Simvastatin against 3-Nitropropionic acid-induced striatal neurodegeneration and mitochondrial dysfunction in Rats. Mol Neurobiol
68. Lin Y, Hubert L Jr, Wilson JH (2009) Transcription destabilizes triplet repeats. Mol Carcinog 48(4):350–361
69. Okazawa H (2003) Polyglutamine diseases: a transcription disorder? Cell Mol Life Sci 60 (7):1427–1439
70. McCampbell A, Taylor JP, Taye AA, et al (2000) CREB-binding protein sequestration by expanded polyglutamine. Hum Mol Genet 9(14):2197–2202
71. Taylor JP, Taye AA, Campbell C, Kazemi-Esfarjani P, Fischbeck KH, Min KT (2003) Aberrant histone acetylation, altered transcription, and retinal degeneration in a Drosophila model of polyglutamine disease are rescued by CREB-binding protein. Genes Dev 17(12): 1463–1468
72. Li F, Macfarlan T, Pittman RN, Chakravarti D (2002) Ataxin-3 is a histone-binding protein with two independent transcriptional corepressor activities. J Biol Chem 277(47):45004–45012
73. Li SH, Cheng AL, Zhou H, et al (2002) Interaction of Huntington disease protein with transcriptional activator Sp1. Mol Cell Biol 22(5):1277–1287
74. Cohen-Carmon D, Meshorer E (2012) Polyglutamine (polyQ) disorders: the chromatin connection. Nucleus 3(5):433–441
75. Chai Y, Wu L, Griffin JD, Paulson HL (2001) The role of protein composition in specifying nuclear inclusion formation in polyglutamine disease. J Biol Chem 276(48):44889–44897
76. Dunah AW, Jeong H, Griffin A, et al (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science 296(5576):2238–2243
77. Grewal SI, Moazed D (2003) Heterochromatin and epigenetic control of gene expression. Science 301(5634):798–802
78. Chou AH, Chen SY, Yeh TH, Weng YH, Wang HL (2011) HDAC inhibitor sodium butyrate reverses transcriptional downregulation and ameliorates ataxic symptoms in a transgenic mouse model of SCA3. Neurobiol Dis 41(2):481–488
79. Esteves S, Duarte-Silva S, Naia L, et al (2015) Limited effect of chronic valproic acid treatment in a mouse model of Machado-Joseph disease. PloS one 10(10):e0141610
80. Tang TS, Tu H, Chan EY, et al (2003) Huntingtin and huntingtin-associated protein 1 influence neuronal calcium signaling mediated by inositol-(1,4,5) triphosphate receptor type 1. Neuron 39(2):227–239
81. Tang TS, Slow E, Lupu V, et al (2005) Disturbed Ca^{2+} signaling and apoptosis of medium spiny neurons in Huntington's disease. Proc Natl Acad Sci USA 102(7):2602–2607
82. Wu J, Tang T, Bezprozvanny I (2006) Evaluation of clinically relevant glutamate pathway inhibitors in in vitro model of Huntington's disease. Neurosci Lett 407(3):219–223

83. Cemal CK, Carroll CJ, Lawrence L, et al (2002) YAC transgenic mice carrying pathological alleles of the MJD1 locus exhibit a mild and slowly progressive cerebellar deficit. Hum mol genet 11(9):1075–1094
84. Krause T, Gerbershagen MU, Fiege M, Weisshorn R, Wappler F (2004) Dantrolene–a review of its pharmacology, therapeutic use and new developments. Anaesthesia 59(4):364–373
85. Chen X, Tang TS, Tu H, Nelson O, Pook M, Hammer R, Nukina N, Bezprozvanny I (2008) Deranged calcium signaling and neurodegeneration in spinocerebellar ataxia type 3. J Neurosci 48:12713–12724
86. Stutzmann GE (2005) Calcium dysregulation, IP3 signaling, and Alzheimer's disease. Neuroscientist: Rev J Bringing Neurobiol, Neurol Psychiatry 11(2):110–115
87. Andrews TC, Weeks RA, Turjanski N, et al (1999) Huntington's disease progression. PET and clinical observations. Brain: J Neurol 122(Pt 12):2353-2363
88. Li H, Li SH, Yu ZX, Shelbourne P, Li XJ (2001) Huntingtin aggregate-associated axonal degeneration is an early pathological event in Huntington's disease mice. J Neurosci: Official J Soc Neurosci 21(21):8473–8481
89. Goncalves N, Simoes AT, Cunha RA, de Almeida LP (2013) Caffeine and adenosine A(2A) receptor inactivation decrease striatal neuropathology in a lentiviral-based model of Machado-Joseph disease. Ann Neurol 73(5):655–666
90. Chen JF, Sonsalla PK, Pedata F, et al (2007) Adenosine A2A receptors and brain injury: broad spectrum of neuroprotection, multifaceted actions and "fine tuning" modulation. Prog Neurobiol 83(5):310–331
91. Gomes CV, Kaster MP, Tome AR, Agostinho PM, Cunha RA (2011) Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. Biochim Biophys Acta 1808 (5):1380–1399
92. Popoli P, Blum D, Martire A, Ledent C, Ceruti S, Abbracchio MP (2007) Functions, dysfunctions and possible therapeutic relevance of adenosine A2A receptors in Huntington's disease. Prog Neurobiol 81(5–6):331–348
93. Rivera-Oliver M, Diaz-Rios M (2014) Using caffeine and other adenosine receptor antagonists and agonists as therapeutic tools against neurodegenerative diseases: a review. Life Sci 101(1–2):1–9
94. Cunha-Santos J, Duarte-Neves J, Carmona V, Guarente L, Pereira de Almeida L, Cavadas C (2016) Caloric restriction blocks neuropathology and motor deficits in Machado-Joseph disease mouse models through SIRT1 pathway. Nat Commun 7:11445
95. Martin A, Tegla CA, Cudrici CD, et al (2015) Role of SIRT1 in autoimmune demyelination and neurodegeneration. Immunol Res 61(3):187–197
96. Jeong H, Cohen DE, Cui L, et al (2012) Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. Nat Med 18 (1):159–165
97. Jiang M, Wang J, Fu J, et al (2012) Neuroprotective role of Sirt1 in mammalian models of Huntington's disease through activation of multiple Sirt1 targets. Nat Med 18(1):153–158
98. Montie HL, Pestell RG, Merry DE (2011) SIRT1 modulates aggregation and toxicity through deacetylation of the androgen receptor in cell models of SBMA. J Neurosci 31 (48):17425–17436
99. Torashima T, Koyama C, Iizuka A, et al (2008) Lentivector-mediated rescue from cerebellar ataxia in a mouse model of spinocerebellar ataxia. EMBO reports 9(4):393–399
100. Walle T (2011) Bioavailability of resveratrol. Ann NY Acad Sci 1215:9–15
101. Teixeira-Castro A, Jalles A, Esteves S, et al (2015) Serotonergic signalling suppresses ataxin 3 aggregation and neurotoxicity in animal models of Machado-Joseph disease. Brain: J Neurol 138(Pt 11):3221–3237
102. Tatum MC, Ooi FK, Chikka MR, et al (2015) Neuronal serotonin release triggers the heat shock response in *C. elegans* in the absence of temperature increase. Curr Biol 25(2): 163–174
103. Koch P, Breuer P, Peitz M, et al (2011) Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. Nature 480(7378):543–546

104. Konno A, Shuvaev AN, Miyake N, Miyake K, et al (2014) Mutant ataxin-3 with an abnormally expanded polyglutamine chain disrupts dendritic development and metabotropic glutamate receptor signaling in mouse cerebellar Purkinje cells. Cerebellum 13(1):29–41
105. Ristori G, Romano S, Visconti A, et al (2010) Riluzole in cerebellar ataxia: a randomized, double-blind, placebo-controlled pilot trial. Neurology 74(10):839–845
106. Romano S, Coarelli G, Marcotulli C, et al (2015) Riluzole in patients with hereditary cerebellar ataxia: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 14 (10):985–991
107. Hockly E, Tse J, Barker AL, et al (2006) Evaluation of the benzothiazole aggregation inhibitors riluzole and PGL-135 as therapeutics for Huntington's disease. Neurobiol Dis 21 (1):228–236
108. Ortega Z, Diaz-Hernandez M, Maynard CJ, Hernandez F, Dantuma NP, Lucas JJ (2010) Acute polyglutamine expression in inducible mouse model unravels ubiquitin/proteasome system impairment and permanent recovery attributable to aggregate formation. J Neurosci 30(10):3675–3688
109. Boy J, Schmidt T, Wolburg H, et al (2009) Reversibility of symptoms in a conditional mouse model of spinocerebellar ataxia type 3. Hum mol genet 18(22):4282–4295
110. Schmidt J, Schmidt T, Golla M, et al (2016) In vivo assessment of riluzole as a potential therapeutic drug for spinocerebellar ataxia type 3. J Neurochem 138(1):150–162
111. Sakai T, Matsuishi T, Yamada S, Komori H, Iwashita H (1995) Sulfamethoxazole-trimethoprim double-blind, placebo-controlled, crossover trial in Machado-Joseph disease: sulfamethoxazole-trimethoprim increases cerebrospinal fluid level of biopterin. J Neural Transm Gen Sect 102(2):159–172
112. Correia M, Coutinho P, Silva MC, Guimaraes J, Amado J, Matos E (1995) Evaluation of the effect of sulphametoxazole and trimethoprim in patients with Machado-Joseph disease. Rev Neurol 23(121):632–634
113. Schulte T, Mattern R, Berger K, et al (2001) Double-blind crossover trial of trimethoprim-sulfamethoxazole in spinocerebellar ataxia type 3/Machado-Joseph disease. Arch Neurol 58(9):1451–1457
114. Chan-Palay V (1977) Indoleamine neurons and their processes in the normal rat brain and in chronic diet-induced thiamine deficiency demonstrated by uptake of 3H-serotonin. J Comp Neurol 176(4):467–493
115. Wenthur CJ, Bennett MR, Lindsley CW (2014) Classics in chemical neuroscience: fluoxetine (Prozac). ACS Chem Neurosci 5(1):14–23
116. Monte TL, Rieder CR, Tort AB, et al (2003) Use of fluoxetine for treatment of Machado-Joseph disease: an open-label study. Acta Neurol Scand 107(3):207–210
117. Lou JS, Goldfarb L, McShane L, Gatev P, Hallett M (1995) Use of buspirone for treatment of cerebellar ataxia. An open-label study. Arch Neurol 52(10):982–988
118. Trouillas P, Brudon F, Adeleine P (1988) Improvement of cerebellar ataxia with levorotatory form of 5-hydroxytryptophan. A double-blind study with quantified data processing. Arch Neurol 45(11):1217–1222
119. Trouillas P, Xie J, Adeleine P (1997) Buspirone, a serotonergic 5-HT1A agonist, is active in cerebellar ataxia. A new fact in favor of the serotonergic theory of ataxia. Prog Brain Res 114:589–599
120. Trouillas P, Xie J, Adeleine P, et al (1997) Buspirone, a 5-hydroxytryptamine1A agonist, is active in cerebellar ataxia. Results of a double-blind drug placebo study in patients with cerebellar cortical atrophy. Arch Neurol 54(6):749–752
121. Friedman JH (1997) Machado-Joseph disease/spinocerebellar ataxia 3 responsive to buspirone. Mov Disord: Official J Mov Disord Soc 12(4):613–614
122. Takei A, Honma S, Kawashima A, et al (2002) Beneficial effects of tandospirone on ataxia of a patient with Machado-Joseph disease. Psychiatry Clin Neurosci 56(2):181–185
123. Takei A, Fukazawa T, Hamada T, et al (2004) Effects of tandospirone on "5-HT1A receptor-associated symptoms" in patients with Machado-Josephe disease: an open-label study. Clin Neuropharmacol 27(1):9–13

124. Lanfumey L, Hamon M (2004) 5-HT1 receptors. Curr Drug Targets CNS Neurol Disord 3 (1):1–10
125. Blier P, Ward NM (2003) Is there a role for 5-HT1A agonists in the treatment of depression? Biol Psychiatry 53(3):193–203
126. Ientile R, Caccamo D, Macaione V, Torre V, Macaione S (2002) NMDA-evoked excitotoxicity increases tissue transglutaminase in cerebellar granule cells. Neuroscience 115 (3):723–729
127. Liu CS, Hsu HM, Cheng WL, Hsieh M (2005) Clinical and molecular events in patients with Machado-Joseph disease under lamotrigine therapy. Acta Neurol Scand 111(6):385–390
128. Zesiewicz TA, Sullivan KL (2008) Treatment of ataxia and imbalance with varenicline (Chantix): report of 2 patients with spinocerebellar ataxia (types 3 and 14). Clin Neuropharmacol 31(6):363–365
129. Zesiewicz TA, Sullivan KL, Freeman A, Juncos JL (2009) Treatment of imbalance with varenicline Chantix®: report of a patient with fragile X tremor/ataxia syndrome. Acta Neurol Scand 119(2):135–138
130. Zesiewicz TA, Sullivan KL, Gooch CL, Lynch DR (2009) Subjective improvement in proprioception in 2 patients with atypical Friedreich ataxia treated with varenicline (Chantix). J Clin Neuromuscul Dis 10(4):191–193
131. Zesiewicz TA, Greenstein PE, Sullivan KL, Wecker L, Miller A, Jahan I, Chen R, Perlman SL (2012) A randomized trial of varenicline (Chantix) for the treatment of spinocerebellar ataxia type 3. Neurology 78(8):545–550
132. Goodwin FK (2003) Rationale for using lithium in combination with other mood stabilizers in the management of bipolar disorder. J Clin Psychiatry 64(Suppl 5):18–24
133. Lin D, Mok H, Yatham LN (2006) Polytherapy in bipolar disorder. CNS Drugs 20(1):29–42
134. Wood NI, Morton AJ (2003) Chronic lithium chloride treatment has variable effects on motor behaviour and survival of mice transgenic for the Huntington's disease mutation. Brain res bull 61(4):375–383
135. Feng HL, Leng Y, Ma CH, Zhang J, Ren M, Chuang DM (2008) Combined lithium and valproate treatment delays disease onset, reduces neurological deficits and prolongs survival in an amyotrophic lateral sclerosis mouse model. Neuroscience 155(3):567–572
136. Jia DD, Zhang L, Chen Z, et al (2013) Lithium chloride alleviates neurodegeneration partly by inhibiting activity of GSK3beta in a SCA3 Drosophila model. Cerebellum 12(6):892–901
137. Fornai F, Longone P, Cafaro L, et al (2008) Lithium delays progression of amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 105(6):2052–2057
138. Watase K, Gatchel JR, Sun Y, et al (2007) Lithium therapy improves neurological function and hippocampal dendritic arborization in a spinocerebellar ataxia type 1 mouse model. PLoS Med 4(5):e182
139. van Gaalen J, Kerstens FG, Maas RP, Harmark L, van de Warrenburg BP (2014) Drug-induced cerebellar ataxia: a systematic review. CNS Drugs 28(12):1139–1153
140. Saute JA, Castilhos RM, Monte TL, et al (2014) A randomized, phase 2 clinical trial of lithium carbonate in Machado-Joseph disease. Mov Disord: Official J Mov Disord Soc
141. Yi J, Zhang L, Tang B, et al (2013) Sodium valproate alleviates neurodegeneration in SCA3/MJD via suppressing apoptosis and rescuing the hypoacetylation levels of histone H3 and H4. PLoS ONE 8(1):e54792
142. Lei LF, Yang GP, Wang JL, et al (2016) Safety and efficacy of valproic acid treatment in SCA3/MJD patients. Parkinsonism Relat Disord 26:55–61
143. Saute JA, Donis KC, Serrano-Munuera C, et al (2012) Ataxia rating scales—psychometric profiles, natural history and their application in clinical trials. Cerebellum 11(2):488–504
144. Kieling C, Morales Saute JA, Jardim LB (2007) When ataxia is not just ataxia. Nat Clin Pract Neurol 3(5):E2

Chapter 20
Gene Therapies for Polyglutamine Diseases

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Abstract Polyglutamine diseases are hereditary degenerative disorders of the nervous system that have remained, to this date, untreatable. Promisingly, investigation into their molecular etiology and the development of increasingly perfected tools have contributed to the design of novel strategies with therapeutic potential. Encouraging studies have explored gene therapy as a means to counteract cell demise and loss in this context. The current chapter addresses the two main focuses of research in the area: the characteristics of the systems used to deliver nucleic acids to cells and the molecular and cellular actions of the therapeutic agents. Vectors used in gene therapy have to satisfyingly reach the tissues and cell types of interest, while eliciting the lowest toxicity possible. Both viral and non-viral systems have been developed for the delivery of nucleic acids to the central nervous system, each with its respective advantages and shortcomings. Since each polyglutamine disease is caused by mutation of a single gene, many gene therapy strategies have tried to halt degeneration by silencing the corresponding protein products, usually recurring to RNA interference. The potential of small interfering RNAs, short hairpin RNAs and microRNAs has been investigated. Overexpression of protective genes has also been evaluated as a means of decreasing mutant protein toxicity and operate beneficial alterations. Recent gene editing tools promise yet

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other ways of interfering with the disease-causing genes, at the most upstream points possible. Results obtained in both cell and animal models encourage further delving into this type of therapeutic strategies and support the future use of gene therapy in the treatment of polyglutamine diseases.

Keywords Polyglutamine diseases · Gene therapy · Gene silencing
RNA interference · Viral and non-viral vectors

20.1 Introduction

Cumulative advances in the fields of molecular cell biology, genetics and biotechnology allow promising new views on the treatment of genetic diseases. The rational design of strategies that have a basis on the molecular events underlying pathogenesis offers exciting, and oftentimes unprecedented, prospects. This is particularly true when the disease cause is a known, discrete, genetic factor, as is the case of polyglutamine (polyQ) diseases. Many of these promising approaches fall under the category of what is commonly designated as gene therapy, since they rely on the delivery of external genetic material (nucleic acids) to cells [1, 2].

The therapeutic action of the genetic material used in gene therapy (or of the molecular products it originates) can be mediated by diverse cell mechanisms. Primarily, these molecular tools may be used to regulate the expression of a gene and thus be employed in directly silencing pathogenic proteins. This strategy is certainly appealing in the case of polyQ diseases, where toxicity is regarded as resulting from a toxic gain-of-function on the part of the expanded protein [3]. On the other hand, it is also possible to supplement cells with beneficial genes. While simple administration of a non-expanded form of a polyQ disease-causing gene may not be applicable to this type of disorders, overexpression of proteins that counter disease-related events or increase degradation of the pathogenic protein presents promising prospects. Finally, novel gene editing tools offer the encouraging possibility of utterly deleting, replacing or even repairing CAG-expanded genes, countering disease at the most upstream point of the pathogenic cascade [4].

Implementation of gene therapy strategies in the treatment of human patients requires not only that their molecular action is proven efficacious, but also that the nucleic acids are properly and safely delivered to the target tissue [1, 5]. In the case of polyQ disease patients, the central nervous system (CNS) is the main region that undergoes cell dysfunction and loss [6] and is thus regarded as the main target for intervention. Vectors including the therapeutic nucleic acid sequences may be delivered directly at a particular brain region such as the striatum or the cerebellum, or be systemically administered into the bloodstream. However, in this last case, the vectors have to be engineered so as to be able to cross the blood-brain barrier (BBB) and also to modify only the target cell populations.

Though everyday usage of gene therapy-based procedures in the treatment of polyQ disease patients is still not a reality, their clinical potential is supported by an

increasing number of studies in cellular and animal models. The current chapter reviews the available literature describing the advances on gene therapy strategies for polyQ diseases.

20.2 Vectors for Gene Therapy

There are two types of delivery systems that have been explored in their capacity to mediate the transfer of exogenous nucleic acid tools—DNA, messenger RNA (mRNA), small interfering RNA (siRNA), short hairpin RNA (shRNA), microRNA (miRNA) or antisense oligonucleotides (ASOs)—into a target tissue: viral and non-viral vectors. Regardless of its origin, a successful vector must be able to deliver the gene therapy molecules to the right cells, at the same time avoiding immune response and toxicity.

20.2.1 Non-viral Vectors

When compared to viral vectors, even though they are often considered less efficient, non-viral vectors are recognized to be safer and are typically easier and less expensive to produce, facilitating industrial scale-up [7]. Furthermore, they involve minimal safety risks, as their transient nature of expression allows an interruption of administration. Additionally, while viral vectors are limited to the delivery of nucleic acids, non-viral vectors can lodge a wider variety of cargoes.

20.2.1.1 Naked Nucleic Acids

Nucleic acids may be administered in an unconjugated, naked, form. However, since naked nucleic acids per se do not have the ability to cross the BBB and access the CNS, they have to be administered intracranially—either by direct intraparenchymal, intracerebroventricular or intrathecal injection. While using naked nucleic acids requires administration by an invasive route, promising results have been obtained with animal models of Huntington's disease (HD). An alleviation of the neurological phenotype was achieved upon direct administration of siRNAs or ASOs in the CNS parenchyma or in the cerebrospinal fluid [8–12].

20.2.1.2 Nanoparticles

Despite the success of the aforementioned studies, the use of nanoparticles presents additional prospects, in that it is expected to overcome the major challenges faced by the non-invasive delivery of nucleic acids to the CNS. Nanoparticles are

expected to be able to promote: (a) biocompatibility and biodistribution; (b) BBB crossing; (c) targeted delivery; and (d) appropriate intracellular delivery.

The strategies discussed below rely on the formation of complexes between nucleic acids and other molecules. The vectors, based on biological molecules such as peptides and/or synthetic materials such as polymers or lipids, are considered nanoparticles as their diameter falls in the nanometer range.

Neuronal-Targeting Peptides

Conjugation of gene therapeutics with ligands that promote receptor-mediated transcytosis is one promising way of delivering gene therapy tools across the BBB. These ligands can be used per se or be further associated with synthetic materials.

One of the peptides employed is derived from the rabies virus glycoprotein (RVG). This 29 amino acid peptide has the ability to bind to the nicotinic acetylcholine receptors present on brain capillary endothelial cells (BCECs) and on neurons and thus promote endothelial permeation and neuronal uptake. When conjugated with nine-arginine residues (RGV-9r), the peptide was able to associate with siRNAs, cross the BBB and promote reduction of a target gene within the brain [13]. Its ability to condense plasmid DNA and induce exogenous gene expression in the brain after intravenous (IV) administration has been demonstrated ([14]; Fig. 20.1a).

Several other ligands and cell-penetrating peptides (CPPs) like Penetratin and TAT are known to elicit increased brain uptake after IV administration [15, 16], but their applicability for the systemic delivery of gene therapeutics is yet to be explored.

Polymer-Derived Nanoparticles

Polyethylenimine (PEI) derivatives and dendrimers are among the most common polymeric nanoparticles used in the delivery of nucleic acids to the brain, after systemic administration.

Branched PEI is able to efficiently condense nucleic acids, deliver gene therapies intracellularly (escaping endosomes), and it is associated with a low immune response [17, 18]. PEI is non-biodegradable and, consequently, dose-dependent cytotoxicity may arise from its use. Nonetheless, this limitation has been overcome through the introduction of a disulfide linkage (SSPEI) that allows the polymer to be degraded [19]. Importantly, SSPEI covalently linked to the RVG peptide enables brain delivery of plasmid DNA [19] and miRNAs [20] after IV administration (Fig. 20.1b). Another derivative from branched PEI—poly(mannitol-co-PEI)—is also efficient in delivering siRNAs to the brain when associated to the RVG peptide and promote gene silencing [21, 22].

Dendrimers are highly organized polymers composed by a central core, branches emanating from the core and terminal functional groups [23]. Associating targeting

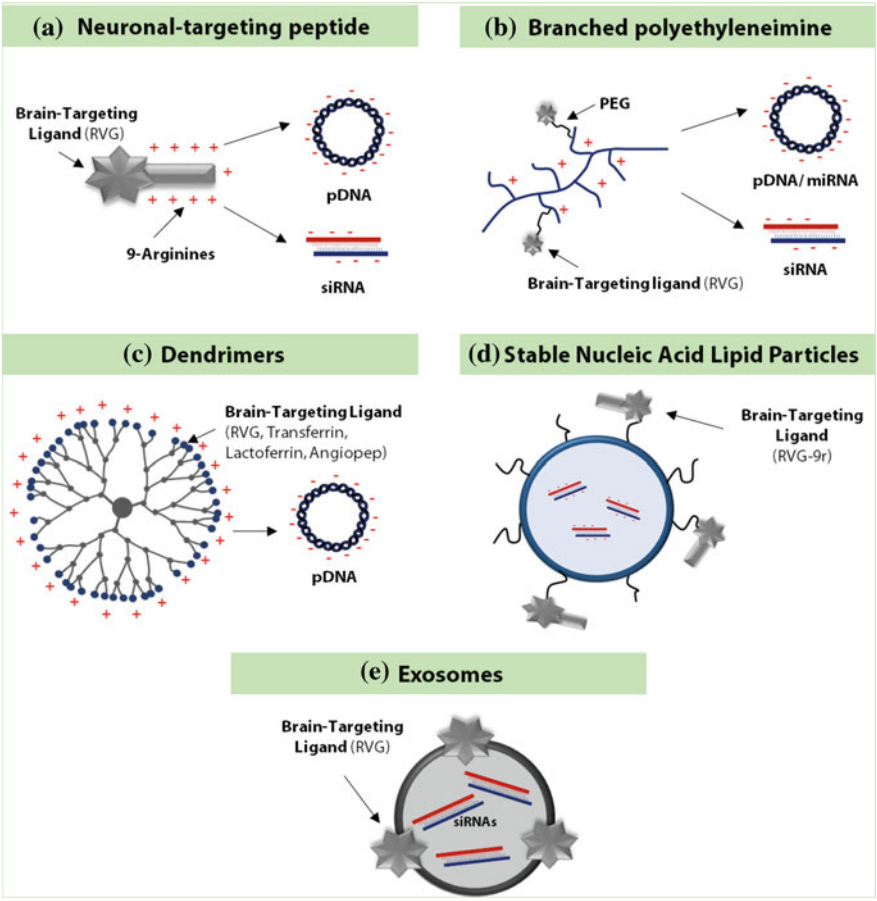

Fig. 20.1 Non-viral systems for systemic delivery of gene therapies to the brain. **a** A short peptide derived from the rabies virus glycoprotein with 29 amino acids (RVG) and 9-arginin residues (RVG-9r), delivers gene therapies across the blood-brain barrier. **b** Branched polyethyleneimine linked to the RVG peptide enables brain delivery of siRNAs, pDNA and miRNAs. **c** Polyamidoamine (PAMAM) dendrimers coupled to specific ligands (RVG, transferrin, lactoferrin or angiopep) enable the delivery of plasmid DNA into the brain. **d** Brain-targeted stable nucleic acid lipid particles (SNALPs) increase the accumulation of siRNAs in the brain after intravenous administration. **e** RVG-targeted exosomes deliver siRNAs to the brain after non-invasive administration

ligands to the periphery masks the high number of amino groups and thereby circumvents their cytotoxicity [24]. Polyamidoamine (PAMAM) dendrimers are the most widely used due to their ability to efficiently encapsulate plasmid DNA. Coupling different brain-targeting ligands such as RVG [25], transferrin [26], lactoferrin [27] or angiopep [28] to the surface enabled the delivery of plasmid DNA to the brain, resulting in widespread expression of exogenous genes upon IV administration (Fig. 20.1c).

Lipid-Based Nanoparticles

Liposomes, the best characterized lipid nanoparticles, have important characteristics for the delivery of nucleic acids: (a) they possess an aqueous core that can encapsulate hydrophilic molecules such as nucleic acids, protecting them from degradation; (b) their surface is easy to modify, allowing conjugation of brain-targeting ligands; (c) they have a good biocompatibility profile; and (d) they elicit low toxicity in comparison with inorganic nanoparticles and viral vectors [29].

The first effective studies that enabled the delivery of gene therapeutics to the brain after IV administration used liposomes conjugated with monoclonal antibodies directed against the transferrin receptor and/or the insulin receptor present in BCECs of the BBB, resulting in receptor-mediated transcytosis of the nanoparticles [30]. The polyethylene glycol-grafted immunoliposomes (PILs) that were employed displayed therapeutic potential in animal models of brain cancer [31–33] and Parkinson's disease [34]. Chronic IV administration of PILs encapsulating plasmid DNA was reported to cause no detectable toxic side effects in rats [35].

Stable nucleic acid lipid particles (SNALPs) are in clinical development for different conditions and represent a promising non-viral approach for the in vivo delivery of siRNAs [36–43]. It has recently been shown that IV administration of brain-targeted SNALPs using the RVG-9r peptide (Fig. 20.1d) is able to alleviate the neurological phenotype of Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD/SCA3) mouse models [36].

