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82

3rd Series

MOTOR NEURON DISORDERS AND RELATED DISEASES

Edited by:
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Foreword

This is the fourth volume in the new (third) series of the *Handbook of Clinical Neurology*. The series was started by Pierre Vinken and George Bruyn in the 1960s and continued under their stewardship until the second series concluded in 2002. The new series, for which we have assumed editorial responsibility, covers advances in clinical neurology and the neurosciences and includes a number of new topics. We have deliberately included the neurobiological aspects of the nervous system in health and disease in order to clarify physiological and pathogenic mechanisms and to provide the underpinning of new therapeutic strategies for neurological disorders. We have also attempted to ensure that data related to epidemiology, imaging, genetics and therapy are emphasized. In addition to being available in print form, the series is also available electronically on Elsevier's Science Direct site, and we hope that this will make it more accessible to readers.

This fourth volume in the new series (volume 82 in the entire series) deals with motor neuron disorders and is edited by Professor Andrew Eisen, from Vancouver, Canada, and Professor Pamela Shaw, from Sheffield, UK. We reviewed all of the chapters in the volume and made suggestions for improvement, but it is clear that they have produced a scholarly and comprehensive account of these disorders that will appeal to clinicians and neuroscientists alike. Remarkable advances have occurred in recent years in our understanding of these disorders and their underlying molecular pathogenesis, and these advances are summarized here. Nevertheless, our understanding remains incomplete, as is clearly emphasized in the text where the limits of our knowledge are defined. An account is also provided of the general clinical features and management of these devastating disorders, which will be of help to all who care for patients affected by them.

The successful preparation of each volume in this new series of the Handbook depends on many people. We are privileged that Andrew Eisen and Pamela Shaw, both of whom are internationally acknowledged experts in the field, agreed to serve as Volume Editors and thank them and the contributing authors whom they assembled for all their efforts. We also thank the editorial staff of the publisher, Elsevier B.V., and especially Ms Lynn Watt and Mr Michael Parkinson in Edinburgh for overseeing all stages in the preparation of this volume.

Michael J. Aminoff
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Dick F. Swaab

Preface

Let us keep looking in spite of everything. Let us keep searching. It is indeed the best method of finding, and perhaps thanks to our efforts, the verdict we will give such a patient tomorrow will not be the same we must give this patient today (*Charcot, 1865*).

This sentiment was well expressed by Charcot in one of his many teaching sessions on amyotrophic lateral sclerosis (ALS). It holds as true today as it did in 1865 and the search must continue but progress has been incredible in recent years. There has been an exponential increase in the number of publications dealing with ALS and motor neuron diseases in the last 50 years, as evidenced by listings in PubMed and related data bases.

The Editors extend their utmost thanks to the internationally renowned experts that have contributed to this volume. They have helped create an in-depth reference on motor neuron diseases that is current and in many aspects should stand the test of time. Nevertheless, we are acutely aware of the escalating rate of progress in ALS and related disorders and certainly some features of these conditions will be viewed differently in years to come.

As is underscored in this volume, disorders of motor neurons are clinically and genetically diverse and many questions remain to be answered with respect to these conditions. Why the highly selective vulnerability, evident pathologically, which determines the unique clinical signatures of these disorders? What is the relationship between different motor neuron diseases? For example, are monomelic amyotrophies of the upper and lower limbs the same or different diseases? Is primary lateral sclerosis (PLS) a unique entity or one end of a spectrum of ALS? To what extent do genetic factors play a role in sporadic disease? Recent studies have identified causative genes in several motor neuron diseases and suspicions are strong that apparently sporadic forms of disease may eventually be proven to have a significant genetic component. For example, the hereditary spastic paraplegias, a diverse group of upper motor neuron diseases, are genetically complex: 28 loci have been mapped and mutations in 11 genes identified to date. This volume attempts to answer some of the questions posed above.

Following an historical introduction, the volume has been divided into five sections. The first, Basic Aspects, covers comparative and developmental aspects of the motor system, molecular mechanisms of motor neuron degeneration and cytopathology of the motor neuron and a chapter on animal models of motor neuron death. The second section covers anterior horn cell disorders and motor neuropathies and the spinal muscular atrophies, with a separate chapter on spinobulbar muscular atrophy, GM_2 gangliosidoses, viral infections affecting motor neurons, focal amyotrophies and multifocal and other motor neuropathies. The next section deals with amyotrophic lateral sclerosis with chapters on classic ALS, familial ALS and juvenile ALS. Section 4, Corticospinal Disorders, has chapters on primary lateral sclerosis, the hereditary spastic paraplegias and toxic disorders of the upper motor neuron. The final section describes therapeutic aspects of motor neuron disorders, with emphasis on modifying therapies and symptomatic and palliative treatment.

Each of the 20 chapters is as current as is possible in a text of this type. There are ample illustrations and the references, although not intended to be exhaustive, are comprehensive and up-to-date.

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Chapter 1

Historical aspects of motor neuron diseases

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Systematic, statistical classification of diseases dates back to the 19th century. Groundwork was done by early medical statisticians William Farr (1807–1883) and Jacques Bertillon (1851–1922). Nevertheless, these classifications largely ignored many neuromuscular diseases which were lumped together in what today would be regarded as a confused fashion. It was not until the International Health Conference held in New York City in 1946 entrusted the Interim Commission of the World Health Organization with the responsibility of preparing a sixth revision of the International Lists of Diseases and Causes of Death that a semblance of neuromuscular classification evolved.

This can be contrasted with knowledge about movement disorders, and in particular Parkinson's disease which was clearly recognized in ancient times with descriptions to be found in the Bible, and the ancient writings of Atreya and Susruta. In addition, classic texts provide information on historical personages, including the dystonia of Alexander the Great (Hornykiewicz, 1977; Keppel Hesselink, 1983; Garcia-Ruiz, 2000). On the other hand Alzheimer's disease was only recognized as such in 1911 (compared to ALS in 1865), when Alois Alzheimer published a detailed report on a peculiar case of the disease that had been named after him by Emil Kraepelin in 1910 (Alzheimer, 1991; Alzheimer et al., 1991, 1995). Achucarro, who had studied with Alois Alzheimer at his Nervenklinik in Munich, Germany described the first American case of Alzheimer's in a 77-year-old in 1910 (Schwartz and Stark, 1992; Graeber et al., 1997).

1.1. Charcot and early descriptions of amyotrophic lateral sclerosis (ALS)

Jean-Martin Charcot was born in Paris, France, late in 1825 (Fig. 1.1). Although he was a 19th century scientist, his influence carried on into the next century, especially in the work of some of his well-known students, amongst them Alfred Binet, Pierre Janet and Sigmund Freud (Ekbom, 1992). He was a professor at the University of Paris for 33 years, and in 1862 he began an association with Paris's Salpêtrière Hospital that lasted throughout his life, ultimately becoming director of the hospital. In 1882, his focus turned to neurology, and he has been called by some the founder of modern neurology. He established a neurological clinic at the Salpêtrière that was unique in Europe, and in so doing established the bases for a neurological classification which have endured (see Fig. 1.2). He described multiple sclerosis [The combination of nystagmus, intention tremor and