Exosomes

Exosomes are small (40–120 nm in diameter) extracellular vesicles that are released by different cell types and mediate intercellular communication [44]. They can transport bioactive lipids, mRNA, miRNAs, proteins and DNA between cells, presenting, beyond their essential role in physiologic activities and pathology, an enormous potential as delivery vehicles for therapeutics [44, 45]. Several features make exosomes attractive over other delivery systems, including: (a) their non-immunogenic properties; (b) their high rates of cellular uptake; (c) the versatility to engineer brain-targeting ligands onto their surface; (d) their ability to simultaneously transport diverse biomolecules; and (e) their inherent anti-inflammatory and tissue regenerative properties, when derived from an adequate cell source.

Systemic administration of exosomes is able to promote delivery of siRNAs into the mouse brain and mediate gene knockdown when their surface is engineered to express the brain-targeting RVG peptide [46, 47]; Fig. 20.1e). Notably, IV administration of siRNA-RVG exosomes was reported to cause no change in cytokine levels, confirming their non-immunogenic profile [46].

20.2.2 Viral Vectors

Although non-viral vectors are relatively safer and their production easier and less expensive, virus-based vectors are, in general, much more efficient at gene delivery in vivo [7]. Over millions of years, many viruses have evolved naturally to efficiently deliver their genomes into mammalian cells [48]. So far, several viral vector systems have been explored and modified for biotechnological purposes, including adenovirus, herpes simplex virus, retrovirus, lentivirus and adeno-associated virus (see Table 20.1). After years of research, the efficacy and safety of viral vectors has been definitely demonstrated in recent clinical trials of inherited diseases of the blood, as well as of the immune and nervous systems [49, 50].

This section focuses on the groups of viral vectors commonly used in preclinical and clinical trials for neurological disorders, which thus are of particular relevance for polyQ disorders.

20.2.2.1 Adenoviral Vectors

Adenoviruses (Ad) are medium-sized (70–100 nm), non-enveloped, double-stranded DNA viruses that belong to the *Adenoviridae* family. Ads infect both dividing and non-dividing cells, remaining in an extrachromosomal form [51].

There are two main categories of adenoviral vectors. The first-generation of Ads had a packaging capacity of 8 kb and was able to produce high levels of the intended transgene. Upon intraparenchymal injection to the brain they transduce a large number of cells, including neurons, astrocytes and microglial cells [52]. However, Ads, particularly the vectors derived from the human type 5 (HAdV-C5), are highly immunogenic, especially in the presence of active peripheral immunization, leading to long-term inflammation and subsequent clearance of transduced cells [53]. The second generation of adenoviral vectors, named high-capacity "gutless" adenoviral vectors (HC-Ad), contain no viral genes, have an increased insert size and have been demonstrated to produce long-term gene expression in the brain, with low toxicity [54]. Nevertheless, it has been difficult to generate high titers of HC-Ads and it has not been possible to eliminate the innate immune response against the viral shell of the commonly used human type 5 virus (HAdV-C5). High doses of HC-Ads can also result in severe inflammation and death, in humans [55]. For these reasons, no clinical trials for neurological disorders have been recently performed using adenoviral vectors, with the exception of brain tumors (Table 20.1). In the last years, non-human Ads, particularly canine adenovirus type 2 (CAV-2) have been engineered and tested with promising reports of low immunogenicity, preferential transduction of neurons, widespread distribution via retrograde axonal transport, and duration of expression in the mammalian brain (see [56] for review).

Table 20.1 Viral vectors for CNS gene therapy, respective clinical trials and preclinical studies for polyQ disorders

Vector	Features of the wild type form	General characteristics	Clinical trials for neurological disorders	In vivo gene transfer applications in animal models of polyQ disorders
Adenovirus	**Viral family**: *Adenoviridae* **Envelope:** No **Diameter**: 70–100 nm **Genome:** dsDNA	**Insert size and titer**: 1st generation: 8 kb (high titer); HC-Ad vectors: 36 kb (low titer) **Infection range**: Mitotic and post-mitotic **Host genome integration**: No **Immunogenicity**: 1st generation: highly immunogenic; HC-Ad vectors: high doses are toxic	**Phase I in vivo gene therapy clinical trials for glioblastoma multiforme/ other gliomas** (NCT00805376, NCT01956734, NCT02197169): direct administration of oncolytic Ad vectors into the brain tumors	**Huntington's disease**: Gutless Ad vectors [57]
Herpes simplex virus—1	**Viral family**: *Herpesviridae* **Envelope**: Yes **Diameter**: 120–300 nm **Genome**: dsDNA	**Insert size and titer**: recombinant vector: 30 kb (high titer); amplicon vector: 150 kb (low titer) **Infection range**: Mitotic and post-mitotic **Host genome integration**: No **Immunogenicity**: Recombinant vectors: highly immunogenic; amplicon vectors: low immunogenicity	**Phase I in vivo gene therapy clinical trials for glioblastoma multiforme/ other gliomas** (NCT02031965, NCT00028158, NCT00157703): direct administration of oncolytic HSV-1 vectors into the brain tumors	No studies

(continued)

Table 20.1 (continued)

Vector	Features of the wild type form	General characteristics	Clinical trials for neurological disorders	In vivo gene transfer applications in animal models of polyQ disorders
Lentivirus	**Viral family**: *Retroviridae* **Envelope**: Yes **Diameter**: 100 nm **Genome**: ssRNA	**Insert size and titer**: 8.5 kb (high titer) **Infection range**: Mitotic and post-mitotic **Host genome integration**: Yes **Immunogenicity**: low	– **Phase I/II ex vivo gene therapy clinical trials for: metachromatic leukodystrophy** [74], **Wiskott-Aldrich syndrome** [73], **X-linked adrenoleukodystrophy** [75] – **Phase I/II in vivo gene therapy clinical trials for Parkinson's disease** (NCT00627588, NCT01856439)	**Huntington's disease**: [76–80] **SCA3**: [81–88, 154]
AAV	**Viral family:** *Parvoviridae* **Envelope:** No **Diameter:** 25 nm **Genome:** ssDNA	**Insert size and titer**: ss: 4.5 kb; sc: 2.25 kb **Infection range**: Mitotic and post-mitotic **Host genome integration:** Mostly non-integrating **Immunogenicity**: very mild	– **Phase I and Phase II in vivo gene therapy clinical trials for: Pompe disease** (NCT02240407, NCT00976352)**, aromatic L-amino acid decarboxylase deficiency** (NCT01395641)**, spinal muscular atrophy type 1** (NCT02122952)**, Canavan disease** [95], **Batten** (NCT00151216, NCT01414985, NCT01161576)**, Sanfilippo syndrome B** (SRCTN19853672), **Alzheimer's disease** (NCT00087789, NCT00876863), and **Parkinson's disease** (NCT00195143, NCT00643890, NCT01301573, NCT00252850, NCT00400634, NCT00985517, NCT01621581, NCT00229736, NCT02418598,	**Huntington's disease**: **AAV1** [104, 109, 115, 119, 120]; **Chimeric AAV1/2** [107, 112]; **AAV2** [105, 108, 111, 114, 116, 117]; **AAV5** [113, 118]; **AAV9**: Intrajugular injection [106], Intraparenchymal injection [110] **SCA1**: **AAV1** [121, 122, 124–127]; **AAV5** [123] **SCA3**: **AAV1** [128, 131]; **Chimeric AAV1/2** [129]; **AAV2** [132]; **AAV9** [130] **SCA6**: **AAV9: i**ntracerebroventricular: [133] **SCA7**: **AAV1** [134, 135]

(continued)

Table 20.1 (continued)

Vector	Features of the wild type form	General characteristics	Clinical trials for neurological disorders	In vivo gene transfer applications in animal models of polyQ disorders
			NCT01973543). **Eye disorders: age-related macular degeneration** (NCT01494805, NCT01024998), **Leber congenital amaurosis** (NCT00749957, NCT00999609, NCT01208389, NCT00516477, NCT00643747), **choroideremia** (NCT01461213, NCT02553135, NCT02407678, NCT02077361, NCT02341807, NCT02671539), **X-linked retinoschisis** (NCT02317887, NCT02416622), **Leber's hereditary optic neuropathy** (NCT02161380, NCT02652780, NCT02652767), and **achromatopsia** (NCT02599922).	

SCA1, 3, 6 and 7 spinocerebellar ataxia type 1, 3, 6 and 7; AAV Adeno-Associated Virus

In the context of polyQ diseases, one preclinical study conducted in two distinct HD mouse models showed that HC-Ads are effective in delivering shRNAs into the brain upon intrastriatal injection [57].

20.2.2.2 Herpes Simplex Viral Vectors

Herpes simplex viruses are enveloped, double-stranded DNA viruses, ranging from 120 to 300 nm in diameter. They belong to the *Herpesviridae* family and infect a wide variety of cells, including mitotic and post-mitotic cells, with a low rate of genomic integration [58].

In particular, herpes simplex virus type 1 (HSV1) is highly neurotropic and capable of retrograde and anterograde transport, which makes it a natural candidate for gene delivery to the CNS [59, 60]. The packaging capacity of HSV1-derived vectors is also large, ranging from 30 kb, in "recombinant" HSV1 vectors, to 150 kb in the HSV1 "amplicon" vectors [61, 62]. However, both types of HSV1-derived vectors present important limitations: "recombinant" vectors induce a strong immune response and "amplicon" vectors, though not containing any viral gene, generate limited viral titers that are insufficient for clinical trials [62]. Similarly to Ads, CNS clinical trials with HSV1-derived vectors have been limited to the treatment of brain tumors.

20.2.2.3 Lentiviral and Adeno-Associated Viral Vectors

Lentiviral and adeno-associated viral vectors are described as the vectors of choice for in vivo CNS gene therapy, in particular for non-oncologic treatments [63]. Although they have a lower gene packaging capacity than Ads and HSV1-derived vectors, both are easier to produce and safer, mediating efficient and long-lasting gene expression in the brain, without toxicity.

Lentiviral and Retroviral Vectors

Lentiviral (LV) and retroviral vectors based on the Moloney murine leukemia virus (MLV) are derived from retroviruses (*Retroviridae* family). Both are enveloped single-stranded RNA vectors with a size of approximately 100 nm and a packing capacity of 8.5 kb [64]. They integrate DNA into the host cell genome; however, while MLV vectors only infect dividing cells, LVs also infect non-dividing cells. As a consequence, the use of MLV vectors has been limited to ex vivo gene therapy [65, 66], whereas LVs can be used in both ex vivo and in vivo approaches (Table 20.1). MLVs are being used to introduce a corrected gene in hematopoietic stem cells, within the first approved ex vivo gene therapy product (Strimvelis), to treat patients with severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID).

LVs have been shown to be particularly efficient for the in vivo transduction of the CNS, providing a stable and long-lasting transduction of neurons with minimal immune response [67–69]. Moreover, LV vectors can be pseudotyped with the vesicular stomatitis virus G envelope protein to increase host-cell range and structural stability, which allows higher viral titers [68, 70]. Some safety issues have been raised, since LVs integrate the host cell genome and derive from the human immunodeficiency virus type 1. However, different strategies have been developed to increase safety, such as the use of non-replicating and self-inactivating vectors [71] or the use of non-integrating variants [72].

LVs have already been used in four CNS clinical trials for neurological disorders. Three involved the successful transplantation of autologous cells, previously transduced ex vivo using LVs carrying functional genes [73–75]; another one was based on the in vivo administration of a LV-mediated gene therapy for Parkinson's disease (Clinical trial nº NCT00627588/NCT01856439).

In the context of polyQ disorders, several in vivo LV-based gene therapies have been successfully tested for HD [76–80] and MJD/SCA3 [81–88].

Adeno-Associated Viral Vectors

Adeno-associated viruses (AAVs) are non-enveloped double-stranded DNA viruses of approximately 25 nm that belong to the *Parvoviridae* family [89]. Like LVs, AAV-derived vectors have the ability to infect both mitotic and post-mitotic cells, but tend to persist in an episomal form and rarely integrate the host genome [90].

AAVs have important advantages over all other viral vectors regarding human CNS gene therapy. Firstly, they are safer, due to the low immunogenicity and non-pathogenic nature; AAVs naturally infect humans but are not associated with disease [91]. They are able to transduce neurons in a stable and long-lasting way without the activation of the immune system [92–94]. In the human brain, for instance, AAV-mediated transgene expression is stable and safe at least for 10 years [95]. Secondly, they induce higher transgene expression and present larger volumetric spread following direct intraparenchymal injection [69, 96]. This is very useful, especially when the target disease affects several brain regions, as is the case of polyQ disorders. Finally, large-scale AAV production protocols result in larger titers and higher purity levels.

Twelve natural AAV serotypes and hundreds of variants have been isolated or engineered so far, each presenting distinct cell and tissue tropisms, as well as different transduction efficiencies [97, 98]. For example, vectors based on AAV serotypes 2, 7, 8 and 9 preferentially infect neurons, while AAV4 transduces ependymal cells and AAV5 targets both neurons and astrocytes [99, 100]. Interestingly, AAV9 shows the highest distribution throughout the brain upon intraparenchymal injection and is also able to cross the BBB, transducing both neurons and astrocytes when systemically administered to rodents or non-human primates [99, 101, 102]. For this reason, AAV9 has become the best candidate vector for non-invasive CNS delivery.

Even though the packaging capacity of AAVs is limited to 5 kb [103], the aforementioned advantageous features make these vectors the most frequently used viral system for in vivo gene transfer to the CNS, including in clinical trials (Table 20.1). At least 20 trials for neurological disorders have been completed or initiated (reviewed in [50]), including trials for neurodegenerative diseases: Alzheimer's and Parkinson's disease.

In the context of polyQ disorders, recombinant AAV vectors have been successfully used as gene delivery vectors in preclinical studies for HD [104–120], spinocerebellar ataxia type 1 (SCA1) [121–127], MJD/SCA3 [128–132], SCA6 [133] and SCA7 [134, 135].

20.3 Gene Therapy Strategies for Polyglutamine Diseases

20.3.1 RNA Interference-Based Approaches

PolyQ diseases are caused by genetic mutations on the codifying region of particular genes. It is thus logical to assume that inhibiting expression of the genes containing the pathogenic mutation—a CAG trinucleotide repeat expansion—hinders disease onset or progression. PolyQ diseases in fact constitute ideal targets for gene silencing strategies, since they are monogenic [136].

RNA interference (RNAi) is an evolutionarily-conserved sequence-specific post-transcriptional gene silencing mechanism that uses non-coding double-stranded RNA (dsRNA) sequences to inhibit mRNA translation [137]. The mechanism of RNAi can be triggered by distinct RNA molecules, including physiological inhibitory microRNAs (miRNAs) or exogenously-introduced small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs) or artificial miRNAs.

Endogenous RNAi mainly occurs via miRNAs encoded in the genome. MiRNAs are small non-coding RNA molecules composed of 18–22 nucleotides that, in a cellular context, act as post-transcriptional regulators of gene expression. MiRNAs are processed in the nucleus from primary-miRNA (pri-miRNAs) transcripts which contain hairpin structures [5]. Upon expression, pri-miRNAs are cleaved by the nuclear endoribonuclease Drosha, originating precursor-miRNAs (pre-miRNAs) [138], which are then translocated to the cytoplasm by exportin-5, where they are further processed by Dicer (another endoribonuclease), giving rise to the mature miRNA duplex [139]. The miRNA antisense strand is then loaded onto the RNA-induced silencing complex (RISC) and recognizes its target mRNA transcript, while the sense strand undergoes degradation [140]. The antisense strand binds mostly to the 3′-untranslated region (3′UTR) of target mRNA molecules, but in some cases also to the coding sequences or the 5′-untranslated region (5′UTR); if there is a perfect complementarity between the miRNA and its target, the transcript is cleaved, consequently inhibiting translation [141, 142]. When it is not the case, imperfect binding leads to translation repression [143].

The RNAi pathway can be artificially harnessed to induce specific gene knockdown. This is commonly achieved using synthetic siRNAs, for instance in combination with non-viral delivery systems, but also through shRNAs, commonly taking advantage of viral delivery systems [144, 145]. Synthetic siRNAs are dsRNA molecules of approximately 19–21 nucleotides with overhanging 3′ ends [137, 146]. They are designed to mimic mature miRNA duplexes entering the RNAi pathway upon Dicer processing. Once in the cytoplasm, the siRNA sense strand is degraded, whereas the antisense strand is loaded into RISC. Activated RISC searches for specific complementary mRNAs, targeting them for degradation [147, 148].

SiRNAs may also be incorporated in expression-based systems that codify the RNA molecules instead of directly delivering them. This is achieved through the inclusion of the sequences into stem-loop structures designed to mimic pri-miRNAs —giving rise to artificial miRNAs (discussed in Sect. 20.3.1.2.)—or pre-miRNAs —originating shRNAs [149]. Once in the cell, shRNAs are expressed within the nucleus and are translocated to the cytoplasm by exportin-5, whereas synthetic siRNAs circumvent this nuclear step. SiRNAs are transient and lasting gene target suppression may require repeated administration. On the other hand, shRNAs and artificial miRNAs typically introduced in cells with viral vectors are more long-standing and, once delivered, are able to permanently silence a target gene after one single administration [150].

20.3.1.1 Strategies Using Small Interfering or Short Hairpin RNAs

Over the last years, gene therapy strategies focused on dominantly-inherited neurodegenerative diseases have experimented great advances. Using RNAi-based approaches, researchers have successfully inhibited the expression of the causative proteins of polyQ diseases in cell and animal models, ameliorating neuropathological features and behavioral phenotypes [151].

The most straightforward approach to RNA-based gene silencing therapy is non-allele-specific silencing. It involves unselective silencing of both the wild-type (WT) and mutant alleles of a gene, admitting that the WT gene is either non-essential or functionally redundant [152]. On the other hand, allele-specific silencing needs to be taken in consideration in the cases where WT protein expression is essential for cellular function. In order to achieve this type of gene silencing, differences that allow distinction between the different transcripts of a gene have to be present in the targetable nucleotide sequence. These include single-nucleotide polymorphisms (SNPs) and differences in the CAG repeat length [153].

Non-allele-Specific Silencing Strategies for Polyglutamine Disorders

In 2004, a pioneering report was published focusing on the therapeutic potential of RNAi in a transgenic mouse model for SCA1. In this study, intracerebellar delivery

of AAV1 encoding shRNA targeting the human mutant *ATXN1* gene led to a significant knockdown of ataxin-1 and to a marked improvement in motor coordination, restoration of cerebellar morphology and reduction of ataxin-1 inclusions in Purkinje cells [127]. It was later shown that delivery of this shRNA cloned into an artificial miRNA expression vector to the deep cerebellar nuclei (DCN) is preferable when compared to the delivery to the cerebellar cortex, mainly due to the broader biodistribution provided by the retrograde trafficking of axons to the Purkinje cell soma [123–125].

Studies on MJD/SCA3 suggested that strategies involving non-allele-specific silencing may be safe and effective in this disease. Knockdown of endogenous *ATXN3* in MJD/SCA3 models did not exacerbate MJD pathology and, in fact, non-allele-specific viral-mediated silencing of *ATXN3* decreased expression of mutant *ATXN3* and substantially reduced signs of neuropathology and the number of ataxin-3 inclusions [154].

AAV-mediated delivery of shRNAs targeting human *HTT* led to the reduction of *HTT* transcript levels and huntingtin protein expression in HD mouse models brains, along with a decrease in the size and number of neuronal intranuclear inclusions. Importantly, motor and neuropathological HD manifestations were improved, halting disease progression [107, 113, 118]. A LV-expressed shRNA was also shown to reduce both human mutant *HTT* and the endogenous HTT gene expression in a rat model of HD, decreasing aggregate formation and preventing motor deficits. Though a disruption in several cellular pathways associated with huntingtin function was detected in WT mice when endogenous HTT was silenced, a partial knockdown of both mutant and WT transcripts seemed to be well-tolerated, with no apparent toxic effects [76].

Transient human mutant *HTT* silencing using siRNAs produced beneficial effects in HD model mice, regarding both motor dysfunction and inclusions formation, and prolonged mice longevity [9, 155]. Intrastriatal injection of hydrophobically-modified siRNA into the mouse brain silenced endogenous HTT mRNA with residual neuronal toxicity [156]. Later, the infusion of siRNA-loaded exosomes into the mouse striatum also resulted in a significant silencing of HTT mRNA [157].

Pre-clinical safety and efficacy of the partial reduction of endogenous HTT has also been evaluated in non-human primates. Sustained six-month partial suppression was well-tolerated in the rhesus monkey striatum [108]. The rapid onset and durability of infused radiolabeled siRNA-mediated gene suppression of HTT in rhesus putamen has also been validated [158, 159].

Allele-Specific Silencing Strategies for Polyglutamine Disorders

Many studies investigating the potential of gene silencing in the treatment of polyQ diseases have exploited the less technically-challenging, non-allele-specific, approach. Nonetheless, allele-specific approaches have also been described.

Allele-specific strategies for MJD/SCA3 have focused on targeting a SNP linked to the CAG repeat expansion occurring in specific patient groups. Specific targeting of the CAG expansion itself is challenging due to the polymorphism and ubiquitous presence of the repeat tract within the genome and the inability of the silencing effectors (<25 nucleotides) to discriminate between WT and expanded CAG tracts in ATXN3 [153]. Taking into consideration that 70% of mutant *ATXN3* gene alleles present a C/G SNP at position 987, immediately after the CAG repeat [160, 161], Li and colleagues have designed a siRNA that successfully reduced the expression of mutant *ATXN3* in cell cultures, with residual decrease in the WT gene [162].

It was then demonstrated that efficient and selective silencing of mutant *ATXN3* in rat and mouse models of MJD/SCA3 is possible employing locally-delivered LVs encoding shRNAs targeting the same SNP. Silencing was accompanied by a decrease in the severity of neuropathological deficits [81, 85]. The study in the rat model represented the first proof-of-principle for allele-specific silencing in the CNS. Nóbrega and colleagues later demonstrated the efficacy of gene silencing in rescuing the MJD/SCA3-associated motor behavior deficits and neuropathological features after disease onset in a severely-impaired transgenic mouse model of MJD [86]. IV administration of SNALPs encapsulating siRNAs targeting mutant *ATXN3* efficiently promoted silencing of the target gene, improving neuropathology and motor behavior readouts in lentiviral and transgenic mouse models of MJD/SCA3 [36].

Primary dermal fibroblasts from SCA7 patients were used to evaluate the efficiency of endogenous allele-specific silencing using siRNAs targeting a common SNP (rs3774729) within the causative gene, *ATXN7*. The treatment promoted selective knockdown of the mutant transcript and amelioration of a disease-relevant phenotype [163].

Although knockdown of WT *HTT* has been proven to be well tolerated in vivo [76, 104], huntingtin is known to play a pivotal functional role in the development and maintenance of the CNS, thus demanding a search for allele-specific silencing approaches for HD [109, 164]. Bilsen and colleagues have performed allele-specific silencing of the mutant *HTT* allele in HD patient-derived fibroblasts [165]. The engineered siRNA was able to selectively target the rs363125 SNP, in which the C-allele was found to be linked to *HTT* expansion. This strategy led to a decrease in mutant mRNA and protein levels, while WT levels were not substantially altered.

In a similar study, a reduction of mutant *HTT* expression was obtained with siRNAs directed to a polymorphic site present in patient fibroblasts [166]. This polymorphism corresponded to a Δ2642 deletion of one among four tandem GAG repeats in exon 58 that was found in 38% of mutant *HTT* alleles and 7% of WT ones [167]. Expression of the Δ2642-marked polyQ-expanded *HTT* was reduced without compromising the expression of its WT counterpart, with improvements in HD-related dysfunctions in donor fibroblasts [166].

Encouraging reports mentioned that as few as five allele-specific siRNAs, corresponding to three SNP sites, are sufficient to treat 75% of the United States and European HD patient population [168]. In order to specifically target the disease-related *HTT* allele, Drouet and colleagues have developed shRNAs directed

at four heterozygous SNPs in the *HTT* exons which are among the most common in the human population [77]. Lentiviral-mediated delivery of these shRNAs promoted an efficient and selective silencing of the mutant *HTT* transcript and prevented neuropathology readouts in HD model rat's striatum expressing mutant *HTT* containing the various SNPs. In transgenic BACHD mice, the mutant *HTT* allele was also silenced by the same strategy.

Finally, Becanovic and collaborators have demonstrated that targeting a non-coding SNP (rs13102260:G > A) in the *HTT* gene promoter that comprises a NF-κB binding site led to an effective reduction of NF-κB binding and reduced huntingtin protein expression in HD transgenic mouse striatum [169].

20.3.1.2 Strategies Using microRNAs

Substantial efforts have been made in order to build tools that control expression of disease-causing genes via regulation of miRNAs. Taking into account that an estimated one third of human genes are regulated by miRNAs and each miRNA can target many transcripts [170, 171], delivering one miRNA may not only silence disease-causing genes but also other genes that contribute to pathogenesis. MiRNAs can be overexpressed as artificial miRNAs [172] or as synthetic double-stranded miRNA mimics [173].

Artificial miRNAs targeting the genes causing HD, SCA1, MJD/SCA3 and SCA7 have been demonstrated to reduce both mRNA and protein levels of huntingtin, ataxin-1, ataxin-3 and ataxin-7, respectively, in cell line cultures and/or transgenic mouse models of disease (Table 20.2; [104, 106, 115, 120, 121, 123–125, 128, 131, 134, 135, 172]).

Administration of a miR-196a mimic, which reduced the mRNA and the protein levels of mutant huntingtin, also decreased huntingtin intranuclear aggregates in a cell and in a mouse model of HD and ameliorated the behavioral phenotype in the animal model, although while not acting on huntingtin directly [174].

In cellular models of SCA1, overexpression of miR-19, miR-101 and miR-130 resulted in a significant decrease on ataxin-1 levels [175, 176]. Delivery of a miR-25 mimic to MJD/SCA3 cell models reduced the levels of ataxin-3, apoptosis, ataxin-3 aggregate formation and increased cell viability [173]. Administration of a miR-124 mimic decreased the expression of lnc-SCA7 and ataxin-7 in N2a cells and in a SCA7 knock-in model [177].

In the case of spinal and bulbar muscular atrophy (SBMA), delivery of miR-196a decreased the mRNA and the protein levels of the androgen receptor (AR) in HEK 293 cells, in human fibroblasts and in SBMA transgenic mice, at the same time reducing CUGBP Elav-like family member 2 (CELF2) mRNA and protein levels. CELF2 siRNA treatment also resulted in similar effects on AR mRNA and protein levels in SBMA patients fibroblasts, suggesting that the interaction between miR-196a and AR is indirect through CELF2 [178].

Regarding SCA6, mir-3191-5p was identified as a miRNA that targets CACNA1A IRES inhibiting α1ACT translation. Moreover, viral delivery of

Table 20.2 MicroRNA-based approaches for polyglutamine disease treatment

PolyQ diseases	microRNAs	Target	Experimental systems	Delivery method	References
HD	Artificial microRNA	*HTT*	HEK 293 cells and HD transgenic mice	AAV-mediated transduction	[104, 106, 115, 120, 172]
	miR-196a mimic	*HTT*	HEK 293 cells, N2a cells, HD transgenic mouse and HD-iPSCs	Lentivirus-mediated transduction	[174]
SCA1	Artificial miRNA	*ATXN1*	C2C12 cells, SCA1 transgenic mouse and non-human primates	AAV-mediated transduction	[121, 123–125]
	miR-19, miR-101 and miR-130 mimic	*ATXN1*	HEK 293, HeLa, and MCF7 cells	Transfection	[175]
	miR-144 mimic	*ATXN1*	HEK 293 cells	Transfection	[176]
SCA3	Artificial miRNA	*ATXN3*	SCA3 transgenic mouse	AAV-mediated transduction	[128, 131, 282]
	miR-25 mimic	*ATXN3*	HEK 293 and SH-SY5Y cells	Transfection	[173]
	miR-9, miR-181a and miR-494 mimics	*ATXN3*	HEK 293 and LV-induced SCA3 mice	LV-mediated transduction	[179]
SCA6	miR-3191-5p	CACNA1A	AAV-induced SCA6 mice	AAV-mediated transduction	[133]
SCA7	Artificial miRNA	*ATXN7*	SCA7 transgenic mouse	AAV-mediated transduction	[134, 135]
	miR-124 mimic	Long non-coding RNA (lnc-SCA7) and ataxin-7	N2a cells	Transfection	[177]
SBMA	miR-196a mimic	Androgen receptor via CELF2	SBMA transgenic mice	AAV-mediated transduction	[178]

Abbreviations: *HD* Huntington's disease; *SCA1,3,6,7* spinocerebellar ataxia type 1, 3, 6 and 7; *SBMA* spinal and bulbar muscular atrophy; *HEK* human embryonic kidney; *iPSCs* induced pluripotent stem cells; *AAV* adeno-associated virus

mir-3191-5p reduced motor deficits and Purkinje cell degeneration in SCA6 mice [133].

More recently, the dysregulation of the endogenous miRNA pathway was identified as an important disease mechanism in MJD/SCA3. Accordingly, the reestablishment of mir-9, mir-181a and mir-494, miRNAs which were found to be downregulated in the context of the disease, resulted in a decrease of mutant ataxin-3 levels and associated neuropathology in vivo [179].

MiRNAs offer several advantages as tools for silencing disease genes involved in polyQ disorders, including: (a) their small sizes; (b) their known and conserved sequences; (c) the putative targeting of several related targets, downstream or upstream in the pathogenic cascade, with just one mRNA molecule. Still, the major concerns for miRNA use in clinical trials are related to their possible off-target effects, their safety and the decreased long-term efficiency of their action.

20.3.2 Antisense Oligonucleotide-Based Approaches

Antisense oligonucleotides (ASOs) are single-stranded DNA molecules that, upon binding to target mRNA molecules, alter their function through multiple mechanisms which can be harnessed in order to artificially modulate gene expression. In general, they have between 8 and 50 nucleotides in length and hybridize to the target mRNA through Watson-Crick base pairing [180].

The mechanisms by which ASOs interfere with RNA function (See Fig. 20.2) can be divided into two groups: RNAse H-dependent mRNA degradation and RNAse H-independent modulation. In the case of RNAse H-dependent mRNA degradation, the ASO is required to have at least a portion of nucleotides that are unmodified at the 2' position of the riboside [181, 182]. Upon recognition of the mRNA:DNA duplex, RNAse H cleaves the target mRNA while leaving the DNA molecule intact. However, completely 2'-modified ASOs are still used for a wide, RNAse H-independent, range of applications when mRNA degradation is not required. For example, ASOs can be used to modulate mRNA splicing events [183]. This is particularly useful for neurodegenerative diseases such as polyQ disorders, where exon skipping of mutated exons might prove beneficial [184]. Moreover, ASOs can modulate mRNA maturation by interfering with 5′cap formation and polyadenylation [185, 186]. Furthermore, ASOs can be designed to interfere with mRNA translation by blocking the attachment of ribosomal subunits to mRNA [187]. Recently, ASOs have been developed to inhibit miRNA function, thereby increasing the levels of the mRNAs they target [188].

Unmodified short oligonucleotides have very low stability, being degraded by intracellular endonucleases and exonucleases [189, 190]. Moreover, they possess weak binding affinities to their target. In order to overcome these limitations and improve the pharmaceutical properties of ASOs (increasing solubility, permeability to the BBB and cellular uptake, as well as reducing toxicity and immune-stimulatory properties) new chemically-modified types have been developed.

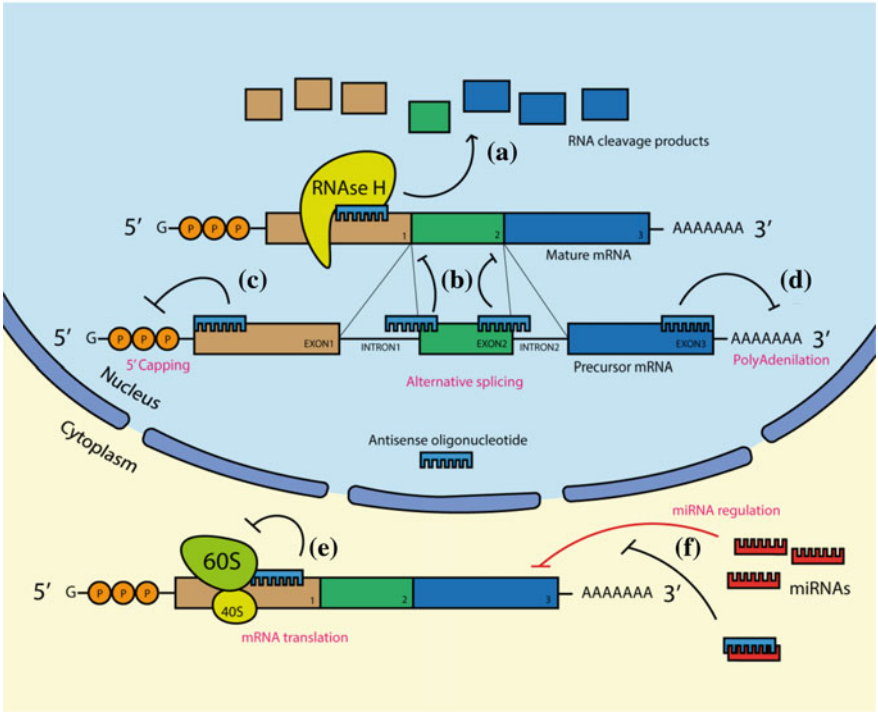

Fig. 20.2 Schematic representation of antisense oligonucleotides mechanisms of action. **a** After binding to their target RNA, ASOs can recruit RNAse-H leading to sequence-specific degradation of target mRNA. **b** Moreover, ASOs designed for splicing junctions can interfere with the endogenous splicing machinery controlling the inclusion or exclusion of interest exons. **c** ASOs can also interfere with 5′ cap formation and **d** with polyadenylation. **e** In the cytoplasm ASOs can block translational machinery reducing protein expression or **f** bind to target miRNAs blocking their action on target mRNAs

Many studies have evaluated different types of chemically-modified ASOs for the treatment of polyQ diseases. Among these, HD has been the one most frequently targeted by antisense therapy and many promising results were obtained in mouse models of the disorder (Table 20.3). Most of these preclinical studies used technology capable of mediating RNAse-H mRNA selective degradation of mutant huntingtin mRNA. In these cases, distinction between WT and expanded CAG mRNA is performed by targeting SNPs existing only in the mutant transcript. Based on these favorable studies, a phase 1/2 clinical trial for the treatment of HD that uses ASOs administered intrathecally is currently underway (NCT02519036).

Regarding the application of ASO technology to other polyQ diseases, reports have been more limited. Nevertheless, in vivo studies in mice have shown promising results in SCA2 and SBMA after intracerebroventricular injection of ASOs [191, 192]. Still regarding SBMA, the administration of ASOs seems to be effective in improving the disease phenotype even when using the subcutaneous

Table 20.3 Antisense oligonucleotide therapeutic approaches in animal models of polyglutamine diseases

Disease	Target	Mechanism	Administration	References
HD	Mutant huntingtin	RNAse-H mediated degradation	Intraparenchimal injection	[8]
	Mutant huntingtin	RNAse-H mediated degradation	ICV	[10]
	Mutant huntingtin	RNAse-H mediated degradation	ICV	[11]
	Mutant huntingtin	RNAse-H mediated degradation	ICV	[283]
	Mutant huntingtin	RNAse-H mediated degradation	ICV	[284]
	Mutant huntingtin	Translation blockage	ICV	[285]
	Huntingtin	Exon-skipping	Intraparenchimal injection	[286]
SCA2	Ataxin-2	RNAse-H mediated degradation	ICV	[191]
SCA3	Ataxin-3	Exon-skipping	ICV	[184]
	Ataxin-3	RNAse-H mediated degradation	ICV	[195]
	Ataxin-3	Exon-skipping	ICV	[287]
SBMA	Androgen receptor	RNAse-H mediated degradation	Subcutaneous	[193]
	Androgen receptor	RNAse-H mediated degradation	ICV	[192]

Abbreviations: *HD* Huntington's disease; *SCA2, 3* spinocerebellar ataxia type 2 and 3; *SBMA* spinal and bulbar muscular atrophy; *ICV* intracerebroventricular

route of administration [193]. Concerning MJD/SCA3, one study has evaluated the potential of ASOs to mediate exon skipping of the exon containing the polyQ tract. Although exon skipping of human mutant ataxin-3 was only evaluated in cell cultures, intracerebroventricular administration of ASOs targeting mouse ataxin-3 was capable of mediating exon skipping in vivo without any apparent toxic effect [184]. Recently, more efforts have been made towards the development of an ASO therapy for MJD/SCA3. One study has taken advantage of ASOs to reduce proteolytic cleavage of ataxin-3 through the removal of exons 8 and 9 which contain multiple predicted cleavage sites [194]. Another study has evaluated the potential of multiple ASOs to reduce the levels of mutant ataxin-3 in vivo. Intracerebroventricular administration resulted in widespread delivery to the brain and reduced ataxin-3 levels in multiple brain regions affected by the disease. Interestingly though, ASOs silencing properties were dependent on the transgenic mice lines used in the study [195].

Another interesting approach that has been investigated in cell cultures is the use of a single ASO to target multiple polyQ mRNAs. This strategy was effective in reducing the levels of atrophin-1, ataxin-1, ataxin-3 and huntingtin. However, for MJD/SCA3 and HD cell models, this strategy failed to demonstrate allele specificity [196].

Although many preclinical studies have shown the efficacy of ASOs in the treatment of polyQ diseases, many questions still arise regarding delivery, long-term efficacy and toxicity of these gene therapy tools.

20.3.3 Overexpression of Therapeutic Proteins

Gene therapy tools offer ways of manipulating the pathogenic cascade of polyQ diseases that go beyond the direct silencing of the CAG-expanded genes. Since its inception, gene therapy has been chiefly regarded as a way of introducing beneficial genes in a cell. In fact, supplementing functional versions of genes that were otherwise defective in patients was the aim of the first gene therapy clinical trials [1]. However, in the case of polyQ diseases this strategy seems to be unviable, considering that they are dominant disorders and pathology admittedly arises from a toxic gain-of-function, even if it might have contribution from a loss-of-function caused by expansion [197]. In a rodent model of MJD/SCA3, co-expression of non-expanded and expanded forms of ataxin-3 has been shown to produce toxicity comparable to what is obtained upon expression of the pathogenic form alone [154].

Nevertheless, studies indicate that expression of certain proteins that counter toxic events involved in polyQ disease pathogenesis may exert a therapeutic effect. Manipulation of particular cell systems in order to correct deficiencies occurring in cells carrying a polyQ expansion so as to operate alterations that decrease the toxic properties of expanded proteins have been suggested to be beneficial. Design of these strategies requires a deep understanding of the pathological mechanisms taking place in affected cells, of the cellular pathways that are negatively affected, and of the players that may produce amelioration. These approaches frequently act downstream of polyQ protein production and target phenomena that are causatively deleterious or that contribute to the persistence and/or increase of toxicity: proteolytic cleavage, aggregation and protein degradation impairment, among others.

20.3.3.1 Preventing Toxic Fragment Formation

Several reports suggest that proteolytic processing of polyQ proteins plays an important part in disease pathogenesis. PolyQ sequence-containing fragments have been described to display increased cytotoxic properties comparing to the correspondent full-length proteins and are thus considered by many to be the actual toxic species at the root of cell demise and loss [198–202].

Along with other proteases, calpains have been suggested to promote the proteolytic fragmentation of huntingtin [203–205] and ataxin-3 [132, 206]. It is possible to block the activity of these enzymes by overexpressing the endogenous calpain inhibitor calpastatin; coexpression of this protein reduced the formation of expanded ataxin-3 in transfected HEK 293 cells [206]. Importantly, AAV-mediated delivery of calpastatin decreased the formation of inclusions and limited neurodegeneration in the brain of MJD model mice expressing expanded ataxin-3 [132], attesting the therapeutic potential of gene therapy strategies aiming at proteolytic cleavage blockade.

20.3.3.2 Reducing Protein Aggregation

The tendency that expanded polyQ tract-carrying proteins have to self-assemble has been repeatedly related to their cell toxicity [207, 208]. Aggregation may generate deleterious oligomeric species, disturb native biologic functions of the polyQ proteins, disrupt important intermolecular interactions or sequester members of vital cell systems [209]. Accordingly, proteins that contribute to proper polypeptide folding and assembly—such as molecular chaperones—may counter aggregation and its adverse effects [136, 198, 210, 211].

Heat shock proteins 40 (Hsp40) and Hsp70 are chaperones that assist the refolding of denaturated proteins and, in vitro, they have been shown to inhibit expanded huntingtin aggregation [212, 213]. In polyQ disease models and patients, inclusions have been described to colocalize with molecular chaperones [214–221], suggesting that these cellular components act in order to counter the toxic transitions experimented by expanded polyQ proteins. Improving their activity, which may be disturbed in a disease context due to redistribution or saturation, may be beneficial.

Overexpression of several heat shock response-related proteins and other chaperones reduced aggregation and/or toxicity in diverse cell models of polyQ diseases, including HD [217–219, 222–227], MJD/SCA3 [214], SCA1 [215] and SBMA [216, 221, 228–230]. In vivo, overexpression of chaperones suppressed neurodegeneration in double-transgenic fly models of HD [231, 232], MJD/SCA3 [233, 234] and SCA1, and mitigated the phenotype of double-transgenic HD [235], SCA1 [236] and SBMA [237] mouse models.

Prominently, lentiviral delivery of yeast Hsp104 and rat Hsp27 to the rat brain was shown to decrease neurodegeneration caused by expanded huntingtin expression [219]. Lentiviral delivery of heat shock factor-1 (which contributes to heat shock protein expression) reduced protein accumulation and neurodegeneration in a transgenic SBMA mouse model [238]. It should be pointed out, however, that not all molecular chaperones exert the same type of protection against every expanded polyQ-containing protein [210, 211]. Selection of an individual chaperone for the treatment of a polyQ disease shall address its efficacy and specify in targeting the particular protein involved.

The beneficial effect of chaperone expression not always correlates with decreased aggregation [234, 236]. It has been suggested that chaperone overexpression favors not only aggregate disassembly, but also (or instead) boosts protein degradation by the proteasome (see below) and rescues proper intermolecular interactions [136, 198, 209, 228, 229]

20.3.3.3 Stimulating Protein Degradation

Another way of countering the cellular defects caused by polyQ-expanded proteins is to decrease their levels by promoting their proteolytic clearance. The two main cellular mechanisms of protein degradation are the ubiquitin-proteasome pathway and autophagy [239–241]. One autophagic pathway—macroautophagy—is considered especially important when dealing with aggregate-prone species, since it allows the bulk degradation of portions of the cytoplasm.

Diverse pharmacological strategies stimulating macroautophagy have been shown to be protective in the context of polyQ protein toxicity [242–244]. Likewise, overexpression of proteins that contribute to the autophagic flux is yet another approach at eliciting protection through this degradation pathway. Macroautophagy stimulation through overexpression of Rab2, histone deacetylase 6 (HDAC6) and several lipocalins attenuated toxicity in fly models of HD [245], SBMA [246] and SCA1 [247], respectively. In double transgenic SBMA mice, p62 reduced mutant AR levels, blocked neurodegeneration and ameliorated motor disease-like phenotype [248]. Subcutaneous delivery of the autophagy-stimulating cytokine granulocyte colony-stimulating factor reduced cell loss and improved motor performance in SCA17 mice [249].

Expression of a fusion protein comprising polyQ-binding peptide 1 and heat shock cognate protein 70 (HSC70)-binding motifs led to degradation of huntingtin by chaperone-mediated autophagy, in cultured cells. Striatal deliver of this chimera by AAVs reduced huntingtin aggregation and ameliorated the disease phenotype in transgenic HD model mice [250].

In cellular models of HD, expression of beclin-1, a protein with a central role in the formation of autophagosomes, has been shown to decrease huntingtin aggregation [251]. Lentiviral delivery of beclin-1 to the striatum or the cerebellum of diverse rodent models of MJD/SCA3 reduced the amount of ataxin-3 aggregates, the extent of degeneration and rescued motor impairments [82, 83]. Lentiviral-mediated overexpression of sirtuin-1 was observed to produce similar ameliorating effects in the neuropathological profile of striatal MJD model mice and this effect was suggested to result from the activation of autophagy and the consequent reduction of mutant ataxin-3 levels [252].

Since the autophagic pathway has been suggested to be disturbed in HD [242, 251], MJD/SCA3 [83] and SCA7 patients [253], as well as in SCA1 [254] and SBMA model animals [246], stimulating this degradation pathway could be

envisioned as a way of normalizing a cellular system that may be disturbed by polyQ toxicity. Autophagy upregulation has also been proposed to protect cells against apoptotic and necrotic cell-death mechanisms [243].

The beneficial effects of autophagy-mediated clearance may be limited to the cytoplasmic population of a particular protein [240]. Potentiating proteasomal degradation of polyQ proteins possibly avoids this constraint. One strategy to upregulate proteasomal activity is to express endogenous proteasome activators; in expanded huntingtin-expressing striatal neurons, lentiviral delivery of proteasome activator PA28-gamma was shown to decrease huntingtin levels and increase cell viability [255, 256]. Enhancing substrate ubiquitination may also potentiate degradation through the ubiquitin-proteasome pathway. Concordantly, co-expression of ataxin-3 and several E3 ubiquitin ligases (including C-terminus of Hsp70-interacting protein—CHIP, E4B, MITOL and parkin) has been shown to increase ataxin-3 ubiquitination and induce its degradation, at the same time decreasing aggregation and toxicity in cell cultures [257–261].

20.3.3.4 Other Methods of Suppressing Toxicity and Increasing Neuronal Survival

Other gene therapy routes for decreasing the toxicity of expanded polyQ proteins may aim at modulating other mechanisms that are known to interfere with deleterious properties and toxic outcomes. Altered post-translational modifications and aberrant intermolecular interactions have been related with the propensity of polyQ proteins to aggregate and cause cell demise. Expressing particular proteins that up-regulate protective alterations and/or down-regulate deleterious transitions constitute promising therapeutic approaches. For example, Akt-mediated phosphorylation of AR reduces its aggregation and toxicity in cultured cells [262]. The insulin-like growth factor 1 (IGF-1), which induces this modification, was shown to ameliorate the disease phenotype when overexpressed in SBMA transgenic mice [263]. Correcting putative effectors of toxicity such as transcriptional aberrations or metabolic imbalances are also conceivably viable strategies.

Finally, available studies suggest that biochemical factors that contribute to cell survival and function, and that are general agents of neuroprotection, produce beneficial effects when delivered to affected brain regions [264, 265]. Molecules such as neurotrophins pose as good candidates; viral delivery of the glial cell line-derived neurotrophic factor (GDNF) and neurturin to the striatum of HD transgenic mice has been shown to protect against cell loss and ameliorate the disease presentation [116, 266]. Local administration of neuropeptide Y through AAV injection was shown to counter neuropathological changes and motor impairment in mouse models of MJD/SCA3 [129].

20.3.4 Gene Editing-Based Approaches

One ideal approach to suppress polyQ pathologies would conceivably rely on the correction of the causative mutation, i.e., the decrease of CAG repeat number in the associated genes to non-pathological lengths. In fact, An and colleagues have reported the generation of genetically-corrected HD patient-derived induced pluripotent stem cells (iPSCs) using traditional homologous recombination approaches. This proof-of-principle study demonstrated the capability of correcting the expanded *HTT* allele to normal lengths, and resulted in the reversal of HD-related phenotypes [267].

Although homologous recombination has been historically used for targeted gene inactivation, replacement or addition, the low efficiency associated with this approach has limited its utility. However, recent advances in genome editing technologies have enormously improved the ability to make precise changes in the genome of eukaryotic cells [4, 268]. These strategies for precise gene correction or transcriptional inactivation rely on programmable nucleases that can be targeted to specific DNA loci. There are mainly four groups of engineered nucleases used as gene editing tools: meganucleases and their derivatives, zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs) and clustered regularly-interspaced short palindromic repeat (CRISPR)/Cas9 systems [269]. Approaches based on these nucleases have been successfully applied to repeat-containing gene loci. Independent studies have demonstrated that different CAG-targeting nucleases are capable of shrinking expanded CAG/CTG repeats in yeast [270, 271], mammalian cells [272] and also in zebrafish [273], as a natural consequence of DNA break repair.

Meganucleases, also named homing endonucleases, are engineered versions of naturally-occurring microbial DNA-cleaving enzymes that are able to recognize long DNA sequences (typically 20 to 30 base pairs; [274]). As the engineering of meganucleases is time-consuming and not sufficiently flexible, this genome editing platform has not been widely used whatsoever [275].

The chimeric enzymes ZFN and TALENs are composed by a DNA-binding domain, assembled in customizable combinations, that is then fused with a non-specific nuclease domain from the *Flavobacterium okeanokoites* restriction enzyme FokI. Two nucleases spaced by a few nucleotides have to be used in combination in order to allow FokI dimerization and DNA double-strand break (DSB) induction [269]. The DNA-binding domain of ZFN, based on eukaryotic transcription factors, is typically composed by three binding arrays, which recognize three consecutive nucleotides each [276]. The DNA-binding domain of TALENs is based on the plant pathogen *Xanthomonas* and consists of tandemly repeated 34 amino acid modules, each recognizing a single base in the target DNA.

In spite of the inherent differences of the described systems, meganucleases, ZFN and TALENs all share the same mode of DNA recognition, which is achieved via protein-DNA interactions [269]. On the other hand, the recently emerged Cas9 nuclease, from the bacterial adaptive immune system CRISPR type II, is recruited

by short RNA guides that bind to a desired DNA sequence, via Watson-Crick base pairing. The existence of a protospacer-adjacent motif (PAM) at the 3′ end of the DNA target site prompts Cas9 to induce DSBs at specific genomic loci [4, 277].

Upon the introduction of targeted DSBs, genome editing is achieved through the activation of endogenous DNA repair machinery: either non-homologous end-joining (NHEJ) or homology-directed repair (HDR). Briefly, in the NHEJ repair pathway the two broken ends are ligated together, leading typically to collateral small insertions or deletions (indels) at the DSB site. As a result, frame-shift mutations are often introduced, creating premature stop codons that suppress gene function. HDR, on the other hand, is a template-dependent pathway of DSB repair. In the presence of a donor template with homology to the break site, this pathway enables the spatially-accurate insertion of transgenes, or nucleotide substitutions, at the target locus [268, 269]. It has been determined that, compared to conventional systems, introducing DSBs using designer nucleases increases the frequency of HDR by several orders of magnitude [268].

Beyond the genome editing possibilities offered by these systems, they also have the potential to regulate endogenous gene expression at specific genome loci in living cells and organisms. This versatility arises from the ability of combining DNA-binding modules with numerous effector domains, including transcriptional activators and repressors, recombinases, transposases, DNA and histone methyltransferases, and histone acetyltransferases [268, 278].

A gene repressor strategy using engineered zinc-finger fusion proteins has been developed for HD. Zinc-finger repressors, designed to recognize the CAG repeat of the mutant *HTT* gene, showed the ability to effectively and selectively inhibit transcription, without affecting WT gene expression, both in cell cultures and in in vivo models [279]. After striatal delivery of zinc-finger repressors to the model mouse brain, significant amelioration in HD-related neuropathology and motor deficits was observed.

The most attractive options for gene therapy would rely on the decrease of mutant allele expression levels, while preserving WT ones. Fink and colleagues have described the construction of TALEs designed to cause mutant *HTT* gene silencing through either SNP targeting or CAG collapse. To achieve allele-specific transcriptional repression, TALEs were conceived to target a SNP in a region near the promoter of the gene and fused with the transcriptional repressor KRAB (Krüppel-associated box). For the gene correction approach, a pair of TALENs was constructed in order to induce a DSB only in long CAG repeats (greater than 15). These two approaches selectively reduced mutant *HTT* expression in patient-derived fibroblasts without affecting the expression of the WT allele [280].

Also aiming at completely inactivating the mutant allele without impacting non-pathogenic allele expression, CRISPR/Cas9 gene editing technology was used to simultaneously target two patient-specific polymorphic sites at the *HTT* mutant-bearing chromosome. Authors initially identified sequences that were present on the mutant chromosome haplotype, but absent from the normal one, in a given HD patient. After the transfection of primary HD fibroblasts with two CRISPR/Cas9 guide RNAs, inactivation of the mutant allele was achieved through

the excision of the promoter region, transcription start site and the CAG expansion in the mutant copy [281].

The interest captured by gene editing and the advances this type of technology is experimenting will surely translate into novel approaches for polyQ disease treatment in the years to come. As with the other types of strategies discussed, the development of increasingly improved nucleic acid delivery tools and the validation of efficacious molecular effects pave the way for the arrival of gene therapy to the clinic in an ever closer future.

References

1. Kaufmann KB, Buning H, Galy A, Schambach A, Grez M (2013) Gene therapy on the move. EMBO Mol Med 5:1642–1661
2. Wirth T, Parker N, Yla-Herttuala S (2013) History of gene therapy. Gene 525:162–169
3. Gatchel JR, Zoghbi HY (2005) Diseases of unstable repeat expansion: mechanisms and common principles. Nat Rev Genet 6:743–755
4. Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157:1262–1278
5. Boudreau RL, Rodriguez-Lebron E, Davidson BL (2011) RNAi medicine for the brain: progresses and challenges. Hum Mol Genet 20:R21–R27
6. Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23:217–247
7. Yin H, Kanasty RL, Eltoukhy AA, Vegas AJ, Dorkin JR, Anderson DG (2014) Non-viral vectors for gene-based therapy. Nat Rev Genet 15:541–555
8. Carroll JB, Warby SC, Southwell AL, Doty CN, Greenlee S, Skotte N, Hung G, Bennett CF, Freier SM, Hayden MR (2011) Potent and selective antisense oligonucleotides targeting single-nucleotide polymorphisms in the huntington disease gene/Allele-specific silencing of mutant Huntingtin. Mol Ther 19:2178–2185
9. DiFiglia M, Sena-Esteves M, Chase K, Sapp E, Pfister E, Sass M, Yoder J, Reeves P, Pandey RK, Rajeev KG, Manoharan M, Sah DW, Zamore PD, Aronin N (2007) Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. Proc Natl Acad Sci U S A 104:17204–17209
10. Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, Artates JW, Weiss A, Cheng SH, Shihabuddin LS, Hung G, Bennett CF, Cleveland DW (2012) Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron 74:1031–1044
11. Østergaard ME, Southwell AL, Kordasiewicz H, Watt AT, Skotte NH, Doty CN, Vaid K, Villanueva EB, Swayze EE, Frank Bennett C, Hayden MR, Seth PP (2013) Rational design of antisense oligonucleotides targeting single nucleotide polymorphisms for potent and allele selective suppression of mutant Huntingtin in the CNS. Nucleic Acids Res 41:9634–9650
12. Yu D, Pendergraff H, Liu J, Kordasiewicz HB, Cleveland DW, Swayze EE, Lima WF, Crooke ST, Prakash TP, Corey DR (2012) Single-stranded RNAs use RNAi to potently and Allele-selectively inhibit mutant Huntingtin expression. Cell 150:895–908
13. Kumar P, Wu H, McBride JL, Jung KE, Kim MH, Davidson BL, Lee SK, Shankar P, Manjunath N (2007) Transvascular delivery of small interfering RNA to the central nervous system. Nature 448:39–43
14. Gong C, Li X, Xu L, Zhang YH (2012) Target delivery of a gene into the brain using the RVG29-oligoarginine peptide. Biomaterials 33:3456–3463

15. Heitz F, Morris MC, Divita G (2009) Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. Br J Pharmacol 157:195–206
16. Zhan C, Yan Z, Xie C, Lu W (2010) Loop 2 of ophiophagus hannah toxin b binds with neuronal nicotinic acetylcholine receptors and enhances intracranial drug delivery. Mol Pharm 7:1940–1947
17. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci USA 92:7297–7301
18. Dunlap DD, Maggi A, Soria MR, Monaco L (1997) Nanoscopic structure of DNA condensed for gene delivery. Nucleic Acids Res 25:3095–3101
19. Son S, Hwang do W, Singha K, Jeong JH, Park TG, Lee DS, Kim WJ (2011) RVG peptide tethered bioreducible polyethylenimine for gene delivery to brain. J Controlled Release Official J Controlled Release Soc 155:18–25
20. Hwang do W, Son S, Jang J, Youn H, Lee S, Lee D, Lee YS, Jeong JM, Kim WJ, Lee DS (2011) A brain-targeted rabies virus glycoprotein-disulfide linked PEI nanocarrier for delivery of neurogenic microRNA. Biomaterials 32:4968–4975
21. Ohno M, Cole SL, Yasvoina M, Zhao J, Citron M, Berry R, Disterhoft JF, Vassar R (2007) BACE1 gene deletion prevents neuron loss and memory deficits in 5XFAD APP/PS1 transgenic mic. Neurobiol Dis 26:134–145
22. Park TE, Singh B, Li H, Lee JY, Kang SK, Choi YJ, Cho CS (2015) Enhanced BBB permeability of osmotically active poly(mannitol-co-PEI) modified with rabies virus glycoprotein via selective stimulation of caveolar endocytosis for RNAi therapeutics in Alzheimer's disease. Biomaterials 38:61–71
23. Tomalia DA, Baker H, Dewald J, Hall M, Kallos G, Martin S, Roeck J, Ryder J, Smith P (1985) A New class of polymers: starburst-dendritic macromolecules. Polym J 17:117–132
24. Duncan R, Izzo L (2005) Dendrimer biocompatibility and toxicity. Adv Drug Deliv Rev 57:2215–2237
25. Liu Y, Huang R, Han L, Ke W, Shao K, Ye L, Lou J, Jiang C (2009) Brain-targeting gene delivery and cellular internalization mechanisms for modified rabies virus glycoprotein RVG29 nanoparticles. Biomaterials 30:4195–4202
26. Huang R-Q, Qu Y-H, Ke W-L, Zhu J-H, Pei Y-Y, Jiang C (2007) Efficient gene delivery targeted to the brain using a transferrin-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. FASEB J 21:1117–1125
27. Huang R, Ke W, Liu Y, Jiang C, Pei Y (2008) The use of lactoferrin as a ligand for targeting the polyamidoamine-based gene delivery system to the brain. Biomaterials 29:238–246
28. Ke W, Shao K, Huang R, Han L, Liu Y, Li J, Kuang Y, Ye L, Lou J, Jiang C (2009) Gene delivery targeted to the brain using an Angiopep-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. Biomaterials 30:6976–6985
29. Pérez-Martínez F, Carrión B, Ceña V (2012) The use of nanoparticles for gene therapy in the nervous system. J Alzheimers Dis 31:697–710
30. Boado R (2007) Blood–brain barrier transport of non-viral gene and RNAi therapeutics. Pharm Res 24:1772–1787
31. Boado RJ (2005) RNA interference and nonviral targeted gene therapy of experimental brain cancer. NeuroRx 2:139–150
32. Zhang Y, Zhang Y-F, Bryant J, Charles A, Boado RJ, Pardridge WM (2004) Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain cancer. Clin Cancer Res 10:3667–3677
33. Zhang Y, Zhu C, Pardridge W (2002) Antisense gene therapy of brain cancer with an artificial virus gene delivery system. Mol Ther 6:67–72
34. Zhang Y, Calon F, Zhu C, Boado R, Pardridge W (2003) Intravenous nonviral gene therapy causes normalization of striatal tyrosine hydroxylase and reversal of motor impairment in experimental parkinsonism. Hum Gene Ther 14:1–12
35. Zhang Y, Boado R, Pardridge W (2003) Absence of toxicity of chronic weekly intravenous gene therapy with pegylated immunoliposomes. Pharm Res 20:1779–1785

36. Conceição M, Mendonca L, Nobrega C, Gomes C, Costa P, Hirai H, Moreira JN, Lima MC, Manjunath N, Pereira de Almeida L (2016) Intravenous administration of brain-targeted stable nucleic acid lipid particles alleviates Machado-Joseph disease neurological phenotype. Biomaterials 82:124–137
37. Costa PM, Cardoso AL, Mendonca LS, Serani A, Custodia C, Conceicao M, Simoes S, Moreira JN, Pereira de Almeida L, Pedroso de Lima MC (2013) Tumor-targeted chlorotoxin-coupled nanoparticles for nucleic acid delivery to glioblastoma cells: a promising system for glioblastoma treatment. Mol Ther Nucleic Acids 2:e100
38. Di Martino MT, Campani V, Misso G, Gallo Cantafio ME, Gulla A, Foresta U, Guzzi PH, Castellano M, Grimaldi A, Gigantino V, Franco R, Lusa S, Cannataro M, Tagliaferri P, De Rosa G, Tassone P, Caraglia M (2014) In vivo activity of miR-34a mimics delivered by stable nucleic acid lipid particles (SNALPs) against multiple myeloma. PLoS ONE 9:e90005
39. Geisbert TW, Hensley LE, Kagan E, Yu EZ, Geisbert JB, Daddario-DiCaprio K, Fritz EA, Jahrling PB, McClintock K, Phelps JR, Lee AC, Judge A, Jeffs LB, MacLachlan I (2006) Postexposure protection of guinea pigs against a lethal ebola virus challenge is conferred by RNA interference. J Infect Dis 193:1650–1657
40. Geisbert TW, Lee AC, Robbins M, Geisbert JB, Honko AN, Sood V, Johnson JC, de Jong S, Tavakoli I, Judge A, Hensley LE, Maclachlan I (2010) Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study. Lancet 375:1896–1905
41. Judge AD, Robbins M, Tavakoli I, Levi J, Hu L, Fronda A, Ambegia E, McClintock K, MacLachlan I (2009) Confirming the RNAi-mediated mechanism of action of siRNA-based cancer therapeutics in mice. J Clin Investig 119:661–673
42. Morrissey DV, Lockridge JA, Shaw L, Blanchard K, Jensen K, Breen W, Hartsough K, Machemer L, Radka S, Jadhav V, Vaish N, Zinnen S, Vargeese C, Bowman K, Shaffer CS, Jeffs LB, Judge A, MacLachlan I, Polisky B (2005) Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nat Biotechnol 23:1002–1007
43. Zimmermann TS, Lee AC, Akinc A, Bramlage B, Bumcrot D, Fedoruk MN, Harborth J, Heyes JA, Jeffs LB, John M, Judge AD, Lam K, McClintock K, Nechev LV, Palmer LR, Racie T, Rohl I, Seiffert S, Shanmugam S, Sood V, Soutschek J, Toudjarska I, Wheat AJ, Yaworski E, Zedalis W, Koteliansky V, Manoharan M, Vornlocher HP, MacLachlan I (2006) RNAi-mediated gene silencing in non-human primates. Nature 441:111–114
44. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 9:654–659
45. El Andaloussi S, Mager I, Breakefield XO, Wood MJA (2013) Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 12:347–357
46. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 29:341–345
47. Cooper JM, Wiklander PB, Nordin JZ, Al-Shawi R, Wood MJ, Vithlani M, Schapira AH, Simons JP, El-Andaloussi S, Alvarez-Erviti L (2014) Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice. Mov Disord Official J Mov Disord Soc 29:1476–1485
48. Dewannieux M, Harper F, Richaud A, Letzelter C, Ribet D, Pierron G, Heidmann T (2006) Identification of an infectious progenitor for the multiple-copy HERV-K human endogenous retroelements. Genome Res 16:1548–1556
49. Naldini L (2015) Gene therapy returns to centre stage. Nature 526:351–360
50. Saraiva J, Nobre RJ, Pereira de Almeida L (2016) Gene therapy for the CNS using AAVs: the impact of systemic delivery by AAV9. J Control Release 241:94–109
51. Rowe WP, Huebner RJ, Bell JA (1957) Definition and outline of contemporary information on the adenovirus group. Ann N Y Acad Sci 67:255–261
52. Le Gal La Salle G, Robert JJ, Berrard S, Ridoux V, Stratford-Perricaudet LD, Perricaudet M, Mallet J (1993) An adenovirus vector for gene transfer into neurons and glia in the brain. Science 259:988-990

53. Byrnes AP, MacLaren RE, Charlton HM (1996) Immunological instability of persistent adenovirus vectors in the brain: peripheral exposure to vector leads to renewed inflammation, reduced gene expression, and demyelination. J Neurosci 16:3045–3055
54. Thomas CE, Schiedner G, Kochanek S, Castro MG, Lowenstein PR (2000) Peripheral infection with adenovirus causes unexpected long-term brain inflammation in animals injected intracranially with first-generation, but not with high-capacity, adenovirus vectors: toward realistic long-term neurological gene therapy for chronic diseases. Proc Natl Acad Sci U S A 97:7482–7487
55. Wilson JM (2009) Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. Mol Genet Metab 96:151–157
56. Junyent F, Kremer EJ (2015) CAV-2–why a canine virus is a neurobiologist's best friend. Curr Opin Pharmacol 24:86–93
57. Huang B, Schiefer J, Sass C, Landwehrmeyer GB, Kosinski CM, Kochanek S (2007) High-capacity adenoviral vector-mediated reduction of huntingtin aggregate load in vitro and in vivo. Hum Gene Ther 18:303–311
58. Oehmig A, Fraefel C, Breakefield XO (2004) Update on herpesvirus amplicon vectors. Mol Ther 10:630–643
59. Chiocca EA, Choi BB, Cai WZ, DeLuca NA, Schaffer PA, DiFiglia M, Breakefield XO, Martuza RL (1990) Transfer and expression of the lacZ gene in rat brain neurons mediated by herpes simplex virus mutants. New Biol 2:739–746
60. Diefenbach RJ, Miranda-Saksena M, Douglas MW, Cunningham AL (2008) Transport and egress of herpes simplex virus in neurons. Rev Med Virol 18:35–51
61. Peruzzi PP, Lawler SE, Senior SL, Dmitrieva N, Edser PA, Gianni D, Chiocca EA, Wade-Martins R (2009) Physiological transgene regulation and functional complementation of a neurological disease gene deficiency in neurons. Mol Ther 17:1517–1526
62. Sena-Esteves M, Saeki Y, Fraefel C, Breakefield XO (2000) HSV-1 amplicon vectors–simplicity and versatility. Mol Ther 2:9–15
63. Nobre RJ, Almeida LP (2011) Gene therapy for Parkinson's and Alzheimer's diseases: from the bench to clinical trials. Curr Pharm Des 17:3434–3445
64. Deglon N, Aebischer P (2002) Lentiviruses as vectors for CNS diseases. Curr Top Microbiol Immunol 261:191–209
65. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, Selz F, Hue C, Certain S, Casanova JL, Bousso P, Deist FL, Fischer A (2000) Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 288:669–672
66. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, Martinache C, Rieux-Laucat F, Latour S, Belohradsky BH, Leiva L, Sorensen R, Debre M, Casanova JL, Blanche S, Durandy A, Bushman FD, Fischer A, Cavazzana-Calvo M (2010) Efficacy of gene therapy for X-linked severe combined immunodeficiency. N Engl J Med 363:355–364
67. Blomer U, Naldini L, Kafri T, Trono D, Verma IM, Gage FH (1997) Highly efficient and sustained gene transfer in adult neurons with a lentivirus vector. J Virol 71:6641–6649
68. Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM, Trono D (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 272:263–267
69. Osten P, Dittgen T, Licznerski P (2006) Lentivirus-based genetic manipulations in neurons in vivo. In: Kittler JT, Moss SJ (eds) The dynamic synapse: molecular methods in ionotropic receptor biology. Boca Raton (FL)
70. Burns JC, Friedmann T, Driever W, Burrascano M, Yee JK (1993) Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. Proc Natl Acad Sci U S A 90:8033–8037
71. Deglon N, Tseng JL, Bensadoun JC, Zurn AD, Arsenijevic Y, Pereira de Almeida L, Zufferey R, Trono D, Aebischer P (2000) Self-inactivating lentiviral vectors with enhanced transgene expression as potential gene transfer system in Parkinson's disease. Hum Gene Ther 11:179–190

72. Philippe S, Sarkis C, Barkats M, Mammeri H, Ladroue C, Petit C, Mallet J, Serguera C (2006) Lentiviral vectors with a defective integrase allow efficient and sustained transgene expression in vitro and in vivo. Proc Natl Acad Sci U S A 103:17684–17689
73. Aiuti A, Biasco L, Scaramuzza S, Ferrua F, Cicalese MP, Baricordi C, Dionisio F, Calabria A, Giannelli S, Castiello MC, Bosticardo M, Evangelio C, Assanelli A, Casiraghi M, Di Nunzio S, Callegaro L, Benati C, Rizzardi P, Pellin D, Di Serio C, Schmidt M, Von Kalle C, Gardner J, Mehta N, Neduva V, Dow DJ, Galy A, Miniero R, Finocchi A, Metin A, Banerjee PP, Orange JS, Galimberti S, Valsecchi MG, Biffi A, Montini E, Villa A, Ciceri F, Roncarolo MG, Naldini L (2013) Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome. Science 341:1233151
74. Biffi A, Montini E, Lorioli L, Cesani M, Fumagalli F, Plati T, Baldoli C, Martino S, Calabria A, Canale S, Benedicenti F, Vallanti G, Biasco L, Leo S, Kabbara N, Zanetti G, Rizzo WB, Mehta NA, Cicalese MP, Casiraghi M, Boelens JJ, Del Carro U, Dow DJ, Schmidt M, Assanelli A, Neduva V, Di Serio C, Stupka E, Gardner J, von Kalle C, Bordignon C, Ciceri F, Rovelli A, Roncarolo MG, Aiuti A, Sessa M, Naldini L (2013) Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. Science 341:1233158
75. Cartier N, Hacein-Bey-Abina S, Bartholomae CC, Veres G, Schmidt M, Kutschera I, Vidaud M, Abel U, Dal-Cortivo L, Caccavelli L, Mahlaoui N, Kiermer V, Mittelstaedt D, Bellesme C, Lahlou N, Lefrere F, Blanche S, Audit M, Payen E, Leboulch P, l'Homme B, Bougneres P, Von Kalle C, Fischer A, Cavazzana-Calvo M, Aubourg P (2009) Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science 326:818–823
76. Drouet V, Perrin V, Hassig R, Dufour N, Auregan G, Alves S, Bonvento G, Brouillet E, Luthi-Carter R, Hantraye P, Deglon N (2009) Sustained effects of nonallele-specific Huntingtin silencing. Ann Neurol 65:276–285
77. Drouet V, Ruiz M, Zala D, Feyeux M, Auregan G, Cambon K, Troquier L, Carpentier J, Aubert S, Merienne N, Bourgois-Rocha F, Hassig R, Rey M, Dufour N, Saudou F, Perrier AL, Hantraye P, Deglon N (2014) Allele-specific silencing of mutant huntingtin in rodent brain and human stem cells. PLoS ONE 9:e99341
78. Francelle L, Galvan L, Gaillard MC, Petit F, Bernay B, Guillermier M, Bonvento G, Dufour N, Elalouf JM, Hantraye P, Deglon N, de Chaldee M, Brouillet E (2015) Striatal long noncoding RNA Abhd11os is neuroprotective against an N-terminal fragment of mutant huntingtin in vivo. Neurobiol Aging 36(1601):e1607–e1616
79. Kleiman RJ, Kimmel LH, Bove SE, Lanz TA, Harms JF, Romegialli A, Miller KS, Willis A, des Etages S, Kuhn M, Schmidt CJ (2011) Chronic suppression of phosphodiesterase 10A alters striatal expression of genes responsible for neurotransmitter synthesis, neurotransmission, and signaling pathways implicated in Huntington's disease. J Pharmacol Exp Ther 336:64–76
80. Taylor DM, Moser R, Regulier E, Breuillaud L, Dixon M, Beesen AA, Elliston L, Silva Santos Mde F, Kim J, Jones L, Goldstein DR, Ferrante RJ, Luthi-Carter R (2013) MAP kinase phosphatase 1 (MKP-1/DUSP1) is neuroprotective in Huntington's disease via additive effects of JNK and p 38 inhibition. J Neurosci 33:2313–2325
81. Alves S, Nascimento-Ferreira I, Auregan G, Hassig R, Dufour N, Brouillet E, Pedroso de Lima MC, Hantraye P, Pereira de Almeida L, Deglon N (2008) Allele-specific RNA silencing of mutant ataxin-3 mediates neuroprotection in a rat model of Machado-Joseph disease. PLoS ONE 3:e3341
82. Nascimento-Ferreira I, Nobrega C, Vasconcelos-Ferreira A, Onofre I, Albuquerque D, Aveleira C, Hirai H, Deglon N, Pereira de Almeida L (2013) Beclin 1 mitigates motor and neuropathological deficits in genetic mouse models of Machado-Joseph disease. Brain 136:2173–2188
83. Nascimento-Ferreira I, Santos-Ferreira T, Sousa-Ferreira L, Auregan G, Onofre I, Alves S, Dufour N, Colomer Gould VF, Koeppen A, Deglon N, Pereira de Almeida L (2011)

Overexpression of the autophagic beclin-1 protein clears mutant ataxin-3 and alleviates Machado-Joseph disease. Brain 134:1400–1415
84. Nóbrega C, Carmo-Silva S, Albuquerque D, Vasconcelos-Ferreira A, Vijayakumar UG, Mendonca L, Hirai H, de Almeida LP (2015) Re-establishing ataxin-2 downregulates translation of mutant ataxin-3 and alleviates Machado-Joseph disease. Brain A J Neurol 138:3537–3554
85. Nóbrega C, Nascimento-Ferreira I, Onofre I, Albuquerque D, Deglon N, de Almeida LP (2014) RNA interference mitigates motor and neuropathological deficits in a cerebellar mouse model of Machado-Joseph disease. PLoS ONE 9:e100086
86. Nóbrega C, Nascimento-Ferreira I, Onofre I, Albuquerque D, Hirai H, Deglon N, de Almeida LP (2013) Silencing mutant ataxin-3 rescues motor deficits and neuropathology in Machado-Joseph disease transgenic mice. PLoS ONE 8:e52396
87. Saida H, Matsuzaki Y, Takayama K, Iizuka A, Konno A, Yanagi S, Hirai H (2014) One-year follow-up of transgene expression by integrase-defective lentiviral vectors and their therapeutic potential in spinocerebellar ataxia model mice. Gene Ther 21:820–827
88. Torashima T, Koyama C, Iizuka A, Mitsumura K, Takayama K, Yanagi S, Oue M, Yamaguchi H, Hirai H (2008) Lentivector-mediated rescue from cerebellar ataxia in a mouse model of spinocerebellar ataxia. EMBO Rep 9:393–399
89. Atchison RW, Casto BC, Hammon WM (1965) Adenovirus-associated defective virus particles. Science 149:754–756
90. McCarty DM, Young SM Jr, Samulski RJ (2004) Integration of adeno-associated virus (AAV) and recombinant AAV vectors. Annu Rev Genet 38:819–845
91. Grossman Z, Mendelson E, Brok-Simoni F, Mileguir F, Leitner Y, Rechavi G, Ramot B (1992) Detection of adeno-associated virus type 2 in human peripheral blood cells. J Gen Virol 73(Pt 4):961–966
92. Bessis N, GarciaCozar FJ, Boissier MC (2004) Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. Gene Ther 11(Suppl 1):S10–S17
93. Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, O'Malley KL, During MJ (1994) Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. Nat Genet 8:148–154
94. McCown TJ, Xiao X, Li J, Breese GR, Samulski RJ (1996) Differential and persistent expression patterns of CNS gene transfer by an adeno-associated virus (AAV) vector. Brain Res 713:99–107
95. Leone P, Shera D, McPhee SW, Francis JS, Kolodny EH, Bilaniuk LT, Wang DJ, Assadi M, Goldfarb O, Goldman HW, Freese A, Young D, During MJ, Samulski RJ, Janson CG (2012) Long-term follow-up after gene therapy for canavan disease. Sci Transl Med 4:165ra163
96. Cetin A, Komai S, Eliava M, Seeburg PH, Osten P (2006) Stereotaxic gene delivery in the rodent brain. Nat Protoc 1:3166–3173
97. Gao G, Vandenberghe LH, Alvira MR, Lu Y, Calcedo R, Zhou X, Wilson JM (2004) Clades of Adeno-associated viruses are widely disseminated in human tissues. J Virol 78:6381–6388
98. Wu Z, Asokan A, Samulski RJ (2006) Adeno-associated virus serotypes: vector toolkit for human gene therapy. Mol Ther J Am Soc Gene Ther 14:316–327
99. Cearley CN, Wolfe JH (2006) Transduction characteristics of adeno-associated virus vectors expressing cap serotypes 7, 8, 9, and Rh10 in the mouse brain. Mol Ther 13:528–537
100. Davidson BL, Stein CS, Heth JA, Martins I, Kotin RM, Derksen TA, Zabner J, Ghodsi A, Chiorini JA (2000) Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. Proc Natl Acad Sci U S A 97:3428–3432
101. Bevan AK, Duque S, Foust KD, Morales PR, Braun L, Schmelzer L, Chan CM, McCrate M, Chicoine LG, Coley BD, Porensky PN, Kolb SJ, Mendell JR, Burghes AH, Kaspar BK (2011) Systemic gene delivery in large species for targeting spinal cord, brain, and peripheral tissues for pediatric disorders. Mol Ther 19:1971–1980

102. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK (2009) Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. Nat Biotechnol 27:59–65
103. Wu Z, Yang H, Colosi P (2010) Effect of genome size on AAV vector packaging. Mol Ther 18:80–86
104. Boudreau RL, McBride JL, Martins I, Shen S, Xing Y, Carter BJ, Davidson BL (2009) Nonallele-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. Mol Ther 17:1053–1063
105. Dai Y, Dudek NL, Li Q, Fowler SC, Muma NA (2009) Striatal expression of a calmodulin fragment improved motor function, weight loss, and neuropathology in the R6/2 mouse model of Huntington's disease. J Neurosci 29:11550–11559
106. Dufour BD, Smith CA, Clark RL, Walker TR, McBride JL (2014) Intrajugular vein delivery of AAV9-RNAi prevents neuropathological changes and weight loss in Huntington's disease mice. Mol Ther 22:797–810
107. Franich NR, Fitzsimons HL, Fong DM, Klugmann M, During MJ, Young D (2008) AAV vector-mediated RNAi of mutant huntingtin expression is neuroprotective in a novel genetic rat model of Huntington's disease. Mol Ther 16:947–956
108. Grondin R, Kaytor MD, Ai Y, Nelson PT, Thakker DR, Heisel J, Weatherspoon MR, Blum JL, Burright EN, Zhang Z, Kaemmerer WF (2012) Six-month partial suppression of Huntingtin is well tolerated in the adult rhesus striatum. Brain A J Neurol 135:1197–1209
109. Harper SQ, Staber PD, He X, Eliason SL, Martins IH, Mao Q, Yang L, Kotin RM, Paulson HL, Davidson BL (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proc Natl Acad Sci U S A 102: 5820–5825
110. Keeler AM, Sapp E, Chase K, Sottosanti E, Danielson E, Pfister E, Stoica L, DiFiglia M, Aronin N, Sena-Esteves M (2016) Cellular analysis of silencing the Huntington's disease gene using AAV9 mediated delivery of artificial micro rna into the striatum of Q140/Q140 mice. J Huntingtons Dis 5:239–248
111. Kells AP, Fong DM, Dragunow M, During MJ, Young D, Connor B (2004) AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. Mol Ther 9:682–688
112. Kells AP, Henry RA, Connor B (2008) AAV-BDNF mediated attenuation of quinolinic acid-induced neuropathology and motor function impairment. Gene Ther 15:966–977
113. Machida Y, Okada T, Kurosawa M, Oyama F, Ozawa K, Nukina N (2006) rAAV-mediated shRNA ameliorated neuropathology in Huntington disease model mouse. Biochem Biophys Res Commun 343:190–197
114. McBride JL, During MJ, Wuu J, Chen EY, Leurgans SE, Kordower JH (2003) Structural and functional neuroprotection in a rat model of Huntington's disease by viral gene transfer of GDNF. Exp Neurol 181:213–223
115. McBride JL, Pitzer MR, Boudreau RL, Dufour B, Hobbs T, Ojeda SR, Davidson BL (2011) Preclinical safety of RNAi-mediated HTT suppression in the rhesus macaque as a potential therapy for Huntington's disease. Mol Ther 19:2152–2162
116. McBride JL, Ramaswamy S, Gasmi M, Bartus RT, Herzog CD, Brandon EP, Zhou L, Pitzer MR, Berry-Kravis EM, Kordower JH (2006) Viral delivery of glial cell line-derived neurotrophic factor improves behavior and protects striatal neurons in a mouse model of Huntington's disease. Proc Natl Acad Sci U S A 103:9345–9350
117. Ramaswamy S, Kordower JH (2012) Gene therapy for Huntington's disease. Neurobiol Dis 48:243–254
118. Rodriguez-Lebron E, Denovan-Wright EM, Nash K, Lewin AS, Mandel RJ (2005) Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. Mol Ther 12:618–633
119. Southwell AL, Ko J, Patterson PH (2009) Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. J Neurosci 29:13589–13602

120. Stanek LM, Sardi SP, Mastis B, Richards AR, Treleaven CM, Taksir T, Misra K, Cheng SH, Shihabuddin LS (2014) Silencing mutant huntingtin by adeno-associated virus-mediated RNA interference ameliorates disease manifestations in the YAC128 mouse model of Huntington's disease. Hum Gene Ther 25:461–474
121. Boudreau RL, Martins I, Davidson BL (2009) Artificial microRNAs as siRNA shuttles: improved safety as compared to shRNAs in vitro and in vivo. Mol Ther: J Am Soc Gene Ther 17:169–175
122. Ito H, Fujita K, Tagawa K, Chen X, Homma H, Sasabe T, Shimizu J, Shimizu S, Tamura T, Muramatsu S, Okazawa H (2014) HMGB1 facilitates repair of mitochondrial DNA damage and extends the lifespan of mutant ataxin-1 knock-in mice. EMBO Mol Med 7:78–101
123. Keiser MS, Boudreau RL, Davidson BL (2014) Broad therapeutic benefit after RNAi expression vector delivery to deep cerebellar nuclei: implications for spinocerebellar ataxia type 1 therapy. Mol Ther 22:588–595
124. Keiser MS, Geoghegan JC, Boudreau RL, Lennox KA, Davidson BL (2013) RNAi or overexpression: alternative therapies for Spinocerebellar Ataxia Type 1. Neurobiol Dis 56:6–13
125. Keiser MS, Kordower JH, Gonzalez-Alegre P, Davidson BL (2015) Broad distribution of ataxin 1 silencing in rhesus cerebella for spinocerebellar ataxia type 1 therapy. Brain A J Neurol 138:3555–3566
126. Taniguchi JB, Kondo K, Fujita K, Chen X, Homma H, Sudo T, Mao Y, Watase K, Tanaka T, Tagawa K, Tamura T, Muramatsu SI, Okazawa H (2016) RpA1 ameliorates symptoms of mutant ataxin-1 knock-in mice and enhances DNA damage repair. Human Mol Genet
127. Xia H, Mao Q, Eliason SL, Harper SQ, Martins IH, Orr HT, Paulson HL, Yang L, Kotin RM, Davidson BL (2004) RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. Nat Med 10:816–820
128. Costa Mdo C, Luna-Cancalon K, Fischer S, Ashraf NS, Ouyang M, Dharia RM, Martin-Fishman L, Yang Y, Shakkottai VG, Davidson BL, Rodriguez-Lebron E, Paulson HL (2013) Toward RNAi therapy for the polyglutamine disease Machado-Joseph disease. Mol Ther J Am Soc Gene Ther 21:1898–1908
129. Duarte-Neves J, Goncalves N, Cunha-Santos J, Simoes AT, den Dunnen WF, Hirai H, Kugler S, Cavadas C, Pereira de Almeida L (2015) Neuropeptide Y mitigates neuropathology and motor deficits in mouse models of Machado-Joseph disease. Hum Mol Genet 24:5451–5463
130. Konno A, Shuvaev AN, Miyake N, Miyake K, Iizuka A, Matsuura S, Huda F, Nakamura K, Yanagi S, Shimada T, Hirai H (2014) Mutant ataxin-3 with an abnormally expanded polyglutamine chain disrupts dendritic development and metabotropic glutamate receptor signaling in mouse cerebellar Purkinje cells. Cerebellum 13:29–41
131. Rodriguez-Lebron E, Costa Mdo C, Luna-Cancalon K, Peron TM, Fischer S, Boudreau RL, Davidson BL, Paulson HL (2013) Silencing mutant ATXN3 expression resolves molecular phenotypes in SCA3 transgenic mice. Mol Ther J Am Soc Gene Ther 21:1909–1918
132. Simões AT, Goncalves N, Koeppen A, Deglon N, Kugler S, Duarte CB, Pereira de Almeida L (2012) Calpastatin-mediated inhibition of calpains in the mouse brain prevents mutant ataxin 3 proteolysis, nuclear localization and aggregation, relieving Machado-Joseph disease. Brain 135:2428–2439
133. Miyazaki Y, Du X, Muramatsu S, Gomez CM (2016) An miRNA-mediated therapy for SCA6 blocks IRES-driven translation of the CACNA1A second cistron. Sci Transl Med 8:347ra394
134. Ramachandran PS, Bhattarai S, Singh P, Boudreau RL, Thompson S, Laspada AR, Drack AV, Davidson BL (2014) RNA interference-based therapy for spinocerebellar ataxia type 7 retinal degeneration. PLoS ONE 9:e95362
135. Ramachandran PS, Boudreau RL, Schaefer KA, La Spada AR, Davidson BL (2014) Nonallele specific silencing of ataxin-7 improves disease phenotypes in a mouse model of SCA7. Mol Ther 22:1635–1642

136. Bauer PO, Nukina N (2009) The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. J Neurochem 110:1737–1765
137. Elbashir SM, Lendeckel W, Tuschl T (2001) RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev 15:188–200
138. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R (2004) The Microprocessor complex mediates the genesis of microRNAs. Nature 432: 235–240
139. Bohnsack MT, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10:185–191
140. Khvorova A, Reynolds A, Jayasena SD (2003) Functional siRNAs and miRNAs exhibit strand bias. Cell 115:209–216
141. Ambros V (2004) The functions of animal microRNAs. Nature 431:350–355
142. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297
143. Doench JG, Petersen CP, Sharp PA (2003) siRNAs can function as miRNAs. Genes Dev 17:438–442
144. Pardridge WM (2007) shRNA and siRNA delivery to the brain. Adv Drug Deliv Rev 59:141–152
145. Thakker DR, Hoyer D, Cryan JF (2006) Interfering with the brain: use of RNA interference for understanding the pathophysiology of psychiatric and neurological disorders. Pharmacol Ther 109:413–438
146. Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494–498
147. Martinez J, Patkaniowska A, Urlaub H, Luhrmann R, Tuschl T (2002) Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. Cell 110:563–574
148. Zamore PD, Tuschl T, Sharp PA, Bartel DP (2000) RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell 101:25–33
149. Paul CP, Good PD, Winer I, Engelke DR (2002) Effective expression of small interfering RNA in human cells. Nat Biotechnol 20:505–508
150. Davidson BL, Harper SQ (2005) Viral delivery of recombinant short hairpin RNAs. Methods Enzymol 392:145–173
151. Watson LM, Wood MJ (2012) RNA therapy for polyglutamine neurodegenerative diseases. Expert Rev Mol Med 14:e3
152. Scholefield J, Wood MJ (2010) Therapeutic gene silencing strategies for polyglutamine disorders. Trends Genet TIG 26:29–38
153. Rodriguez-Lebron E, Paulson HL (2006) Allele-specific RNA interference for neurological disease. Gene Ther 13:576–581
154. Alves S, Nascimento-Ferreira I, Dufour N, Hassig R, Auregan G, Nobrega C, Brouillet E, Hantraye P, Pedroso de Lima MC, Deglon N, de Almeida LP (2010) Silencing ataxin-3 mitigates degeneration in a rat model of Machado-Joseph disease: no role for wild-type ataxin-3? Hum Mol Genet 19:2380–2394
155. Wang YL, Liu W, Wada E, Murata M, Wada K, Kanazawa I (2005) Clinico-pathological rescue of a model mouse of Huntington's disease by siRNA. Neurosci Res 53:241–249
156. Alterman JF, Hall LM, Coles AH, Hassler MR, Didiot MC, Chase K, Abraham J, Sottosanti E, Johnson E, Sapp E, Osborn MF, Difiglia M, Aronin N, Khvorova A (2015) Hydrophobically modified siRNAs silence Huntingtin mRNA in primary neurons and mouse brain. Mol Ther Nucleic Acids 4:e266
157. Didiot MC, Hall LM, Coles AH, Haraszti RA, Godinho BM, Chase K, Sapp E, Ly S, Alterman JF, Hassler MR, Echeverria D, Raj L, Morrissey DV, DiFiglia M, Aronin N, Khvorova A (2016) Exosome-mediated delivery of hydrophobically modified siRNA for Huntingtin mRNA silencing. Mol Ther
158. Grondin R, Ge P, Chen Q, Sutherland JE, Zhang Z, Gash DM, Stiles DK, Stewart GR, Sah DW, Kaemmerer WF (2015) Onset time and durability of Huntingtin suppression in

Rhesus Putamen after direct infusion of antihuntingtin siRNA. Mol Ther Nucleic Acids 4: e245
159. Stiles DK, Zhang Z, Ge P, Nelson B, Grondin R, Ai Y, Hardy P, Nelson PT, Guzaev AP, Butt MT, Charisse K, Kosovrasti V, Tchangov L, Meys M, Maier M, Nechev L, Manoharan M, Kaemmerer WF, Gwost D, Stewart GR, Gash DM, Sah DW (2012) Widespread suppression of huntingtin with convection-enhanced delivery of siRNA. Exp Neurol 233:463–471
160. Gaspar C, Lopes-Cendes I, DeStefano AL, Maciel P, Silveira I, Coutinho P, MacLeod P, Sequeiros J, Farrer LA, Rouleau GA (1996) Linkage disequilibrium analysis in Machado-Joseph disease patients of different ethnic origins. Hum Genet 98:620–624
161. Gaspar C, Lopes-Cendes I, Hayes S, Goto J, Arvidsson K, Dias A, Silveira I, Maciel P, Coutinho P, Lima M, Zhou YX, Soong BW, Watanabe M, Giunti P, Stevanin G, Riess O, Sasaki H, Hsieh M, Nicholson GA, Brunt E, Higgins JJ, Lauritzen M, Tranebjaerg L, Volpini V, Wood N, Ranum L, Tsuji S, Brice A, Sequeiros J, Rouleau GA (2001) Ancestral origins of the Machado-Joseph disease mutation: a worldwide haplotype study. Am J Hum Genet 68:523–528
162. Li Y, Yokota T, Matsumura R, Taira K, Mizusawa H (2004) Sequence-dependent and independent inhibition specific for mutant ataxin-3 by small interfering RNA. Ann Neurol 56:124–129
163. Scholefield J, Watson L, Smith D, Greenberg J, Wood MJ (2014) Allele-specific silencing of mutant Ataxin-7 in SCA7 patient-derived fibroblasts. Eur J Human Genet EJHG 22: 1369–1375
164. Cattaneo E, Rigamonti D, Goffredo D, Zuccato C, Squitieri F, Sipione S (2001) Loss of normal huntingtin function: new developments in Huntington's disease research. Trends Neurosci 24:182–188
165. van Bilsen PH, Jaspers L, Lombardi MS, Odekerken JC, Burright EN, Kaemmerer WF (2008) Identification and allele-specific silencing of the mutant huntingtin allele in Huntington's disease patient-derived fibroblasts. Hum Gene Ther 19:710–719
166. Zhang Y, Engelman J, Friedlander RM (2009) Allele-specific silencing of mutant Huntington's disease gene. J Neurochem 108:82–90
167. Ambrose CM, Duyao MP, Barnes G, Bates GP, Lin CS, Srinidhi J, Baxendale S, Hummerich H, Lehrach H, Altherr M et al (1994) Structure and expression of the Huntington's disease gene: evidence against simple inactivation due to an expanded CAG repeat. Somat Cell Mol Genet 20:27–38
168. Pfister EL, Kennington L, Straubhaar J, Wagh S, Liu W, DiFiglia M, Landwehrmeyer B, Vonsattel JP, Zamore PD, Aronin N (2009) Five siRNAs targeting three SNPs may provide therapy for three-quarters of Huntington's disease patients. Curr Biol CB 19:774–778
169. Becanovic K, Norremolle A, Neal SJ, Kay C, Collins JA, Arenillas D, Lilja T, Gaudenzi G, Manoharan S, Doty CN, Beck J, Lahiri N, Portales-Casamar E, Warby SC, Connolly C, De Souza RA, REGISTRY Investigators of the European Huntington's Disease Network, Tabrizi SJ, Hermanson O, Langbehn DR, Hayden MR, Wasserman WW, Leavitt BR (2015) A SNP in the HTT promoter alters NF-kappaB binding and is a bidirectional genetic modifier of Huntington disease. Nat Neurosci 18:807–816
170. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP (2008) The impact of microRNAs on protein output. Nature 455:64–71
171. Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120:15–20
172. Monteys AM, Wilson MJ, Boudreau RL, Spengler RM, Davidson BL (2015) Artificial miRNAs targeting mutant Huntingtin show preferential silencing in vitro and in vivo. Mol Ther Nucleic Acids 4:e234
173. Huang F, Zhang L, Long Z, Chen Z, Hou X, Wang C, Peng H, Wang J, Li J, Duan R, Xia K, Chuang DM, Tang B, Jiang H (2014) MiR-25 alleviates polyQ-mediated cytotoxicity by silencing ATXN3. FEBS Lett 588:4791–4798

174. Cheng PH, Li CL, Chang YF, Tsai SJ, Lai YY, Chan AWS, Chen CM, Yang SH (2013) MiR-196a ameliorates phenotypes of huntington disease in cell, transgenic mouse, and induced pluripotent stem cell models. Am J Hum Genet 93:306–312
175. Lee Y, Samaco RC, Gatchel JR, Thaller C, Orr HT, Zoghbi HY (2008) miR-19, miR-101 and miR-130 co-regulate ATXN1 levels to potentially modulate SCA1 pathogenesis. Nat Neurosci 11:1137–1139
176. Persengiev S, Kondova I, Otting N, Koeppen AH, Bontrop RE (2011) Genome-wide analysis of miRNA expression reveals a potential role for miR-144 in brain aging and spinocerebellar ataxia pathogenesis. Neurobiol Aging 32:2316.e2317–2327
177. Tan JY, Vance KW, Varela MA, Sirey T, Watson LM, Curtis HJ, Marinello M, Alves S, Steinkraus BR, Cooper S, Nesterova T, Brockdorff N, Fulga TA, Brice A, Sittler A, Oliver PL, Wood MJ, Ponting CP, Marques AC (2014) Cross-talking noncoding RNAs contribute to cell-specific neurodegeneration in SCA7. Nat Struct Mol Biol 21:955–961
178. Miyazaki Y, Adachi H, Katsuno M, Minamiyama M, Jiang Y-M, Huang Z, Doi H, Matsumoto S, Kondo N, Iida M, Tohnai G, Tanaka F, Muramatsu S-I, Sobue G (2012) Viral delivery of miR-196a ameliorates the SBMA phenotype via the silencing of CELF2. Nat Med 18:1136–1141
179. Carmona V, Cunha-Santos J, Onofre I, Simoes AT, Vijayakumar U, Davidson BL, Pereira de Almeida L (2017) Unravelling endogenous microrna system dysfunction as a new pathophysiological mechanism in Machado-Joseph disease. Mol Ther: J Am Soc Gene Ther
180. Zamecnik PC, Stephenson ML (1978) Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc Natl Acad Sci U S A 75:280–284
181. Monia BP, Lesnik EA, Gonzalez C, Lima WF, McGee D, Guinosso CJ, Kawasaki AM, Cook PD, Freier SM (1993) Evaluation of 2'-modified oligonucleotides containing 2'-deoxy gaps as antisense inhibitors of gene expression. J Biol Chem 268:14514–14522
182. Walder RY, Walder JA (1988) Role of RNase H in hybrid-arrested translation by antisense oligonucleotides. Proc Natl Acad Sci U S A 85:5011–5015
183. Sazani P, Kole R (2003) Therapeutic potential of antisense oligonucleotides as modulators of alternative splicing. J Clin Investig 112:481–486
184. Evers MM, Tran HD, Zalachoras I, Pepers BA, Meijer OC, den Dunnen JT, van Ommen GJ, Aartsma-Rus A, van Roon-Mom WM (2013) Ataxin-3 protein modification as a treatment strategy for spinocerebellar ataxia type 3: removal of the CAG containing exon. Neurobiol Dis 58:49–56
185. Baker BF, Lot SS, Kringel J, Cheng-Flournoy S, Villiet P, Sasmor HM, Siwkowski AM, Chappell LL, Morrow JR (1999) Oligonucleotide-europium complex conjugate designed to cleave the 5′ cap structure of the ICAM-1 transcript potentiates antisense activity in cells. Nucleic Acids Res 27:1547–1551
186. Vickers TA, Wyatt JR, Burckin T, Bennett CF, Freier SM (2001) Fully modified 2′ MOE oligonucleotides redirect polyadenylation. Nucleic Acids Res 29:1293–1299
187. Johansson HE, Belsham GJ, Sproat BS, Hentze MW (1994) Target-specific arrest of mRNA translation by antisense 2'-O-alkyloligoribonucleotides. Nucleic Acids Res 22:4591–4598
188. Esau CC (2008) Inhibition of microRNA with antisense oligonucleotides. Methods 44: 55–60
189. Akhtar S, Kole R, Juliano RL (1991) Stability of antisense DNA oligodeoxynucleotide analogs in cellular extracts and sera. Life Sci 49:1793–1801
190. Wickstrom E (1986) Oligodeoxynucleotide stability in subcellular extracts and culture media. J Biochem Biophys Methods 13:97–102
191. Pulst SM (2016) Degenerative ataxias, from genes to therapies: the 2015 Cotzias Lecture. Neurology 86:2284–2290
192. Sahashi K, Katsuno M, Hung G, Adachi H, Kondo N, Nakatsuji H, Tohnai G, Iida M, Bennett CF, Sobue G (2015) Silencing neuronal mutant androgen receptor in a mouse model of spinal and bulbar muscular atrophy. Hum Mol Genet 24:5985–5994
193. Lieberman AP, Yu Z, Murray S, Peralta R, Low A, Guo S, Yu XX, Cortes CJ, Bennett CF, Monia BP, La Spada AR, Hung G (2014) Peripheral androgen receptor gene suppression

rescues disease in mouse models of spinal and bulbar muscular atrophy. Cell reports 7: 774–784
194. Toonen LJ, Schmidt I, Luijsterburg MS, van Attikum H, van Roon-Mom WM (2016) Antisense oligonucleotide-mediated exon skipping as a strategy to reduce proteolytic cleavage of ataxin-3. Sci Rep 6:35200
195. Moore LR, Rajpal G, Dillingham IT, Qutob M, Blumenstein KG, Gattis D, Hung G, Kordasiewicz HB, Paulson HL, McLoughlin HS (2017) Evaluation of antisense oligonucleotides targeting ATXN3 in SCA3 mouse models. Mol Ther Nucleic Acids 7:200–210
196. Evers MM, Pepers BA, van Deutekom JC, Mulders SA, den Dunnen JT, Aartsma-Rus A, van Ommen GJ, van Roon-Mom WM (2011) Targeting several CAG expansion diseases by a single antisense oligonucleotide. PLoS ONE 6:e24308
197. Orr HT (2012) Polyglutamine neurodegeneration: expanded glutamines enhance native functions. Curr Opin Genet Dev 22:251–255
198. Ferrigno P, Silver PA (2000) Polyglutamine expansions: proteolysis, chaperones, and the dangers of promiscuity. Neuron 26:9–12
199. Matos CA, de Almeida LP, Nobrega C (2016) Proteolytic cleavage of polyglutamine disease-causing proteins: revisiting the toxic fragment hypothesis. Curr Pharm Des
200. Tarlac V, Storey E (2003) Role of proteolysis in polyglutamine disorders. J Neurosci Res 74:406–416
201. Walsh R, Storey E, Stefani D, Kelly L, Turnbull V (2005) The roles of proteolysis and nuclear localisation in the toxicity of the polyglutamine diseases. A Rev Neurotox Res 7: 43–57
202. Weber JJ, Sowa AS, Binder T, Hubener J (2014) From pathways to targets: understanding the mechanisms behind polyglutamine disease. Biomed Res Int 2014:701758
203. Gafni J, Ellerby LM (2002) Calpain activation in Huntington's disease. J Neurosci 22:4842–4849
204. Goffredo D, Rigamonti D, Tartari M, De Micheli A, Verderio C, Matteoli M, Zuccato C, Cattaneo E (2002) Calcium-dependent cleavage of endogenous wild-type huntingtin in primary cortical neurons. J Biol Chem 277:39594–39598
205. Kim YJ, Yi Y, Sapp E, Wang Y, Cuiffo B, Kegel KB, Qin ZH, Aronin N, DiFiglia M (2001) Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. Proc Natl Acad Sci U S A 98:12784–12789
206. Haacke A, Hartl FU, Breuer P (2007) Calpain inhibition is sufficient to suppress aggregation of polyglutamine-expanded ataxin-3. J Biol Chem 282:18851–18856
207. Takahashi T, Katada S, Onodera O (2010) Polyglutamine diseases: where does toxicity come from? what is toxicity? where are we going? J Mol Cell Biol 2:180–191
208. Todd TW, Lim J (2013) Aggregation formation in the polyglutamine diseases: protection at a cost? Mol Cells 36:185–194
209. Williams AJ, Paulson HL (2008) Polyglutamine neurodegeneration: protein misfolding revisited. Trends Neurosci 31:521–528
210. Robertson AL, Bottomley SP (2010) Towards the treatment of polyglutamine diseases: the modulatory role of protein context. Curr Med Chem 17:3058–3068
211. Ross CA, Tabrizi SJ (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. Lancet Neurol 10:83–98
212. Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hartl MK, Hartl FU (2000) Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. Proc Natl Acad Sci U S A 97:7841–7846
213. Wacker JL, Zareie MH, Fong H, Sarikaya M, Muchowski PJ (2004) Hsp70 and Hsp40 attenuate formation of spherical and annular polyglutamine oligomers by partitioning monomer. Nat Struct Mol Biol 11:1215–1222
214. Chai Y, Koppenhafer SL, Bonini NM, Paulson HL (1999) Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. J Neurosci 19:10338–10347

215. Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. Nat Genet 19:148–154
216. Ishihara K, Yamagishi N, Saito Y, Adachi H, Kobayashi Y, Sobue G, Ohtsuka K, Hatayama T (2003) Hsp105alpha suppresses the aggregation of truncated androgen receptor with expanded CAG repeats and cell toxicity. J Biol Chem 278:25143–25150
217. Jana NR, Tanaka M, Wang G, Nukina N (2000) Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminal huntingtin: their role in suppression of aggregation and cellular toxicity. Hum Mol Genet 9:2009–2018
218. Mitsui K, Nakayama H, Akagi T, Nekooki M, Ohtawa K, Takio K, Hashikawa T, Nukina N (2002) Purification of polyglutamine aggregates and identification of elongation factor-1alpha and heat shock protein 84 as aggregate-interacting proteins. J Neurosci 22:9267–9277
219. Perrin V, Regulier E, Abbas-Terki T, Hassig R, Brouillet E, Aebischer P, Luthi-Carter R, Deglon N (2007) Neuroprotection by Hsp104 and Hsp27 in lentiviral-based rat models of Huntington's disease. Mol Ther J Am Soc Gene Ther 15:903–911
220. Schmidt T, Lindenberg KS, Krebs A, Schols L, Laccone F, Herms J, Rechsteiner M, Riess O, Landwehrmeyer GB (2002) Protein surveillance machinery in brains with spinocerebellar ataxia type 3: redistribution and differential recruitment of 26S proteasome subunits and chaperones to neuronal intranuclear inclusions. Ann Neurol 51:302–310
221. Stenoien DL, Cummings CJ, Adams HP, Mancini MG, Patel K, DeMartino GN, Marcelli M, Weigel NL, Mancini MA (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. Hum Mol Genet 8:731–741
222. Carmichael J, Chatellier J, Woolfson A, Milstein C, Fersht AR, Rubinsztein DC (2000) Bacterial and yeast chaperones reduce both aggregate formation and cell death in mammalian cell models of Huntington's disease. Proc Natl Acad Sci U S A 97:9701–9705
223. Carra S, Sivilotti M, Chavez Zobel AT, Lambert H, Landry J (2005) HspB8, a small heat shock protein mutated in human neuromuscular disorders, has in vivo chaperone activity in cultured cells. Hum Mol Genet 14:1659–1669
224. Kitamura A, Kubota H, Pack CG, Matsumoto G, Hirayama S, Takahashi Y, Kimura H, Kinjo M, Morimoto RI, Nagata K (2006) Cytosolic chaperonin prevents polyglutamine toxicity with altering the aggregation state. Nat Cell Biol 8:1163–1170
225. Tam S, Geller R, Spiess C, Frydman J (2006) The chaperonin TRiC controls polyglutamine aggregation and toxicity through subunit-specific interactions. Nat Cell Biol 8:1155–1162
226. Wyttenbach A, Sauvageot O, Carmichael J, Diaz-Latoud C, Arrigo AP, Rubinsztein DC (2002) Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. Hum Mol Genet 11:1137–1151
227. Zhou H, Li SH, Li XJ (2001) Chaperone suppression of cellular toxicity of huntingtin is independent of polyglutamine aggregation. J Biol Chem 276:48417–48424
228. Bailey CK, Andriola IF, Kampinga HH, Merry DE (2002) Molecular chaperones enhance the degradation of expanded polyglutamine repeat androgen receptor in a cellular model of spinal and bulbar muscular atrophy. Hum Mol Genet 11:515–523
229. Howarth JL, Kelly S, Keasey MP, Glover CP, Lee YB, Mitrophanous K, Chapple JP, Gallo JM, Cheetham ME, Uney JB (2007) Hsp40 molecules that target to the ubiquitin-proteasome system decrease inclusion formation in models of polyglutamine disease. Mol Ther J Am Soc Gene Ther 15:1100–1105
230. Kobayashi Y, Kume A, Li M, Doyu M, Hata M, Ohtsuka K, Sobue G (2000) Chaperones Hsp70 and Hsp40 suppress aggregate formation and apoptosis in cultured neuronal cells expressing truncated androgen receptor protein with expanded polyglutamine tract. J Biol Chem 275:8772–8778
231. Branco J, Al-Ramahi I, Ukani L, Perez AM, Fernandez-Funez P, Rincon-Limas D, Botas J (2008) Comparative analysis of genetic modifiers in Drosophila points to common and

distinct mechanisms of pathogenesis among polyglutamine diseases. Hum Mol Genet 17:376–390
232. Kazemi-Esfarjani P, Benzer S (2000) Genetic suppression of polyglutamine toxicity in Drosophila. Science 287:1837–1840
233. Bilen J, Bonini NM (2007) Genome-wide screen for modifiers of ataxin-3 neurodegeneration in Drosophila. PLoS Genet 3:1950–1964
234. Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM (1999) Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. Nat Genet 23:425–428
235. Vacher C, Garcia-Oroz L, Rubinsztein DC (2005) Overexpression of yeast hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease. Hum Mol Genet 14:3425–3433
236. Cummings CJ, Sun Y, Opal P, Antalffy B, Mestril R, Orr HT, Dillmann WH, Zoghbi HY (2001) Over-expression of inducible HSP70 chaperone suppresses neuropathology and improves motor function in SCA1 mice. Hum Mol Genet 10:1511–1518
237. Adachi H, Katsuno M, Minamiyama M, Sang C, Pagoulatos G, Angelidis C, Kusakabe M, Yoshiki A, Kobayashi Y, Doyu M, Sobue G (2003) Heat shock protein 70 chaperone overexpression ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model by reducing nuclear-localized mutant androgen receptor protein. J Neurosci 23:2203–2211
238. Kondo N, Katsuno M, Adachi H, Minamiyama M, Doi H, Matsumoto S, Miyazaki Y, Iida M, Tohnai G, Nakatsuji H, Ishigaki S, Fujioka Y, Watanabe H, Tanaka F, Nakai A, Sobue G (2013) Heat shock factor-1 influences pathological lesion distribution of polyglutamine-induced neurodegeneration. Nat Commun 4:1405
239. Banerjee R, Beal MF, Thomas B (2010) Autophagy in neurodegenerative disorders: pathogenic roles and therapeutic implications. Trends Neurosci 33:541–549
240. Jimenez-Sanchez M, Thomson F, Zavodszky E, Rubinsztein DC (2012) Autophagy and polyglutamine diseases. Prog Neurobiol 97:67–82
241. Williams A, Jahreiss L, Sarkar S, Saiki S, Menzies FM, Ravikumar B, Rubinsztein DC (2006) Aggregate-prone proteins are cleared from the cytosol by autophagy: therapeutic implications. Curr Top Dev Biol 76:89–101
242. Ghavami S, Shojaei S, Yeganeh B, Ande SR, Jangamreddy JR, Mehrpour M, Christoffersson J, Chaabane W, Moghadam AR, Kashani HH, Hashemi M, Owji AA, Los MJ (2014) Autophagy and apoptosis dysfunction in neurodegenerative disorders. Prog Neurobiol 112:24–49
243. Harris H, Rubinsztein DC (2012) Control of autophagy as a therapy for neurodegenerative disease. Nat Rev Neurol 8:108–117
244. Sarkar S, Rubinsztein DC (2008) Huntington's disease: degradation of mutant huntingtin by autophagy. FEBS J 275:4263–4270
245. Ravikumar B, Imarisio S, Sarkar S, O'Kane CJ, Rubinsztein DC (2008) Rab5 modulates aggregation and toxicity of mutant huntingtin through macroautophagy in cell and fly models of Huntington disease. J Cell Sci 121:1649–1660
246. Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, Schwartz SL, DiProspero NA, Knight MA, Schuldiner O, Padmanabhan R, Hild M, Berry DL, Garza D, Hubbert CC, Yao TP, Baehrecke EH, Taylor JP (2007) HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature 447:859–863
247. del Cano-Espinel M, Acebes JR, Sanchez D, Ganfornina MD (2015) Lazarillo-related Lipocalins confer long-term protection against type I Spinocerebellar Ataxia degeneration contributing to optimize selective autophagy. Mol Neurodegeneration 10:11
248. Doi H, Adachi H, Katsuno M, Minamiyama M, Matsumoto S, Kondo N, Miyazaki Y, Iida M, Tohnai G, Qiang Q, Tanaka F, Yanagawa T, Warabi E, Ishii T, Sobue G (2013) p62/SQSTM1 differentially removes the toxic mutant androgen receptor via autophagy and inclusion formation in a spinal and bulbar muscular atrophy mouse model. J Neurosci 33:7710–7727

249. Chang YC, Lin CW, Hsu CM, Lee-Chen GJ, Su MT, Ro LS, Chen CM, Huang HJ, Hsieh-Li HM (2016) Targeting the prodromal stage of spinocerebellar ataxia type 17 mice: G-CSF in the prevention of motor deficits via upregulating chaperone and autophagy levels. Brain Res 1639:132–148
250. Bauer PO, Goswami A, Wong HK, Okuno M, Kurosawa M, Yamada M, Miyazaki H, Matsumoto G, Kino Y, Nagai Y, Nukina N (2010) Harnessing chaperone-mediated autophagy for the selective degradation of mutant huntingtin protein. Nat Biotechnol 28:256–263
251. Shibata M, Lu T, Furuya T, Degterev A, Mizushima N, Yoshimori T, MacDonald M, Yankner B, Yuan J (2006) Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1. J Biol Chem 281:14474–14485
252. Cunha-Santos J, Duarte-Neves J, Carmona V, Guarente L, Pereira de Almeida L, Cavadas C (2016) Caloric restriction blocks neuropathology and motor deficits in Machado-Joseph disease mouse models through SIRT1 pathway. Nat Commun 7:11445
253. Alves S, Cormier-Dequaire F, Marinello M, Marais T, Muriel MP, Beaumatin F, Charbonnier-Beaupel F, Tahiri K, Seilhean D, El Hachimi K, Ruberg M, Stevanin G, Barkats M, den Dunnen W, Priault M, Brice A, Durr A, Corvol JC, Sittler A (2014) The autophagy/lysosome pathway is impaired in SCA7 patients and SCA7 knock-in mice. Acta Neuropathol 128:705–722
254. Vig PJ, Shao Q, Subramony SH, Lopez ME, Safaya E (2009) Bergmann glial S100B activates myo-inositol monophosphatase 1 and Co-localizes to purkinje cell vacuoles in SCA1 transgenic mice. Cerebellum 8:231–244
255. Li X, Liu H, Fischhaber PL, Tang TS (2015) Toward therapeutic targets for SCA3: Insight into the role of Machado-Joseph disease protein ataxin-3 in misfolded proteins clearance. Prog Neurobiol 132:34–58
256. Seo H, Sonntag KC, Kim W, Cattaneo E, Isacson O (2007) Proteasome activator enhances survival of Huntington's disease neuronal model cells. PLoS ONE 2:e238
257. Jana NR, Dikshit P, Goswami A, Kotliarova S, Murata S, Tanaka K, Nukina N (2005) Co-chaperone CHIP associates with expanded polyglutamine protein and promotes their degradation by proteasomes. J Biol Chem 280:11635–11640
258. Matsumoto M, Yada M, Hatakeyama S, Ishimoto H, Tanimura T, Tsuji S, Kakizuka A, Kitagawa M, Nakayama KI (2004) Molecular clearance of ataxin-3 is regulated by a mammalian E4. EMBO J 23:659–669
259. Miller VM, Nelson RF, Gouvion CM, Williams A, Rodriguez-Lebron E, Harper SQ, Davidson BL, Rebagliati MR, Paulson HL (2005) CHIP suppresses polyglutamine aggregation and toxicity in vitro and in vivo. J Neurosci 25:9152–9161
260. Sugiura A, Yonashiro R, Fukuda T, Matsushita N, Nagashima S, Inatome R, Yanagi S (2010) A mitochondrial ubiquitin ligase MITOL controls cell toxicity of polyglutamine-expanded protein. Mitochondrion 11:139–146
261. Tsai YC, Fishman PS, Thakor NV, Oyler GA (2003) Parkin facilitates the elimination of expanded polyglutamine proteins and leads to preservation of proteasome function. J Biol Chem 278:22044–22055
262. Palazzolo I, Burnett BG, Young JE, Brenne PL, La Spada AR, Fischbeck KH, Howell BW, Pennuto M (2007) Akt blocks ligand binding and protects against expanded polyglutamine androgen receptor toxicity. Hum Mol Genet 16:1593–1603
263. Palazzolo I, Stack C, Kong L, Musaro A, Adachi H, Katsuno M, Sobue G, Taylor JP, Sumner CJ, Fischbeck KH, Pennuto M (2009) Overexpression of IGF-1 in muscle attenuates disease in a mouse model of spinal and bulbar muscular atrophy. Neuron 63:316–328
264. Alberch J, Perez-Navarro E, Canals JM (2004) Neurotrophic factors in Huntington's disease. Prog Brain Res 146:195–229
265. Demeestere J, Vandenberghe W (2011) Experimental surgical therapies for Huntington's disease. CNS Neurosci Ther 17:705–713
266. Ramaswamy S, McBride JL, Han I, Berry-Kravis EM, Zhou L, Herzog CD, Gasmi M, Bartus RT, Kordower JH (2009) Intrastriatal CERE-120 (AAV-Neurturin) protects striatal

and cortical neurons and delays motor deficits in a transgenic mouse model of Huntington's disease. Neurobiol Dis 34:40–50
267. An MC, Zhang N, Scott G, Montoro D, Wittkop T, Mooney S, Melov S, Ellerby LM (2012) Genetic correction of Huntington's disease phenotypes in induced pluripotent stem cells. Cell Stem Cell 11:253–263
268. Gaj T, Gersbach CA, Barbas CF 3rd (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31:397–405
269. Cox DB, Platt RJ, Zhang F (2015) Therapeutic genome editing: prospects and challenges. Nat Med 21:121–131
270. Richard GF, Dujon B, Haber JE (1999) Double-strand break repair can lead to high frequencies of deletions within short CAG/CTG trinucleotide repeats. Mol Gen Genet MGG 261:871–882
271. Richard GF, Viterbo D, Khanna V, Mosbach V, Castelain L, Dujon B (2014) Highly specific contractions of a single CAG/CTG trinucleotide repeat by TALEN in yeast. PLoS ONE 9: e95611
272. Mittelman D, Moye C, Morton J, Sykoudis K, Lin Y, Carroll D, Wilson JH (2009) Zinc-finger directed double-strand breaks within CAG repeat tracts promote repeat instability in human cells. Proc Natl Acad Sci U S A 106:9607–9612
273. Huang W, Zheng J, He Y, Luo C (2013) Tandem repeat modification during double-strand break repair induced by an engineered TAL effector nuclease in zebrafish genome. PLoS ONE 8:e84176
274. Stoddard BL (2011) Homing endonucleases: from microbial genetic invaders to reagents for targeted DNA modification. Structure 19:7–15
275. Puchta H, Fauser F (2014) Synthetic nucleases for genome engineering in plants: prospects for a bright future. Plant J Cell Mol Biol 78:727–741
276. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD (2010) Genome editing with engineered zinc finger nucleases. Nat Rev Genet 11:636–646
277. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8:2281–2308
278. Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32:347–355
279. Garriga-Canut M, Agustin-Pavon C, Herrmann F, Sanchez A, Dierssen M, Fillat C, Isalan M (2012) Synthetic zinc finger repressors reduce mutant huntingtin expression in the brain of R6/2 mice. Proc Natl Acad Sci U S A 109:E3136–E3145
280. Fink KD, Deng P, Gutierrez J, Anderson JS, Torrest A, Komarla A, Kalomoiris S, Cary W, Anderson JD, Gruenloh W, Duffy A, Tempkin T, Annett G, Wheelock V, Segal DJ, Nolta JA (2016) Allele-specific reduction of the mutant Huntingtin Allele using transcription activator-like effectors in human Huntington's disease fibroblasts. Cell Transplant 25: 677–686
281. Shin JW, Kim KH, Chao MJ, Atwal RS, Gillis T, MacDonald ME, Gusella JF, Lee JM (2016) Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. Human Mol Genet
282. Rodriguez-Lebron E, Liu G, Keiser M, Behlke MA, Davidson BL (2013) Altered Purkinje cell miRNA expression and SCA1 pathogenesis. Neurobiol Dis 54:456–463
283. Stanek LM, Yang W, Angus S, Sardi PS, Hayden MR, Hung GH, Bennett CF, Cheng SH, Shihabuddin LS (2013) Antisense oligonucleotide-mediated correction of transcriptional dysregulation is correlated with behavioral benefits in the YAC128 mouse model of Huntington's disease. J Huntington's Dis 2:217–228
284. Southwell AL, Skotte NH, Kordasiewicz HB, Ostergaard ME, Watt AT, Carroll JB, Doty CN, Villanueva EB, Petoukhov E, Vaid K, Xie Y, Freier SM, Swayze EE, Seth PP, Bennett CF, Hayden MR (2014) In vivo evaluation of candidate allele-specific mutant huntingtin gene silencing antisense oligonucleotides. Mol Ther J Am Soc Gene Ther 22:2093–2106

285. Sun X, Marque LO, Cordner Z, Pruitt JL, Bhat M, Li PP, Kannan G, Ladenheim EE, Moran TH, Margolis RL, Rudnicki DD (2014) Phosphorodiamidate morpholino oligomers suppress mutant huntingtin expression and attenuate neurotoxicity. Hum Mol Genet 23:6302–6317
286. Evers MM, Tran HD, Zalachoras I, Meijer OC, den Dunnen JT, van Ommen GJ, Aartsma-Rus A, van Roon-Mom WM (2014) Preventing formation of toxic N-terminal huntingtin fragments through antisense oligonucleotide-mediated protein modification. Nucleic acid Ther 24:4–12
287. Toonen LJA, Rigo F, van Attikum H, van Roon-Mom WMC (2017) Antisense oligonucleotide-mediated removal of the polyglutamine repeat in spinocerebellar ataxia type 3 mice. Mol Ther Nucleic Acids 8:232–242

Chapter 21
Stem Cell-Based Therapies for Polyglutamine Diseases

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Abstract Polyglutamine (polyQ) diseases are a family of neurodegenerative disorders with very heterogeneous clinical presentations, although with common features such as progressive neuronal death. Thus, at the time of diagnosis patients might present an extensive and irreversible neuronal death demanding cell replacement or support provided by cell-based therapies. For this purpose stem cells, which include diverse populations ranging from embryonic stem cells (ESCs), to fetal stem cells, mesenchymal stromal cells (MSCs) or induced pluripotent stem cells (iPSCs) have remarkable potential to promote extensive brain regeneration and recovery in neurodegenerative disorders. This regenerative potential has been demonstrated in exciting pre and clinical assays. However, despite these promising results, several drawbacks are hampering their successful clinical implementation. Problems related to ethical issues, quality control of the cells used and the lack of reliable models for the efficacy assessment of human stem cells. In this chapter the main advantages and disadvantages of the available sources of stem cells as well as their efficacy and potential to improve disease outcomes are discussed.

Keywords NSCs · ESCs · MSCs · iPSCs and polyQ diseases

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21.1 Introduction

Polyglutamine (polyQ) diseases are progressive neurodegenerative diseases caused by abnormal overexpression of the CAG triplet, which encodes the aminoacid glutamine, in a specific gene to each disease. This results in a polyQ-expanded protein that causes numerous neuropathological and motor impairments. To date, 9 polyglutamine diseases have been identified: Huntington's disease, Spinobulbar Muscular Atrophy (SBMA), Dentatorubral-Palidoluisiana Atrophy (DRPLA), and the Spinocerebellar Ataxias (SCAs) type 1, 2, 3, 6, 7 and 17 [1, 2].

Stem cells comprise all the undifferentiated cells that have the capacity to self-renew and differentiate into different cell lineages. Thus, through continuous proliferation these cells maintain the pool of stem cells and undergo differentiation originating mature functional cells upon stimulation [3]. Stem cells are distinguished by their origin and the variety of cell types in which they can differentiate (Fig. 21.1). Thus, concerning to the variety of cells originated upon differentiation, these cells can be classified as (i) totipotent stem cells, which give rise to all the cells of the three germ layers (endoderm, ectoderm and mesoderm), as well as the support structures needed for embryo/fetal development, such as the placenta; (ii) pluripotent stem cells, which can also originate cells of the three germ layers, are not able to form the support structures, and therefore, per se do not originate a living being; (iii) multipotent stem cells, which are more compromised in a cellular lineage and consequently can only give rise to cells of one germ layer; finally (iv) unipotent cells, which differentiate into only one cell type [3, 4]. Relatively to the origin, totipotent stem cells are formed after fertilization and can be obtained from the morula. Pluripotent stem cells can be found in the inner cell mass of the embryos' blastocyst or can be obtained by cell reprogramming of differentiated cells into induced pluripotent stem cells (iPSCs). Multipotent neural stem cells (NSCs) can be obtained by neural induction of pluripotent cells and can also be directly isolated from fetal or adult nervous system, however in adults the amount of available stem cells is much smaller [3, 5, 6]. Thus, there are several sources of stem cells available to be used in research and hopefully with potential to be used in clinical practice in a near future.

In fact, stem cell-based therapies have been growing in the past years as one of the most promising approaches for therapy of neurodegenerative diseases as a result of significant accelerating factors, such as major studies on neurodevelopment and adult neurogenesis conducted in mammals [7–11] and specially since the discovery of the presence of neural stem cells in the human adult brain [12], and their role in neural plasticity and patterning [13]. The implementation of sources of stem cells capable of generating NSCs, such as human embryonic stem cells (ESCs) [14] and more recently human iPSCs [15] has also increased the therapeutic potential. Moreover, mesenchymal stromal cells application in neurodegenerative disorders in the past ten years revealed promising effects, mainly through the secretion of supportive factors [16].

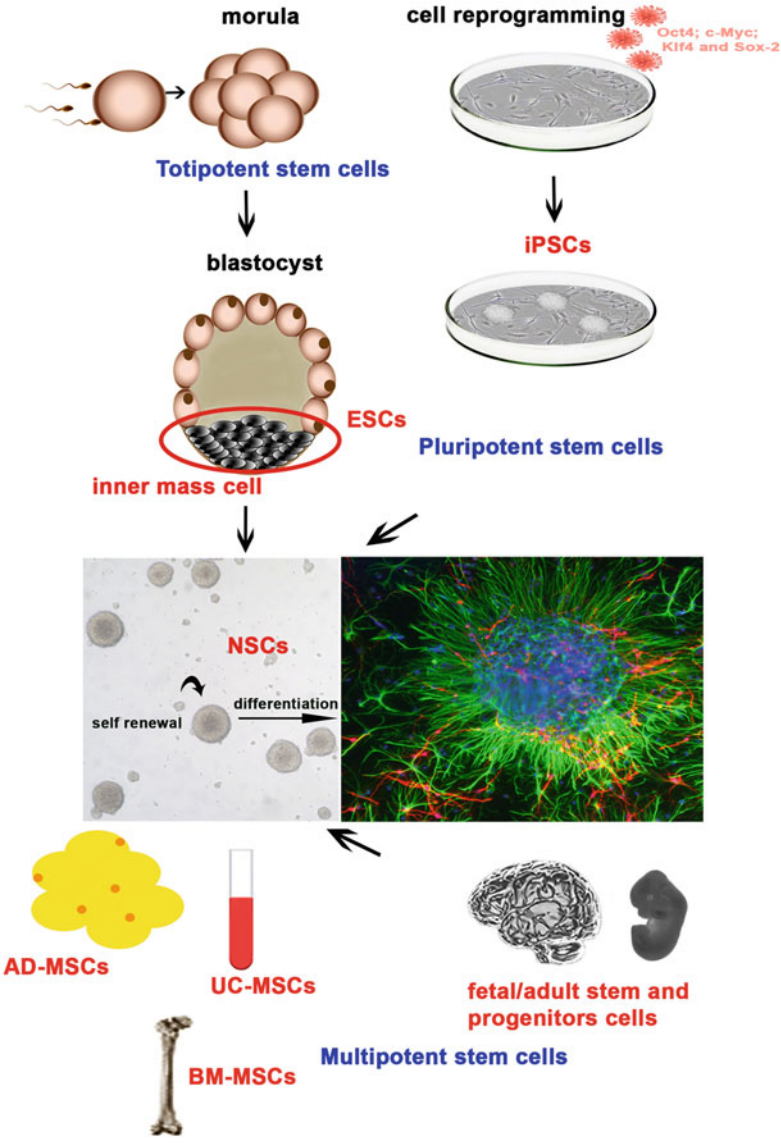

Fig. 21.1 Different sources of stem cells currently available. Totipotent stem cells can be extracted from the morula; Embryonic stem cells (ESCs) are pluripotent stem cells found in the inner cell mass of blastocysts; Induced pluripotent stem cells (iPSCs) are pluripotent stem cells obtained by cell reprogramming of differentiated cells; Neural stem cells (NSCs) are multipotent stem cells that can be obtained by neural induction of pluripotent stem cells or isolated from fetal or adult nervous system; Mesenchymal stromal cells are multipotent adult stem cells of the mesoderm lineage, present in several sources, such as adipose tissue (AD-MSCs), Bone marrow (BM-MSCs) and umbilical cord (UC-MSCs)

The first clinical trials using stem cells were performed in the mid-1980s and a total of 300–400 Parkinson's disease (PD) patients worldwide have now been submitted to fetal cell transplantation, showing an improvement in symptoms after the surgery, as well as increased dopaminergic neuronal function [17, 18]. The success of these experiments greatly increased the hopes and expectations laid on these cell-based strategies.

Therefore, in this chapter, different sources of stem cells, namely, embryonic, fetal, mesenchymal and iPSCs are discussed in terms of their advantages and disadvantages as tools for stem-cell based approaches aiming at the development of new disease models and therapeutic strategies for polyQ diseases. Moreover, several pre-clinical and clinical studies conducted using stem cell-based approaches are evaluated and the main outcomes and conclusions are highlighted, namely graft survival and functional integration after transplantation, the impact of the transplantation in the host neuropathology and motor impairments, as well as significant secondary effects.

21.2 Neural Stem and Precursor Cells

Neural stem cells are multipotent stem cells committed to the neuro-ectoderme lineage. Therefore, upon differentiation give rise to neural cells, such as neurons, astrocytes and oligodendrocytes. These cells can be isolated from the nervous system of fetuses and adults, namely from the canonical neural stem cell niches: the subventricular zone (SVZ) [19], and the subgranular zone (SGZ) within the dentate gyrus of the hippocampus or other regions, such as the hypothalamus [20] or the cerebellum [21].

This source of stem cells has been used in several clinical and pre-clinical assays. Chintawar and collaborators transplanted neural precursor cells isolated from SVZ into SCA1 mice and observed an improvement of motor impairments as well as reduction of Purkinje cells (PCs) loss and cellular layer thickness preservation [22]. Mendonça and colleagues recently evaluated the effect of adult cerebellar NSCs transplantation into the cerebellum of Machado-Joseph disease (MJD) transgenic mice. Significant and robust improvement of the MJD-associated neuropathology, namely reduction of PCs loss, reduction of cellular layer shrinkage and mutant ataxin-3 aggregates as well as alleviation of motor behaviour impairments were observed. Moreover, a significant reduction of neuroinflammation and an increase of neurotrophic factors levels were observed, indicating that transplantation of cerebellar NSCs triggers important neuroprotective effects [21].

In clinical research the preferential source of neural stem cells used so far were isolated from fetuses. In HD, for example, the main source region of the fetal brain is the lateral ganglionic eminence, given the fact that these cells can spontaneously differentiate into striatal medium spiny neurons MSNs, which suffer extensive degeneration in HD [23]; reviewed in [24]. Indeed, a number of preclinical studies initially reported the efficacy of human fetal striatal tissue to provide functional

recovery in a variety of rodent and nonhuman primate models of striatal neuronal loss [25–28]. The first report regarding fetal transplantation in HD, dates back from 1995 by Madrazo and colleagues, who performed fetal striatal transplantations in two young HD patients [29]. Patients were implanted into the ventricular wall of the right caudate nucleus with striata from human fetuses. The main reported outcome of this study was a slower progression of disease in both patients comparing to their preoperative state [29]. Later, other successive implants were carried out in three moderately advanced HD patients, who received striatal tissue of 8–10 week old human fetuses, selectively obtained from the lateral ganglionic eminence [30, 31]. Each patient was transplanted with bilateral grafts from 5 to 8 donors, placed into the caudate nucleus and the putamen. One year after transplantation, graft survival and growth within the striatum was observed by magnetic resonance imaging (MRI) in all the three patients. Furthermore, none of the patients showed major side effects. The patients died 74, 79 and 121 months after transplantation and their autopsies showed that the grafts survived well in two patients, with evidence of mature donor-derived neuronal differentiation and no signs of intranuclear inclusions in grafted neurons, although with little integration into the host striatum and a limited descriptive assessment of clinical outcomes [30, 32, 33].

Bachoud-Lévi and colleagues undertook a remarkable clinical trial in this field in 2000. This study was the first to follow the CAPIT-HD (the Core Assessment Protocol for Intracerebral Transplantation in HD), which defines a standardized set of neurological, neuropsychiatric and imaging assessments at defined intervals over a minimum of 1 year pre-operatively and 2 years post-operatively [34]. Five HD patients were transplanted with grafts composed of whole ganglionic eminence dissections from 1 to 2 human fetuses with 7.5–9.5 week-old, implanted bilaterally as tissue fragments into multiple caudate and putamen sites. Approximately 1-year interval separated the surgeries on the two sides [35]. Three of the five tested HD patients revealed an effective improvement on motor and cognitive functions and a clinical stabilization two years after the last transplantation, associated with a positive graft signal detected by MRI and fluorodeoxyglucose positron emission tomography (FDG-PET) and restoration of lost sensory-evoked potentials in electrophysiological tests [36]. A fourth patient exhibited no positive response and showed no signs of graft survival in the physiological and imaging tests. The fifth patient initially showed signs of recovery, however suffered a complete relapse immediately after an acute fever, followed by loss of MRI signal in the grafted region and loss of physiological signs of graft survival, raising the possibility of its rejection. In the case of these patients, PET imaging of glucose has given important clinical information, revealing a reduction on striatal and cortical hypometabolism in the three patients that showed functional stabilization, while in the other two, who did not respond to graft transplantation, there was a progression of the metabolic abnormalities [36]. Nevertheless, 4–6 years after neural grafting, the early benefits from the treatment had largely been lost [37].

Less positive results have been reported in another trial involving seven symptomatic advanced HD patients, who were bilaterally transplanted with micro-dissected lateral ganglionic eminence from 8 to 12 human fetal brains into

the posterior putamen [38]. From these seven HD transplanted patients, six-showed moderate improvement in Unified Huntington's Disease Rating Scale (UHDRS) scores, while one patient presented the characteristic decline associated to the disease. Three patients developed subdural hematomas after the surgery, which may be correlated with the advanced stage of the disease in comparison to other studies. This suggested that the implantation of tissue into the already extensively degenerated basal ganglia might increase the risk of severe side effects compared to surgeries in patients at an earlier stage of the disease [35]. One of the patients died after 18 months of causes unrelated to the surgery. This allowed a detailed anatomical analysis of the *post-mortem* brain, which revealed healthy bilaterally surviving grafts, with differentiation of the grafted cells into a mature striatal-like tissue mass containing all striatal cell phenotypes examined. However, despite the use of multiple donors, only 8–10% of the striatal region transplanted was engrafted [38, 39]. Moreover, in general no clinical improvements suggestive of a functional integration of the graft within the host striatum were observed in the transplanted patients [40]. A decade after the neural transplants, the brains of three subjects with HD were evaluated at autopsy by immunohistochemistry and electron microscopy for markers of projection neurons, inflammatory cells, abnormal huntingtin protein and host-derived connectivity [41]. In brief, surviving grafts were identified in two of the patients and presented classic striatal projection neurons and interneurons, with no genetic markers of HD being detected withing the graft. However, the authors reported that: (i) long-term graft survival was decreased; (ii) grafts underwent disease-like neuronal loss with a preferential degeneration of projection neurons; (iii) immunologically unrelated cells died faster comparing to the patient's neurons subtype and (iv) microglial inflammatory in the grafts specifically targeted the neuronal components of the grafts. The combination of these observations raised important challenges about the potential therapeutic of this approach for the treatment of HD [41].

The European Network for Striatal Transplantation in HD accomplished a clinical safety study in a United Kingdom-based multicenter trial [42]. This study aimed to evaluate the safety of unilateral intrastriatal dissociated cell suspension of whole human fetal ganglionic eminence in the caudate and the putamen of four patients with mild to moderate HD. Six months after transplantation, MRI did not show any feature of uncontrolled or invasive growth of the implanted cells in any of the grafted patients. Moreover, no major adverse effects related to cell transplantation were observed [42]. The long-term follow up over 3–10 years after the surgery, showed that there were no differences found over time between the grafted and non-grafted patients in any sub score of the UHDRS, although a tendency to a slower progression on some motor tasks in transplanted patients was observed. The main conclusion of this study was that fetal striatal allografting in HD is safe, however, without observable functional gains to the patients [43].

A pilot study was also undertaken to address the utility of fetal cell grafts involving two patients with moderate HD [44]. Both patients were transplanted with bilateral fetal striatal allografts of 2–3 ganglionic eminences. One of the patients showed prolonged clinical motor amelioration during a 5-year period and

increased striatal D2 receptor binding, evident with raclopride (RAC-) PET, suggesting long term survival and efficacy of the graft. However, the other patient did not demonstrate evidence of either clinical improvement or functional grafting, with a similar decline in motor function to that of 6 untreated controls [44].

More recently, Gallina and colleagues reported the results of a clinical study involving four patients with moderate to highly advanced HD that were bilaterally transplanted with human fetal striatal tissues (9–12 weeks gestation) [45]. Small blocks of whole ganglionic eminencies were dissected to obtain a cell suspension that was stereotaxicly grafted into the caudate head and the putamen of these patients. The follow-up period allowed concluding that all patients showed stabilization or improvement in some neurological indices, as well as neo-generation of metabolically active tissue with striatal-like MRI features in 6 out of 8 grafts. In addition, the increase in D2 receptor binding was suggestive of striatal differentiation of the newly-generated tissue in 3 patients. Remarkably, new tissue connecting the developing grafts with the frontal cortex and, in one case, with the ventral striatum, was also detected. No clinical and imaging signs indicating uncontrolled graft growth were observed [45]. This study provided some reliable results supporting both the reconstructive potential of fetal tissue and the capacity of the adult brain to retain plasticity.

Overall, transplantation of fetal stem cells triggers several beneficial mechanisms such as the upregulation of intrinsic mechanisms of cell proliferation and neuroprotection. Furthermore, through the integration into the endogenous host network these cells can replace or repair the lost/damaged neurons. Nevertheless, the use of fetal tissue has raised ethical concerns with respect to the donor and the recipient [46], as well as safety issues based on adverse effects of neurografting [47], namely grafting overgrowth, cyst development and the occurrence of subdural hematomas [32, 38]. In addition, the limited number of HD patients that have been treated with fetal neural tissue transplantation and particularly, the differences in the employed protocols, brought even more difficulties to make a definitive conclusion about the value and long-term safety of these procedures for HD [24]. Moreover, there is a considerable heterogeneity on the obtained outcomes and the benefits from these implants seem to be temporary [32, 37, 41]. As a result, there is a need for larger clinical trials in these patients and urgent efforts on the standardization of techniques regarding isolation, handling and transplantation of the intended cell type [48].

21.3 Embryonic Stem Cells

Problems such as the insufficient availability of fetal donor cells and their heterogeneity have been hindering the progression of cell replacement therapies for neurodegenerative diseases. This led to the use of alternative stem cell sources. For that reason, human ESCs have been considered a renewable potential source for these neurons and their progenitors.

ESCs are pluripotent stem cells derived and expanded from the inner cell mass of the blastocyst stage embryo and have the potential to proliferate extensively in vitro and differentiate into any cell type of the human body, including neurons [49–51].

Aubry and colleagues performed the first studies in the transplantation of human neural cells differentiated from ESCs in animal models of HD, aiming at disclosing the best cellular differentiation stage for grafting [52]. To achieve this goal, the authors transplanted ESC-derived neural cells at different phases, ranging from neural precursor rosettes to differentiated striatal MSNs, into a quinolinic acid (QA)-induced HD rat model. The main outcome of this study was the development and validation of a protocol to direct the differentiation of human ESCs into neurons that exhibited phenotypic features of MSNs, both in vitro and in vivo following xenografting. Despite the huge amount of interesting data, the authors showed that cells transplanted at the early stages of the differentiation protocol presented a strong tendency to originate teratoma-like masses, while differentiated cells showed a reduced survival [52].

In 2012, Ma and co-workers conducted a notable study, where human ESCs-derived GABAergic neuronal progenitors were transplanted into the QA-induced HD mice. Four months later, the authors observed that approximately 60% of the total grafted cells were positive for the neuronal marker DARPP32. Remarkably, the transplanted cells received dopaminergic and glutamatergic inputs and projected correctly to the *substantia nigra*, which was associated with an improvement in the motor performance of these animals [53].

Kaemmerer and colleagues assessed ESCs therapeutic potential in heterozygous SCA-1 transgenic mice that received bilateral cerebellar transplants of dissociated cerebellar cells from E13 to E15 WT embryos of a syngeneic mouse brain. Results showed that engrafted Purkinje cells were identified in 9 of the 12 transplanted animals for as long as 20 weeks, which targeted the deep cerebellar nuclei, even though with a variable localization between them. Interestingly, data from behavioural tests showed that the cerebellar grafts provided a beneficial outcome in motor performance of the animals where the engraftment occurred for 20 weeks [54].

Carri and collaborators have undertaken a study where human ESC-derived striatal precursors exposed to sonic hedgehog (SHH) and dickkopf-1 (DKK-1; a WNT signaling pathway inhibitor) were grafted into the QA-induced HD rats. The authors confirmed the differentiation of the transplanted cells into DARPP32-positive neurons and observed temporary improvements in apomorphine-induced rotational behaviour, concomitant with the presence of the human cells at 9 weeks post-transplantation [55].

Recently, Arber et al. [56] showed in the QA-induced HD rat model that neuronal progenitors derived from human ESCs exposed to activin-A [a multifunctional TGFβ family protein that has been shown to induce forebrain neurogenesis [57, 58], readily differentiated into postmitotic neurons expressing DARPP32, both in culture and following transplantation. Furthermore, 16 weeks post-transplantation, immunoreactivity for human DARPP32 and GABBA was still

observed, as well as dopaminergic and glutamatergic fibres surrounding the grafted cells. Small amounts of human neural cell adhesion molecule (hNCAM) positive fibers in the *globus pallidus* and midbrain were also found [56]. Despite the encouraging pre-clinical results obtained with ESCs, to the best of our knowledge, no clinical trials were or are being performed with ESCs or ESC-derived cells for polyQ diseases.

In summary, ESCs allow adequate cell expansion, and upon transplantation trigger mechanisms of neuroprotection as well as cell-replacement, allowing the change or repair of lost/damaged neurons. However, several issues need to be addressed that may hinder the clinical use of human ESC-derived cells at this current level of technology. This is mainly related to the formation of teratomas or neuroepithelial tumours induced by ESC-derived grafts, which seems to be dependent on cell's differentiation degree prior to transplantation [52]. Several questions remain open, such as the maximum acceptable number of pluripotent cells in the graft to reach a negligible probability of tumor formation. Furthermore, we should take into account that these studies were conducted in rodents and did not include long survival periods necessary to rigorously assess tumorigenic potential. Thus, a detailed characterization and comparison of graft behaviour according to the differentiation level of transplants should also be included in future studies. Finally, the functional effect of the grafts needs to be carefully explored in order to evaluate the therapeutic potential of the transplants and future applications in neurodegenerative disorders.

21.4 Induced Pluripotent Stem Cells

In 2006 Takashi and Yamanaka [6] tested 24 genes, selected for their pivotal roles in the maintenance of ESCs identity, and found that the overexpression of a combination of 4 reprogramming factors (RF) was sufficient to trigger a cascade of events that gave rise to pluripotent stem cells, the so-called iPSCs. This successful derivation of iPSCs from adult mouse fibroblasts through the ectopic co-expression of only 4 reprogramming factors (OCT4/Pou5f1, SOX2, KLF4 and c-MYC) revolutionized the field of pluripotent stem cells. For this discovery Shinya Yamanaka has been awarded, in 2012, with the Nobel Prize in physiology or medicine jointly with John Gurdon. Cell reprogramming was quickly applied to human cells and, in 2007, human iPSCs were generated by the same group [15] through transducing human dermal fibroblasts with retroviral vectors carrying the same reprogramming factors. In the same year, Yu and colleagues [59] also generated human iPSCs by transducing human fibroblasts with lentiviral vectors encoding the factors OCT4, SOX2, NANOG and LIN28. The generated iPSCs shared the main properties of hESCs, including the unlimited self-renewal and the potential to differentiate into cells of the three germ layers, being molecularly and functionally very similar [6, 59].

Many iPSCs-derived neuronal models have been developed to study and characterize monogenic neurodegenerative diseases in the last years. The first iPSCs-based disease models for neurodegenerative disorders were generated for amyotrophic lateral sclerosis (ALS) [60] and spinal muscular atrophy (SMA) [61] and since then many other models of neurodegenerative disorders, particularly of the PolyQ diseases, have been produced, as presented in Table 21.1.

Neuronal models of PolyQ diseases obtained from differentiation of patients' iPSCs prove to be accurate human in vitro models if the expression of the mutant protein associated with the genetic mutation linked to the disease pathogenesis is conserved. Most of the disease phenotypes reported for the obtained neuronal models were early phenotypes, associated with increased neuronal sensitivity to cellular stress, failure of survival pathways as autophagy and apoptosis and impaired maturation and complexity of neuronal processes. Drug screening, evaluation of molecular therapy approaches involving gene silencing, overexpression or correction along with disease modeling are the most immediate applications of these models as they prove to be valuable human in vitro predictive tools to elucidate disease mechanisms and to obtain therapeutic tools efficacy and toxicity

Table 21.1 iPSCs-derived in vitro neuronal models

Neurodegenerative disease	Reprogramming method	iPSC-derived cell subtype	Reported phenotype	References
Huntington's disease	RV: OSKM SF Episomal OSKML siRNA p53 SF	NPCs, neurons, Striatal neurons	Increased lysosomal activity and susceptibility to stress and toxicity Huntingtin aggregates upon proteasome inhibition	[68, 73, 76, 81, 140, 141, 145]
SBMA/DRPLA	LV: OSKM SF	Motor neurons	Increased acetylated α-tubulin AR aggregation	[65, 66]
SCA2	RV: OSKM SF	Neurons	Decreased survival of neurons	[142, 143]
(SCA3/MJD)	RV: OSKM SF LV: OSKM	Neurons	Insoluble ataxin-3 aggregates upon glutamate excitation Autophagy impairment microRNA dysregulation	[67, 78, 79]
SCA7	RV: OSKM SF	Neurons	NA	[144]

Legend: *RV* retrovirus; *OSKM* Oct4, Sox2, Klf4, c-Myc; *SF* skin fibroblasts; *LV* lentivirus; *AR* androgen receptor; *NA* not applicable

data in preclinical testing of candidate therapies [62–64]. Several compounds have been tested in iPSCs-derived neuronal models, as in the cases of SBMA [65, 66] and MJD [67].

Neurons derived from patients with late onset disorders can reveal early mechanisms prior to the development of "downstream" phenotypes, such as the altered gene expression and protein processing/aggregation that may only be observed in fully mature and aged neurons. Several methods are employed to accelerate the maturation and aging of human iPSCs-derived neurons enabling the study of disease phenotypes in manageable time frames. The use of stressors [68], the overexpression of proteins implicated in cellular aging regulation [69] and the induction of excitotoxicity [67] are the most common techniques that have been successfully used in several studies [70].

Ageing is associated with increased oxidative stress [71] and therefore in an effort to mimic stress-induced changes that occur during normal aging, oxidative stressors were used in iPSCs-derived neuronal models of late-onset diseases to produce the ageing effect in vitro and to induce age-dependent phenotypes. Hydrogen peroxide and sodium arsenite have been used to induce the production of reactive oxygen species in HD [68] iPSCs-derived neuronal models. In these studies, disease-related susceptibility phenotypes were uncovered, where patient-specific neurons were more susceptible to stress-induced death than control neurons. Other classes of stressors used to unveil late-onset neuronal phenotypes are the proteasome inhibitors such as MG132 [72–75] and autophagy inhibitors such as 3-methyladenine [68], chloroquine [76] and ammonium chloride [77] that contribute to protein aggregation and proteotoxicity, hallmarks of neurodegeneration in the established neuronal models.

Our group has shown that early cultures of MJD iPSCs-derived neurons reveal autophagy impairment, upon treatment with the autophagy inhibitor chloroquine [78]. We found increased levels of p62 and LC3 for both control and MJD cultures, being this accumulation more pronounced and significative in MJD cultures, suggesting failure in clearance mechanism as reported for other neurodegenerative in iPSCs-derived in vitro models [77]. The same cultures also presented a dysfunctional phenotype, regarding microRNA dysregulation (downregulation of mir-181a and mir-494) [79].

Excitotoxicity is another pathological hallmark of neurodegenerative diseases and neurotoxicity can be induced by excessive concentrations of glutamate, the major excitatory neurotransmitter in the central nervous system [80]. Therefore, another method to mimic in vivo ageing in vitro is the exposure of iPSCs-derived neurons to high extracellular concentrations of glutamate, as demonstrated using iPSCs derived from HD [68, 81] and MJD patients [67, 82]. The neurons derived from patients' iPSCs presented higher sensitivity and cell death dependent on glutamate treatment as compared to controls. In the MJD model, it was demonstrated that glutamate-induced excitotoxicity mediated by increased levels of Ca^{2+} activated proteolysis and aggregation of the ataxin-3 protein, reinforcing the role of aggregation in MJD pathogenesis [67, 82].

For future research, it is important to improve the assessment of the in vitro maturation and aging of neurons to further define the disease-specific molecular signature and correlate it with emerging disease phenotypes. The improved predictability of these iPSCs-derived neuronal models is an essential requirement and will help to support these models as essential tools to provide significant understanding of disease mechanisms and to identify new therapies for patients.

Cellular therapy based on the transplantation of iPSCs or their neuronal derivatives is still on early stages, with few published works on polyQ diseases. Some studies on HD indicate that the transplantation of iPSCs [83, 84] was successful in the recovery of striatal lesions caused by 3-NP and QA in rat models. Moreover, the intrastriatal transplantation of rat iPSCs in the 3-NP Huntington's rat model [83] led to preservation of the neuronal population and motor and metabolic functions. Similar results were found for the QA-induced HD rat model [84], where it was demonstrated that mouse iPSCs could survive, migrate and differentiate into neurons and glia after transplantation alleviating striatal atrophy and improving functional recovery.

There is some controversy surrounding the transplantation of iPSCs due to the propensity to form teratomas or the emergence of other tumors [85, 86] and some common strategies are used to prevent this safety issue as the use of compounds that inhibit teratoma formation or the transplantation of no pluripotent iPSCs-derived cells as NSCs. Some small compounds such as quercetin and YM155 proved to be efficient for inhibiting teratoma formation after transplantation of human pluripotent stem cells (PSCs) and PSCs-derived cells without compromising the differentiation capability [85]. The most used strategy to overcome this issue and also direct the differentiation process towards the neuronal fate is the use of NSCs or NPCs taking inspiration from works in PD [87–89]. Also, the genetic stability of human iPSCs and derived cells is a major concern in the cell therapy application. Most of the aneuploidies found are associated to long-term cultures [90, 91] and karyotypes should be analyzed as close as possible to the actual differentiation/transplantation to avoid compromising the capability of differentiation or engraftment.

Taken together, the role of iPSCs and its neuronal derivatives in cellular therapy of neurodegenerative diseases presents itself as an opportunity, overcoming pressing issues as the immunological rejection and opening the possibility of autologous transplantation but with some challenges, mainly regarding the potential tumorigenicity and genetic stability of the transplants. Currently, the most immediate use of those cells is the creation of disease model platforms. In fact, there is an ongoing clinical trial (NCT00874783), focused on the development of iPSCs lines for several neurodegenerative diseases, including PolyQ diseases as HD and MJD, which highlights the importance of iPSCs and their neuronal derivatives as reliable in vitro models and source of stem cells for cell-based therapies.

21.5 Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) are adult multipotent cells that were initially identified in the bone marrow as a heterogeneous and small non-hematopoietic population of cells, which gives structural and physiological support for hematopoiesis [92]. They derive from the mesenchyme, the connective tissue that is originated from the mesoderm, and can then differentiated into tissues of mesoderm origin, such as bone, cartilage, tendon, adipose tissue, and muscle [93].

MSCs can in theory be isolated from almost all adult connective tissues that derive from the mesenchyme, but the most frequently used sources for MSCs isolation are the bone marrow (BM-MSCs), the adipose tissue (AD-MSCs) and the umbilical cord blood (UC-MSCs) [94]. The designation "mesenchymal stem cells" is also commonly used in the literature, but in 2005 the committee of the International Society for Cytotherapy suggested "mesenchymal stromal cells" as the most appropriate designation for these cells [95]. MSCs have no major ethical concerns regarding their use in clinics and are considered safe, given that they have low immunogenicity, due to low expression of major histocompatibility complex antigens [96], and are not prone to form tumors upon transplantation [97]. Their boundless therapeutic potential comes essentially from their strong paracrine effects, as MSCs can secrete factors capable of helping injured tissue to recover or otherwise to protect neuronal cells from death and damage. Among other features, MSCs can provide trophic support, control immunological processes, thereby decreasing neuroinflammation [98], and secrete extracellular vesicles such as exosomes, that are thought to carry miRNAs and other molecules from MSCs to neuronal cells, thus controlling important cellular processes such as growth, death, transcription or synaptogenesis [99].

21.5.1 AD-MSCs

The therapeutic potential of AD-MSCs in polyQ diseases, has been reported in three studies using HD models. Lee and collaborators [100] observed that human AD-MSCs could slow striatal degeneration and alleviate behavioral impairments in the QA-induced rat model and R6/2 transgenic mice. Additionally, multiple growth factors such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), and ciliary neurotrophic factor (CNTF) were increased. In order to evaluate AD-MSCs transplantation effect in long-lived HD mice the same group used the YAC128 transgenic model [101]. Comparison of the efficacy of AD-MSCs isolated from the subcutaneous adipose tissue of an HD patient and a healthy donor showed reduction of striatal atrophy and motor improvements for the latter, but not upon transplantation of HD AD-MSCs. Hosseini and collaborators used AD-MSCs purified from liposuction of healthy donors that were injected 7 days post lesion into the lesioned

striatum of QA-induced HD rats [102]. A significant decrease of motor impairments and anxiety levels was observed in treated rats.

Thus, these studies demonstrated that AD-MSCs transplantation triggers promoted both neuropathological and phenotypic alleviation in different HD models. Nevertheless, their potential is dependent on the time window of administration [100, 101]. Moreover, AD-MSCs isolated from HD patients may not be as effective as AD-MSCs isolated from healthy donors, probably due to a lower paracrine effect in vivo in the case of HD AD-MSCs [101].

21.5.2 BM-MSCs

The potential of BM-MSCs has also been assessed by Edalatmanesh and colleagues in a QA-induced lesion in the cerebellum (folia VI) of rats [103]. Transplantation of rat BM-MSCs was performed directly into the lesion site, 48 h after QA injection. Deficits in motor and cognitive functions were diminished in BM-MSCs treated rats, from two to six weeks after transplantation. Transplantation of human BM-MSCs from healthy donors in the right side of the striatum of 2-months old transgenic N171-82Q HD mice [104] mediated preservation of the volume of the striatum and increased expression of the neurotrophic factors (fibroblast growth factor (FGF); CNTF; vascular endothelial growth factor (VEGF) and NGF). Moreover, induced neurogenesis and neural differentiation of the host endogenous NSC was also observed.

Ex vivo gene therapy approaches have also been explored by engineering BM-MSCs to overexpress BDNF and glial cell line-derived neurotrophic factor (GDNF) (NTF^+ cells) [105, 106], which promoted improvement of both neuropathological and behavioral impairments [16]. Identical results were obtained with human BM-MSCs isolated from healthy donors and from HD patients, in opposition to what was shown for AD-MSCs [101]. Additionally, NTF^+ MSCs were more effective in preserving striatal volume, as compared to naïve MSCs, and in decreasing apomorphine induced rotational behavior. Using a similar approach, Dey and collaborators engineered BM-MSCs to overexpress BDNF (BDNF-BM-MSCs) or NGF (NGF-BM-MSCs) and each of these cell types or a mix of both, were injected into the striatum of 4-month old YAC128 transgenic and wild type mice [107]. Nine months after transplantation, mice treated with BDNF-BM-MSCs showed less neuronal loss and better motor performance.

Lin and collaborators investigated the potential alleviation of HD by human BM-MSCs immortalized by retrovirus-mediated gene transfer of human telomerase reverse transcriptase (hTERT) combined with human papillomavirus E6 and E7 [108]. Two different HD mouse models, the QA-induced model and the R6/2 transgenic mice, were used. In the QA-induced model, BM-MSCs injection in the lesioned striatum, mediated alleviation on motor impairments, induction of neural proliferation and differentiation, as well as recruitment of microglia, neuroblasts and bone marrow-derived cells to the lesion site. Furthermore, a partial recovery of the

striatum volume, decrease in apoptosis and increased life-span was observed. In the R6/2-J2 mice, BM-MSCs were transplanted in the striatum at 12 weeks of age. The observed effects were very similar to the effects observed in the QA-induced model, except for the motor performance, which did not present any significant improvement.

A second chemically-induced HD model—the 3NP rat model was also used to evaluate the therapeutic potential of BM-MScs [109]. BM-MSCs were isolated from adult non-syngeneic Sprague–Dawley rat bone marrow and transplanted into the striatum, 28 days after the first administration of 3NP. The treatment with low (3NP + TPlow, 200,000 MSCs) or high number of BM-MSCs (3NP + TPhigh, 400,000 MSCs) was compared. All treated groups had the lateral ventricles less enlarged than 3NP rats. The 3NP + TPlow group showed improved motor performance. Surprisingly, the 3NP + TPhigh group did not achieve any improvements in the motor performance. The authors explain this result suggesting that above an optimal number of BM-MSCs, the striatal cytoarchitecture might be disrupted and cause additional neuronal injury, thus diminishing the beneficial net effects of BM-MSCs. No neuronal differentiation was observed, in opposition to previous reports [108]. Furthermore, BM-MSCs transplantation promoted over-expression of BDNF, collagen type-I and fibronectin. Thus, the improvements achieved in this HD rat model seem to be due to paracrine factors secretion. Therefore, BDNF appears to be a common potential neurotrophic factor behind the action of both BM-MSCs and AD-MSCs in HD models [16, 100, 107].

Recently, the same authors investigated the role of passage number on treatment efficacy. MSCs isolated from mice bone marrow were grown for low (3 to 8) or high (40 to 50) number of passages and transplanted into the striatum of 5-week-old R6/2 mice. As previously reported [109], no neuronal or glial differentiation was observed. Higher mRNA levels of BDNF were present in the striatum of transplanted BM-MSCs mice. However, no difference in the mRNA BDNF levels was observed between mice transplanted with low or high number of passages. Nevertheless, improved striatal metabolic activity and behavioral performance were observed for the mice transplanted with the cells at higher of passage number. Therefore, this report demonstrates that the time that BM-MSCs are kept in culture before transplantation can alter their efficacy [110].

The relevance of the administration pathway has also been investigated by transplanting human BM-MSCs intravenously or intracranially in SCA-2 transgenic mice [111]. Several intravenous infusions of human BM-MSCs alleviated motor impairments, while intracranial administration of these cells failed to produce beneficial effects. Moreover, intravenous treatment was more effective in inducing PCs survival than intracranial transplantations. To assess the potential of BM-MSCs in treating SCA-1 a single intrathecal injection of BM-MSCs derived from C57/Bl6 was performed into SCA-1 transgenic mice [112]. The results showed that this approach could alleviate motor phenotype and neuropathology, namely by partially restoring the PCs layer organization, 15 weeks after the treatment.

21.5.3 UC-MSCs

UC-MSCs application to polyQ diseases have been assessed, to best of our knowledge, in a single study, in which UC-MSCs of C57/BL6 mice pups with low- (3 to 8) or high-passage (40 to 50) MSCs were transplanted into 5-week-old R6/2 HD mice [113]. Six weeks after transplantation, a higher amount of transplanted cells were detected in R6/2 mice transplanted with high-passage UC-MSCs than in the group that received low-passage UC-MSCs. Thus, the augmentation of culture time increases the UC-MSCs survival rate. Moreover, in opposition to the previously described work of Rossignol and colleagues [109], both low- or high-passage UC-MSCs transplantation triggered less brain damage, as well as increased striatum metabolic activity. Nevertheless, no motor coordination improvements were observed, indicating that UC-MSCs might not be as effective as BM-MSCs in treating this HD model.

21.5.4 Clinical Trials with MSCs

Considering the promising pre-clinical results with MSCs in several polyQ diseases, clinical trials using MSCs have been performed to assess the safety and the efficacy of these cells transplantation. Most of the clinical trials already completed or that are running used UC-MSCs (Table 21.2).

One of the completed studies enrolled 14 SCA patients and 10 multiple system atrophy-cerebellar type patients (MSA-C). The UC-MSCs were weekly administered during 4 weeks via intrathecal injection [114]. Motor skills and daily life quality were assessed during 6–15 months and compared with the initial scores. The results showed that 1 month after, symptoms were alleviated in all patients, except for one case, in which no response was observed. A follow-up evaluation reported that 10 patients' improved conditions persisted for half a year or more, nevertheless in 14 cases patients' conditions returned to the initial status.

In a Phase I/II clinical trial (NCT01360164), 16 patients with SCA1, SCA2, or SCA3 received UC-MSCs, first by intravenous injection, followed by 3 consecutive intravenous and intrathecal simultaneous injections at 1-week intervals [115]. A follow-up of 12 months proved the safety of this therapeutic approach. Moreover, 7 patients showed continuous improvement of motor deficits for at least 6 months after receiving the therapy, although 5 patients suffered from disease aggravation.

Another clinical trial, enrolled patients from several neurodegenerative disorders, including 8 SCA patients, and aimed at testing the safety and feasibility of intrathecal UC-MSCs injection via lumbar puncture [116]. Four to 6 administrations of UC-MSCs were made between the L4 and L5 interspace within an interval of 5–7 days. All patients were followed-up for more than 1 year and no significant

Table 21.2 Clinical trials with MSCs in PolyQ diseases

Disease	Title	Trial ID	Sponsor	Phase
SCA and multiple system atrophy-cerebellar type patients (MSA-C)	Intrathecal injection of UC-MSC is safe and can delay the progression of neurologic deficits for SCA and MSA-C patients	Unknown [114]	Unknown	I/II
SCA1, SCA2 and SCA3	Safety and efficacy of umbilical cord mesenchymal stem cell therapy for patients with Hereditary Ataxia	NCT01360164 [115]	Shenzhen Beike Bio-Technology Co., Ltd.	I/II
Several neurodegenerative disorders, including SCAs	Umbilical cord mesenchymal stem cells in neurological disorders: a clinical study	Unknown [116]	Unknown	I/II
SCA1	A new method to treat Hereditary Cerebellar Ataxia—Umbilical cord mesenchymal stem cells transplantation (SCA)	NCT01489267	General Hospital of Chinese Armed Police Forces	II
SCA3 or MSA-C	Treatment of Cerebellar Ataxia with Mesenchymal stem cells	NCT01649687	National Yang Ming University	I/II
Cerebellar Ataxias	Efficacy and safety study of Mesenchymal stem cells (Stemchymal®) in Polyglutamine Spinocerebellar Ataxia	NCT02540655	Steminent Biotherapeutics Inc.	II
HD	Safety evaluation of Cellavita HD administered intravenously in participants with Huntington's disease (SAVE-DH)	NCT02728115	Azidus Brasil	I

side effects were reported. Moreover, 3 of the 8 patients showed motor function improvement with the treatment.

Other clinical trials are running or have already finished, but no results have been reported so far (NCT01489267, NCT01958177, NCT01649687, NCT02540655). The therapeutic effect of AD-MSCs in SCAs will be evaluated in a phase II clinical trial (NCT02540655), which will test the safety and efficacy of Stemchymal® (allogeneic AD-MSCs). In HD, a dose escalation, non-randomized, phase I clinical trial will evaluate the safety of Cellavita (mesenchymal stem cells isolated from the dental pulp). Six participants with HD will receive 3 intravenous injections of one of three doses of the cells, one every month for three months and will be followed by 5 years (NCT02728115).

In conclusion, clinical trials have been showing that MSCs are safe and can delay disease progression in some patients. Nevertheless, the beneficial effect promoted by MSCs treatment seems not to be transversal to all the patients, given that, a considerable percentage of the patients returned to the disease stage on which they were before the treatment had started. Another important issue is the survival of MSCs upon transplantation. The majority of the studies herein discussed generally refer that MSCs can survive for a relatively short time after transplantation, ranging from a couple of weeks [104] up to 10 weeks after transplantation [109]. The studies that tracked MSCs for longer periods of time have not found the cells at the lesion sites [107, 111]. Moreover, a very low percentage of the injected cells can usually be found engrafted in the lesion tissues [16, 100, 107]. Therefore, several issues in the application of MSCs to clinical practice need a deeper investigation, such as the appropriate cell source, timing, dosage, method of injection and the feasibility of a continued treatment to obtain more sustained benefits. Moreover, the need for standardization of the protocols used by different institutions, as well as performing longer follow-ups of the patients to evaluate treatment efficacy and safety are also important issues [117, 118].

21.6 Strengths, Obstacles and Future Perspectives

The use of stem cells for therapeutic and research purposes deals with several issues, namely the ethical controversies associated to some of the most promising sources of stem cells. Particularly, the use of human fetal tissues derived from abortion and the use of human stem cell lines derived from human embryos generated during in vitro fertilization programs has raised ethical concerns in the field. The major ethical problem is related to the way these cells are derived, given that to establish the ESCs lines, the embryos have to be destroyed. Therefore, moral and scientific discussion has been debating the point in the developing human embryo or fetuses in which humanness start [119, 120]. Despite this debate, the use of human stem cells for research and therapeutic applications has been regulated and legislated and is conducted under the rules and guidelines of several ethical committee and legal entities, such as the International Society for Stem Cell Research, that in 2006 and 2016 established the Guidelines for Stem Cell Research and Clinical Translation. Nevertheless, there are some countries where the use of human fetal tissues and embryonic stem cells remains illegal, which constrained research in this field, increasing, consequently, the research efforts made with other sources of cells such as adult stem cells and iPSCs, the latter is potentially an unlimited source of cells without major ethical problems.

Another challenge for stem cell-based therapies is the guarantee of viability, purity, reproducibility and safety of the different batches of cells. Therefore, the standardization of production procedures and the control of cell viability and purity are mandatory for the successful use of stem cells and to reduce the heterogeneity of the results obtained both in pre- and clinical studies. In fact, the clinical translation

process of cell-based therapies have specific requirements for characterization, manufacturing, and safety assessments, namely contamination with infectious disease agents, instability due to extensive expansion and tumorigenicity testing [120–122].

The lack of reliable in vivo models is another important limitation in human stem cells transplantation research. In fact, most of the used and available in vivo models for neurodegenerative diseases are rodent models. The use of rodents to study human neural stem cells transplantation have several drawbacks, namely, the central nervous system of primates is much more complex, neural circuitry is different from rodents, and the human stem cells have different developmental programs [123]. Thus, it is inevitable to question if rodent brains contain the instructive cues necessary to truly assess the engraftment potential of human cells. Additionally, long term studies of the transplanted cells (>3 years), namely studies to assess the genetic stability of the cells upon transplantation are not possible in rodents [123]. Moreover, the need to administrate immunosuppressive drugs to avoid immune rejection of the transplanted human cells interferes with the study outcomes because these drugs are known to activate neurogenesis [124, 125]. Furthermore, there is an important interplay between the immune system and NSCs [126–128], which can be imbalanced by the immunosuppressors use.

Therefore, new cellular models based on the use of patients cells' obtained through the iPSCs technology have been developed aiming at creating more reliable models [123, 129] that will enable to improve the understanding of the pathologies and to better evaluate the outcomes of new therapeutic approaches. Some of these very promising models and technologies are the organoids, microfluidic systems and 3D bioprinting. Organoids are 3D-organ resembling structures derived from stem or progenitors cells that undergo in vitro self-organization and differentiation into different functional cell types replicating the complexity of organs like the brain in several aspects, such as the cell types present and the 3D structural tissue organization [130–132]. This research field is in the early beginning. Therefore, important limitations still have to be solved, such as the poor maturation of these systems, a consequence of the lack of vascularization and absence of essential developmental and patterning cues, during the in vitro culturing, which are fundamental for the development of mature organs. Thus, these very promising systems recapitulate the early brain development but fail to originate a proper circuitry recapitulating more mature stages [131].

Microfluidic devices also named "organs in chip" are automated 3D culture systems that mimic tissues and organs, comprising either single or multiple organs, which are integrated in a single chip. In multiple organ systems, different cell types representing different organs are cultured in separated chambers communicating through microfluidic networks, which transport fluids and soluble factors between the different chambers. This automated technique integrates biosensors with microscopy-based read-outs allowing a real time follow-up and control of the cellular microenvironment and processes [133–135]. This automated microscale cell culture systems are much more cost-effective reducing cell culturing expenses. Moreover, automation increases reproducibility and quality control, which is a

central requirement of regulating agencies for cell manufacturing and, presently, is an important problem of the conventional in vitro stem cell culturing. Thus, in a near future, these microfluidic devices might play a decisive role in the implementation of personalized stem-cell therapies, enabling the expansion of patient-derived stem cells in vitro [123] in an automated, reproducible and cost-effective manner. Finally, the 3D bioprinting is a powerful technique that allows building tissues through embedding cells into biomaterials [136] and printing them in 3D tissue-like structures increasing this way cell survival, migration and differentiation [137–139]. This technique has been used in neural tissue engineering to develop biological substitutes enabling functional engraftment and neural repair [138].

21.7 Conclusion

The human adult brain has a small cell turnover and regeneration, and, consequently a poor self-repairing ability. Thus, the use of stem cell-based therapies in neurodegenerative diseases such as the polyQ diseases holds the promise to promote the brain regeneration that an adult brain can no longer activate. The golden goal of cell-based therapies is the replacement of dead or damaged cells and the integration of new cells in the affected tissues and organs restoring the impaired structures and functions. An efficient brain repair process has two distinct, but interdependent, processes. First, the cells to be integrated in the brain have to be generated in the tissue or transplanted, and then these cells must migrate, differentiate and integrate into the host neural network, becoming functional in the complex cellular network that constitute the brain. However, the integration of new mature, functional neurons into the adult host neuronal network is very demanding and in fact, in most of the cases, only a very small number of the transplanted cells become functionally integrated. Therefore, most of the beneficial outcomes triggered by stem and neuroprogenitor cells transplantation are driven by neuroprotective effects such as production of neurotransmitters, trophic factors or neuroprotective agents, rather than actual cell-replacement of the loss neural cells.

Currently, there are different types of stem cells available for cell-replacement and/or neuroprotection strategies, such as the iPSCs, ESCs, MSCs, fetal and progenitors neural stem cells. These different sources of stem cells have different advantages and disadvantages. IPSCs can originate patient-specific neural cells overcoming ethical and immunological rejection issues but are associated to genetic instability, triggered by the cell reprogramming, and consequently to tumor formation. MSCs can be easily isolated, when transplanted migrate to the injured tissue secreting neuroprotective factors but, preclinical and clinical trials show a limited time window of action of these cells upon transplantation. Transplantation of neural stem cells isolated from fetus led to very encouraging results with graft functional integration and motor improvements observed for long times upon transplantation. However, there is a big heterogeneity of the outcomes obtained for

different patients and the use of NSCs obtained from human fetus are associated to ethical controversies. Moreover, these cells need to be pooled from several fetuses, raising immunological problems, and cells preparation has to be synchronized with surgery in a very narrow time window.

Overall, we can conclude that important issues remain unsolved to achieve functional long-lasting and safe integration of the grafted stem cells. Nevertheless, the unmet need of effective therapies for neurodegenerative disorders and the promising results obtained so far reinforce our confidence and need to further investigate stem-cell-based therapies for polyglutamine disorders.

References

1. Takahashi T, Katada S, Onodera O (2010) Polyglutamine diseases: where does toxicity come from? what is toxicity? where are we going? J Mol Cell Biol 2:180–191
2. Teive HA (2009) Spinocerebellar ataxias. Arq Neuropsiquiatr 67:1133–1142
3. Ostenfeld T, Svendsen CN (2003) Recent advances in stem cell neurobiology. Adv Tech Stand Neurosurg 28:3–89
4. Dulak J, Szade K, Szade A, Nowak W, Jozkowicz A (2015) Adult stem cells: hopes and hypes of regenerative medicine. Acta Biochim Pol 62:329–337
5. Kim TG, Yao R, Monnell T, Cho JH, Vasudevan A, Koh A, Peeyush KT, Moon M, Datta D, Bolshakov VY et al (2014) Efficient specification of interneurons from human pluripotent stem cells by dorsoventral and rostrocaudal modulation. Stem Cells 32:1789–1804
6. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
7. Anderson DJ (1989) The neural crest cell lineage problem: neuropoiesis? Neuron 3:1–12
8. Cattaneo E, McKay R (1990) Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor. Nature 347:762–765
9. Davis AA, Temple S (1994) A self-renewing multipotential stem cell in embryonic rat cerebral cortex. Nature 372:263–266
10. Kilpatrick TJ, Bartlett PF (1993) Cloning and growth of multipotential neural precursors: requirements for proliferation and differentiation. Neuron 10:255–265
11. Temple S (1989) Division and differentiation of isolated CNS blast cells in microculture. Nature 340:471–473
12. Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nat Med 4:1313–1317
13. Gage FH, Temple S (2013) Neural stem cells: generating and regenerating the brain. Neuron 80:588–601
14. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282:1145–1147
15. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872
16. Sadan O, Shemesh N, Barzilay R, Dadon-Nahum M, Blumenfeld-Katzir T, Assaf Y, Yeshurun M, Djaldetti R, Cohen Y, Melamed E et al (2012) Mesenchymal stem cells induced to secrete neurotrophic factors attenuate quinolinic acid toxicity: a potential therapy for Huntington's disease. Exp Neurol 234:417–427
17. Lindvall O, Kokaia Z (2009) Prospects of stem cell therapy for replacing dopamine neurons in Parkinson's disease. Trends Pharmacol Sci 30:260–267

18. Lindvall O, Kokaia Z, Martinez-Serrano A (2004) Stem cell therapy for human neurodegenerative disorders-how to make it work. Nat Med 10(Suppl):S42–S50
19. O'Donoghue K, Fisk NM (2004) Fetal stem cells. Best Pract Res Clin Obstet Gynaecol 18:853–875
20. Sousa-Ferreira L, Aveleira C, Botelho M, Alvaro AR, Pereira de Almeida L, Cavadas C (2014) Fluoxetine induces proliferation and inhibits differentiation of hypothalamic neuroprogenitor cells in vitro. PLoS ONE 9:e88917
21. Mendonca LS, Nobrega C, Hirai H, Kaspar BK, Pereira de Almeida L (2015) Transplantation of cerebellar neural stem cells improves motor coordination and neuropathology in Machado-Joseph disease mice. Brain J Neurol 138:320–335
22. Chintawar S, Hourez R, Ravella A, Gall D, Orduz D, Rai M, Bishop DP, Geuna S, Schiffmann SN, Pandolfo M (2009) Grafting neural precursor cells promotes functional recovery in an SCA1 mouse model. J Neurosci Official J Soc Neurosci 29:13126–13135
23. Benraiss A, Goldman SA (2011) Cellular therapy and induced neuronal replacement for Huntington's disease. Neurother J Am Soc Exp Neurother 8:577–590
24. Golas MM, Sander B (2016) Use of human stem cells in Huntington disease modeling and translational research. Exp Neurol 278:76–90
25. Borlongan CV, Koutouzis TK, Poulos SG, Saporta S, Sanberg PR (1998) Bilateral fetal striatal grafts in the 3-nitropropionic acid-induced hypoactive model of Huntington's disease. Cell Transplant 7:131–135
26. Hurelbrink CB, Armstrong RJ, Dunnett SB, Rosser AE, Barker RA (2002) Neural cells from primary human striatal xenografts migrate extensively in the adult rat CNS. Eur J Neurosci 15:1255–1266
27. Palfi S, Conde F, Riche D, Brouillet E, Dautry C, Mittoux V, Chibois A, Peschanski M, Hantraye P (1998) Fetal striatal allografts reverse cognitive deficits in a primate model of Huntington disease. Nat Med 4:963–966
28. Wictorin K, Ouimet CC, Bjorklund A (1989) Intrinsic organization and connectivity of intrastriatal striatal transplants in rats as revealed by DARPP-32 immunohistochemistry: specificity of connections with the lesioned host brain. Eur J Neurosci 1:690–701
29. Madrazo I, Franco-Bourland RE, Castrejon H, Cuevas C, Ostrosky-Solis F (1995) Fetal striatal homotransplantation for Huntington's disease: first two case reports. Neurol Res 17:312–315
30. Kopyov OV, Jacques S, Lieberman A, Duma CM, Eagle KS (1998) Safety of intrastriatal neurotransplantation for Huntington's disease patients. Exp Neurol 149:97–108
31. Philpott LM, Kopyov OV, Lee AJ, Jacques S, Duma CM, Caine S, Yang M, Eagle KS (1997) Neuropsychological functioning following fetal striatal transplantation in Huntington's chorea: three case presentations. Cell Transplant 6:203–212
32. Keene CD, Chang RC, Leverenz JB, Kopyov O, Perlman S, Hevner RF, Born DE, Bird TD, Montine TJ (2009) A patient with Huntington's disease and long-surviving fetal neural transplants that developed mass lesions. Acta Neuropathol 117:329–338
33. Keene CD, Sonnen JA, Swanson PD, Kopyov O, Leverenz JB, Bird TD, Montine TJ (2007) Neural transplantation in Huntington disease: long-term grafts in two patients. Neurology 68:2093–2098
34. Quinn N, Brown R, Craufurd D, Goldman S, Hodges J, Kieburtz K, Lindvall O, MacMillan J, Roos R (1996) Core Assessment Program for Intracerebral Transplantation in Huntington's Disease (CAPIT-HD). Movement disorders: official journal of the Movement Disorder Society 11:143–150
35. Bachoud-Levi AC, Remy P, Nguyen JP, Brugieres P, Lefaucheur JP, Bourdet C, Baudic S, Gaura V, Maison P, Haddad B et al (2000) Motor and cognitive improvements in patients with Huntington's disease after neural transplantation. Lancet 356:1975–1979
36. Gaura V, Bachoud-Levi AC, Ribeiro MJ, Nguyen JP, Frouin V, Baudic S, Brugieres P, Mangin JF, Boisse MF, Palfi S et al (2004) Striatal neural grafting improves cortical metabolism in Huntington's disease patients. Brain J Neurol 127:65–72

37. Bachoud-Levi AC, Gaura V, Brugieres P, Lefaucheur JP, Boisse MF, Maison P, Baudic S, Ribeiro MJ, Bourdet C, Remy P et al (2006) Effect of fetal neural transplants in patients with Huntington's disease 6 years after surgery: a long-term follow-up study. Lancet Neurol 5:303–309
38. Hauser RA, Furtado S, Cimino CR, Delgado H, Eichler S, Schwartz S, Scott D, Nauert GM, Soety E, Sossi V et al (2002) Bilateral human fetal striatal transplantation in Huntington's disease. Neurology 58:687–695
39. Freeman TB, Cicchetti F, Hauser RA, Deacon TW, Li XJ, Hersch SM, Nauert GM, Sanberg PR, Kordower JH, Saporta S et al (2000) Transplanted fetal striatum in Huntington's disease: phenotypic development and lack of pathology. Proc Natl Acad Sci USA 97:13877–13882
40. Furtado S, Sossi V, Hauser RA, Samii A, Schulzer M, Murphy CB, Freeman TB, Stoessl AJ (2005) Positron emission tomography after fetal transplantation in Huntington's disease. Ann Neurol 58:331–337
41. Cicchetti F, Saporta S, Hauser RA, Parent M, Saint-Pierre M, Sanberg PR, Li XJ, Parker JR, Chu Y, Mufson EJ et al (2009) Neural transplants in patients with Huntington's disease undergo disease-like neuronal degeneration. Proc Natl Acad Sci USA 106:12483–12488
42. Rosser AE, Barker RA, Harrower T, Watts C, Farrington M, Ho AK, Burnstein RM, Menon DK, Gillard JH, Pickard J et al (2002) Unilateral transplantation of human primary fetal tissue in four patients with Huntington's disease: NEST-UK safety report ISRCTN no 36485475. J Neurol Neurosurg Psychiatry 73:678–685
43. Barker RA, Mason SL, Harrower TP, Swain RA, Ho AK, Sahakian BJ, Mathur R, Elneil S, Thornton S, Hurrelbrink C et al (2013) The long-term safety and efficacy of bilateral transplantation of human fetal striatal tissue in patients with mild to moderate Huntington's disease. J Neurol Neurosurg Psychiatry 84:657–665
44. Reuter I, Tai YF, Pavese N, Chaudhuri KR, Mason S, Polkey CE, Clough C, Brooks DJ, Barker RA, Piccini P (2008) Long-term clinical and positron emission tomography outcome of fetal striatal transplantation in Huntington's disease. J Neurol Neurosurg Psychiatry 79:948–951
45. Gallina P, Paganini M, Lombardini L, Mascalchi M, Porfirio B, Gadda D, Marini M, Pinzani P, Salvianti F, Crescioli C et al (2010) Human striatal neuroblasts develop and build a striatal-like structure into the brain of Huntington's disease patients after transplantation. Exp Neurol 222:30–41
46. Boer GJ (1999) Ethical issues in neurografting of human embryonic cells. Theor Med Bioeth 20:461–475
47. Freeman TB, Cicchetti F, Bachoud-Levi AC, Dunnett SB (2011) Technical factors that influence neural transplant safety in Huntington's disease. Exp Neurol 227:1–9
48. Bachoud-Levi AC, Perrier AL (2014) Regenerative medicine in Huntington's disease: current status on fetal grafts and prospects for the use of pluripotent stem cell. Revue Neurologique 170:749–762
49. Barberi T, Klivenyi P, Calingasan NY, Lee H, Kawamata H, Loonam K, Perrier AL, Bruses J, Rubio ME, Topf N et al (2003) Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. Nat Biotechnol 21:1200–1207
50. Fan HC, Ho LI, Chi CS, Chen SJ, Peng GS, Chan TM, Lin SZ, Harn HJ (2014) Polyglutamine (PolyQ) diseases: genetics to treatments. Cell Transplant 23:441–458
51. Okabe S, Forsberg-Nilsson K, Spiro AC, Segal M, McKay RD (1996) Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. Mech Dev 59:89–102
52. Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL (2008) Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. Proc Natl Acad Sci U S A 105:16707–16712
53. Ma L, Hu B, Liu Y, Vermilyea SC, Liu H, Gao L, Sun Y, Zhang X, Zhang SC (2012) Human embryonic stem cell-derived GABA neurons correct locomotion deficits in quinolinic acid-lesioned mice. Cell Stem Cell 10:455–464

54. Kaemmerer WF, Low WC (1999) Cerebellar allografts survive and transiently alleviate ataxia in a transgenic model of spinocerebellar ataxia type-1. Exp Neurol 158:301–311
55. Delli Carri A, Onorati M, Lelos MJ, Castiglioni V, Faedo A, Menon R, Camnasio S, Vuono R, Spaiardi P, Talpo F et al (2013) Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation toward authentic DARPP-32+ medium-sized spiny neurons. Development 140:301–312
56. Arber C, Precious SV, Cambray S, Risner-Janiczek JR, Kelly C, Noakes Z, Fjodorova M, Heuer A, Ungless MA, Rodriguez TA et al (2015) Activin A directs striatal projection neuron differentiation of human pluripotent stem cells. Development 142:1375–1386
57. Abdipranoto-Cowley A, Park JS, Croucher D, Daniel J, Henshall S, Galbraith S, Mervin K, Vissel B (2009) Activin A is essential for neurogenesis following neurodegeneration. Stem Cells 27:1330–1346
58. Sekiguchi M, Hayashi F, Tsuchida K, Inokuchi K (2009) Neuron type-selective effects of activin on development of the hippocampus. Neurosci Lett 452:232–237
59. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R et al (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917–1920
60. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R et al (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science 321:1218–1221
61. Ebert AD, Yu J, Rose FF Jr, Mattis VB, Lorson CL, Thomson JA, Svendsen CN (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature 457:277–280
62. Grskovic M, Javaherian A, Strulovici B, Daley GQ (2011) Induced pluripotent stem cells—opportunities for disease modelling and drug discovery. Nat Rev Drug Discov 10:915–929
63. Merkle FT, Eggan K (2013) Modeling human disease with pluripotent stem cells: from genome association to function. Cell Stem Cell 12:656–668
64. Pankevich DE, Altevogt BM, Dunlop J, Gage FH, Hyman SE (2014) Improving and accelerating drug development for nervous system disorders. Neuron 84:546–553
65. Grunseich C, Zukosky K, Kats IR, Ghosh L, Harmison GG, Bott LC, Rinaldi C, Chen KL, Chen G, Boehm M et al (2014) Stem cell-derived motor neurons from spinal and bulbar muscular atrophy patients. Neurobiol Dis 70:12–20
66. Nihei Y, Ito D, Okada Y, Akamatsu W, Yagi T, Yoshizaki T, Okano H, Suzuki N (2013) Enhanced aggregation of androgen receptor in induced pluripotent stem cell-derived neurons from spinal and bulbar muscular atrophy. J Biol Chem 288:8043–8052
67. Koch P, Breuer P, Peitz M, Jungverdorben J, Kesavan J, Poppe D, Doerr J, Ladewig J, Mertens J, Tuting T et al (2011) Excitation-induced ataxin-3 aggregation in neurons from patients with Machado-Joseph disease. Nature 480:543–546
68. Consortium, H.D.i (2012) Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. Cell Stem Cell 11:264–278
69. Liu GH, Ding Z, Izpisua Belmonte JC (2012) iPSC technology to study human aging and aging-related disorders. Curr Opin Cell Biol 24:765–774
70. Sandoe J, Eggan K (2013) Opportunities and challenges of pluripotent stem cell neurodegenerative disease models. Nat Neurosci 16:780–789
71. Ward RJ, Zucca FA, Duyn JH, Crichton RR, Zecca L (2014) The role of iron in brain ageing and neurodegenerative disorders. Lancet Neurol 13:1045–1060
72. Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T et al (2012) Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. Sci Transl Med 4, 141ra190
73. Jeon I, Lee N, Li JY, Park IH, Park KS, Moon J, Shim SH, Choi C, Chang DJ, Kwon J et al (2012) Neuronal properties, in vivo effects, and pathology of a Huntington's disease patient-derived induced pluripotent stem cells. Stem Cells 30:2054–2062
74. Mitne-Neto M, Machado-Costa M, Marchetto MC, Bengtson MH, Joazeiro CA, Tsuda H, Bellen HJ, Silva HC, Oliveira AS, Lazar M et al (2011) Downregulation of VAPB

expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. Hum Mol Genet 20:3642–3652
75. Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, Kee K, Schule B, Dolmetsch RE, Langston W et al (2011) LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 8:267–280
76. Camnasio S, Delli Carri A, Lombardo A, Grad I, Mariotti C, Castucci A, Rozell B, Lo Riso P, Castiglioni V, Zuccato C et al (2012) The first reported generation of several induced pluripotent stem cell lines from homozygous and heterozygous Huntington's disease patients demonstrates mutation related enhanced lysosomal activity. Neurobiol Dis 46:41–51
77. Sanchez-Danes A, Richaud-Patin Y, Carballo-Carbajal I, Jimenez-Delgado S, Caig C, Mora S, Di Guglielmo C, Ezquerra M, Patel B, Giralt A et al (2012) Disease-specific phenotypes in dopamine neurons from human iPS-based models of genetic and sporadic Parkinson's disease. EMBO Mol Med 4:380–395
78. Onofre I (2016) Dissecting the pathogenesis of Machado-Joseph Disease in a new human disease model derived from induced pluripotent stem cells (Doctoral Dissertation). In University of Coimbra
79. Carmona V, Cunha-Santos J, Onofre I, Simoes AT, Vijayakumar U, Davidson BL, Pereira de Almeida L (2017) Unravelling endogenous microRNA system dysfunction as a new pathophysiological mechanism in Machado-Joseph disease. Mol Ther J Am Soc Gene Ther 25:1038–1055
80. Mattson MP, Magnus T (2006) Ageing and neuronal vulnerability. Nat Rev Neurosci 7:278–294
81. Mattis VB, Tom C, Akimov S, Saeedian J, Ostergaard ME, Southwell AL, Doty CN, Ornelas L, Sahabian A, Lenaeus L et al (2015) HD iPSC-derived neural progenitors accumulate in culture and are susceptible to BDNF withdrawal due to glutamate toxicity. Hum Mol Genet 24:3257–3271
82. Hansen SK, Borland H, Hasholt LF, Tumer Z, Nielsen JE, Rasmussen MA, Nielsen TT, Stummann TC, Fog K, Hyttel P (2016) Generation of spinocerebellar ataxia type 3 patient-derived induced pluripotent stem cell line SCA3.B11. Stem Cell Res 16:589–592
83. Fink KD, Crane AT, Leveque X, Dues DJ, Huffman LD, Moore AC, Story DT, Dejonge RE, Antcliff A, Starski PA et al (2014) Intrastriatal transplantation of adenovirus-generated induced pluripotent stem cells for treating neuropathological and functional deficits in a rodent model of Huntington's disease. Stem Cells Transl Med 3:620–631
84. Mu S, Wang J, Zhou G, Peng W, He Z, Zhao Z, Mo C, Qu J, Zhang J (2014) Transplantation of induced pluripotent stem cells improves functional recovery in Huntington's disease rat model. PLoS ONE 9:e101185
85. Lee MO, Moon SH, Jeong HC, Yi JY, Lee TH, Shim SH, Rhee YH, Lee SH, Oh SJ, Lee MY et al (2013) Inhibition of pluripotent stem cell-derived teratoma formation by small molecules. Proc Natl Acad Sci U S A 110:E3281–E3290
86. Miura K, Okada Y, Aoi T, Okada A, Takahashi K, Okita K, Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M et al (2009) Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol 27:743–745
87. Hargus G, Cooper O, Deleidi M, Levy A, Lee K, Marlow E, Yow A, Soldner F, Hockemeyer D, Hallett PJ et al (2010) Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. Proc Natl Acad Sci U S A 107:15921–15926
88. Peng SP, Copray S (2016) Comparison of human primary with human iPS cell-derived dopaminergic neuron grafts in the rat model for Parkinson's Disease. Stem Cell Rev 12:105–120
89. Wang S, Zou C, Fu L, Wang B, An J, Song G, Wu J, Tang X, Li M, Zhang J et al (2015) Autologous iPSC-derived dopamine neuron transplantation in a nonhuman primate Parkinson's disease model. Cell Discov 1:15012
90. Ben-David U, Benvenisty N (2011) The tumorigenicity of human embryonic and induced pluripotent stem cells. Nat Rev Cancer 11:268–277

91. Ben-David U, Benvenisty N, Mayshar Y (2010) Genetic instability in human induced pluripotent stem cells: classification of causes and possible safeguards. Cell Cycle 9:4603–4604
92. Friedenstein AJ, Chailakhjan RK, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 3:393–403
93. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
94. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24:1294–1301
95. Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A (2005) Clarification of the nomenclature for MSC: the international society for cellular therapy position statement. Cytotherapy 7:393–395
96. Lee M, Jeong SY, Ha J, Kim M, Jin HJ, Kwon SJ, Chang JW, Choi SJ, Oh W, Yang YS et al (2014) Low immunogenicity of allogeneic human umbilical cord blood-derived mesenchymal stem cells in vitro and in vivo. Biochem Biophys Res Commun 446:983–989
97. Choumerianou DM, Dimitriou H, Perdikogianni C, Martimianaki G, Riminucci M, Kalmanti M (2008) Study of oncogenic transformation in ex vivo expanded mesenchymal cells, from paediatric bone marrow. Cell Prolif 41:909–922
98. Caplan AI, Dennis JE (2006) Mesenchymal stem cells as trophic mediators. J Cell Biochem 98:1076–1084
99. Zhang Y, Chopp M, Liu XS et al (2016) Exosomes derived from mesenchymal stromal cells promote axonal growth of cortical neurons. Mol Neurobiol 54(4):2659–2673
100. Lee ST, Chu K, Jung KH, Im WS, Park JE, Lim HC, Won CH, Shin SH, Lee SK, Kim M et al (2009) Slowed progression in models of Huntington disease by adipose stem cell transplantation. Ann Neurol 66:671–681
101. Im W, Lee ST, Park JE, Oh HJ, Shim J, Lim J, Chu K, Kim M (2010) Transplantation of patient-derived adipose stem cells in YAC128 Huntington's disease transgenic mice. PLoS currents 2
102. Hosseini M, Moghadas M, Edalatmanesh MA, Hashemzadeh MR (2015) Xenotransplantation of human adipose derived mesenchymal stem cells in a rodent model of Huntington's disease: motor and non-motor outcomes. Neurol Res 37:309–319
103. Edalatmanesh MA, Bahrami AR, Hosseini E, Hosseini M, Khatamsaz S (2011) Bone marrow derived mesenchymal stem cell transplantation in cerebellar degeneration: a behavioral study. Behav Brain Res 225:63–70
104. Snyder BR, Chiu AM, Prockop DJ, Chan AW (2010) Human multipotent stromal cells (MSCs) increase neurogenesis and decrease atrophy of the striatum in a transgenic mouse model for Huntington's disease. PLoS ONE 5:e9347
105. Sadan O, Bahat-Stromza M, Barhum Y, Levy YS, Pisnevsky A, Peretz H, Ilan AB, Bulvik S, Shemesh N, Krepel D et al (2009) Protective effects of neurotrophic factor-secreting cells in a 6-OHDA rat model of Parkinson disease. Stem Cells Dev 18:1179–1190
106. Sadan O, Shemesh N, Barzilay R, Bahat-Stromza M, Melamed E, Cohen Y, Offen D (2008) Migration of neurotrophic factors-secreting mesenchymal stem cells toward a quinolinic acid lesion as viewed by magnetic resonance imaging. Stem Cells 26:2542–2551
107. Dey ND, Bombard MC, Roland BP, Davidson S, Lu M, Rossignol J, Sandstrom MI, Skeel RL, Lescaudron L, Dunbar GL (2010) Genetically engineered mesenchymal stem cells reduce behavioral deficits in the YAC 128 mouse model of Huntington's disease. Behav Brain Res 214:193–200
108. Lin YT, Chern Y, Shen CK, Wen HL, Chang YC, Li H, Cheng TH, Hsieh-Li HM (2011) Human mesenchymal stem cells prolong survival and ameliorate motor deficit through trophic support in Huntington's disease mouse models. PLoS ONE 6:e22924

109. Rossignol J, Boyer C, Leveque X, Fink KD, Thinard R, Blanchard F, Dunbar GL, Lescaudron L (2011) Mesenchymal stem cell transplantation and DMEM administration in a 3NP rat model of Huntington's disease: morphological and behavioral outcomes. Behav Brain Res 217:369–378
110. Rossignol J, Fink KD, Crane AT, Davis KK, Bombard MC, Clerc S, Bavar AM, Lowrance SA, Song C, Witte S et al (2015) Reductions in behavioral deficits and neuropathology in the R6/2 mouse model of Huntington's disease following transplantation of bone-marrow-derived mesenchymal stem cells is dependent on passage number. Stem Cell Res Ther 6:9
111. Chang YK, Chen MH, Chiang YH, Chen YF, Ma WH, Tseng CY, Soong BW, Ho JH, Lee OK (2011) Mesenchymal stem cell transplantation ameliorates motor function deterioration of spinocerebellar ataxia by rescuing cerebellar Purkinje cells. J Biomed Sci 18
112. Matsuura S, Shuvaev AN, Iizuka A, Nakamura K, Hirai H (2014) Mesenchymal stem cells ameliorate cerebellar pathology in a mouse model of spinocerebellar ataxia type 1. Cerebellum 13:323–330
113. Fink KD, Rossignol J, Crane AT, Davis KK, Bombard MC, Bavar AM, Clerc S, Lowrance SA, Song C, Lescaudron L et al (2013) Transplantation of umbilical cord-derived mesenchymal stem cells into the striata of R6/2 mice: behavioral and neuropathological analysis. Stem Cell Res Ther 4:130
114. Dongmei H, Jing L, Mei X, Ling Z, Hongmin Y, Zhidong W, Li D, Zikuan G, Hengxiang W (2011) Clinical analysis of the treatment of spinocerebellar ataxia and multiple system atrophy-cerebellar type with umbilical cord mesenchymal stromal cells. Cytotherapy 13:913–917
115. Jin JL, Liu Z, Lu ZJ, Guan DN, Wang C, Chen ZB, Zhang J, Zhang WY, Wu JY, Xu Y (2013) Safety and efficacy of umbilical cord mesenchymal stem cell therapy in hereditary spinocerebellar ataxia. Curr Neurovascular Res 10:11–20
116. Miao X, Wu X, Shi W (2015) Umbilical cord mesenchymal stem cells in neurological disorders: a clinical study. Indian J Biochem Biophys 52:140–146
117. Fink KD, Deng P, Torrest A, Stewart H, Pollock K, Gruenloh W, Annett G, Tempkin T, Wheelock V, Nolta JA (2015) Developing stem cell therapies for juvenile and adult-onset Huntington's disease. Regenerative Med 10:623–646
118. Maucksch C, Vazey EM, Gordon RJ, Connor B (2013) Stem cell-based therapy for Huntington's disease. J Cell Biochem 114:754–763
119. Barker RA, de Beaufort I (2013) Scientific and ethical issues related to stem cell research and interventions in neurodegenerative disorders of the brain. Prog Neurobiol 110:63–73
120. Dunnett SB, Rosser AE (2014) Challenges for taking primary and stem cells into clinical neurotransplantation trials for neurodegenerative disease. Neurobiol Dis 61:79–89
121. Aboody K, Capela A, Niazi N, Stern JH, Temple S (2011) Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta stone. Neuron 70:597–613
122. Council EPa (2004) Directive 2004/23/EC of 31 March 2004, on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. J Eur Union, pp 48–58
123. Qiu Z, Farnsworth SL, Mishra A, Hornsby PJ (2013) Patient-specific induced pluripotent stem cells in neurological disease modeling: the importance of nonhuman primate models. Stem Cells Cloning Adv App 6:19–29
124. Chow A, Morshead CM (2016) Cyclosporin A enhances neurogenesis in the dentate gyrus of the hippocampus. Stem Cell Res 16:79–87
125. Erlandsson A, Lin CH, Yu F, Morshead CM (2011) Immunosuppression promotes endogenous neural stem and progenitor cell migration and tissue regeneration after ischemic injury. Exp Neurol 230:48–57
126. Dooley D, Vidal P, Hendrix S (2014) Immunopharmacological intervention for successful neural stem cell therapy: new perspectives in CNS neurogenesis and repair. Pharmacol Ther 141:21–31

127. Pluchino S, Zanotti L, Brambilla E, Rovere-Querini P, Capobianco A, Alfaro-Cervello C, Salani G, Cossetti C, Borsellino G, Battistini L et al (2009) Immune regulatory neural stem/precursor cells protect from central nervous system autoimmunity by restraining dendritic cell function. PLoS ONE 4:e5959
128. Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, Martinello M, Cattalini A, Bergami A, Furlan R et al (2005) Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature 436:266–271
129. Han SS, Williams LA, Eggan KC (2011) Constructing and deconstructing stem cell models of neurological disease. Neuron 70:626–644
130. Huch M, Koo BK (2015) Modeling mouse and human development using organoid cultures. Development 142:3113–3125
131. Kelava I, Lancaster MA (2016) Dishing out mini-brains: current progress and future prospects in brain organoid research. Dev Biol
132. Lancaster MA, Knoblich JA (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. Science 345:1247125
133. Ertl P, Sticker D, Charwat V, Kasper C, Lepperdinger G (2014) Lab-on-a-chip technologies for stem cell analysis. Trends Biotechnol 32:245–253
134. Ghaemmaghami AM, Hancock MJ, Harrington H, Kaji H, Khademhosseini A (2012) Biomimetic tissues on a chip for drug discovery. Drug Discov Today 17:173–181
135. Neuzi P, Giselbrecht S, Lange K, Huang TJ, Manz A (2012) Revisiting lab-on-a-chip technology for drug discovery. Nat Rev Drug Discovery 11:620–632
136. Maclean FL, Rodriguez AL, Parish CL, Williams RJ, Nisbet DR (2016) Integrating biomaterials and stem cells for neural regeneration. Stem Cells Dev 25:214–226
137. Gu Q, Tomaskovic-Crook E, Lozano R, Chen Y, Kapsa RM, Zhou Q, Wallace GG, Crook JM (2016) Functional 3D neural mini-tissues from printed gel-based bioink and human neural stem cells. Adv Healthc Mater 5:1429–1438
138. Hsieh FY, Lin HH, Hsu SH (2015) 3D bioprinting of neural stem cell-laden thermoresponsive biodegradable polyurethane hydrogel and potential in central nervous system repair. Biomaterials 71:48–57
139. Murphy SV, Atala A (2014) 3D bioprinting of tissues and organs. Nat Biotechnol 32:773–785
140. Juopperi TA, Kim WR, Chiang CH, Yu H, Margolis RL, Ross CA, Ming GL, Song H (2012) Astrocytes generated from patient induced pluripotent stem cells recapitulate features of Huntington's disease patient cells. Mol Brain 5:17
141. Zhang N, An MC, Montoro D, Ellerby LM (2010) Characterization of human Huntington's disease cell model from induced pluripotent stem cells. PLoS Curr 2, RRN1193
142. Marthaler AG, Tubsuwan A, Schmid B, Poulsen UB, Engelbrecht AF, Mau-Holzmann UA, Hyttel P, Nielsen TT, Nielsen JE, Holst B (2016) Generation of an isogenic, gene-corrected control cell line of the spinocerebellar ataxia type 2 patient-derived iPSC line H266. Stem Cell Res 16:202–205
143. Xia G, Santostefano K, Hamazaki T, Liu J, Subramony SH, Terada N, Ashizawa T (2013) Generation of human-induced pluripotent stem cells to model spinocerebellar ataxia type 2 in vitro. J Mol Neurosci 51:237–248
144. Luo Y, Fan Y, Zhou B, Xu Z, Chen Y, Sun X (2012) Generation of induced pluripotent stem cells from skin fibroblasts of a patient with olivopontocerebellar atrophy. Tohoku J Exp Med 226:151–159
145. An MC, Zhang N, Scott G, Montoro D, Wittkop T, Mooney S, Melov S, Ellerby LM (2012) Genetic correction of Huntington's disease phenotypes in induced pluripotent stem cells. Cell Stem Cell 11:253–263

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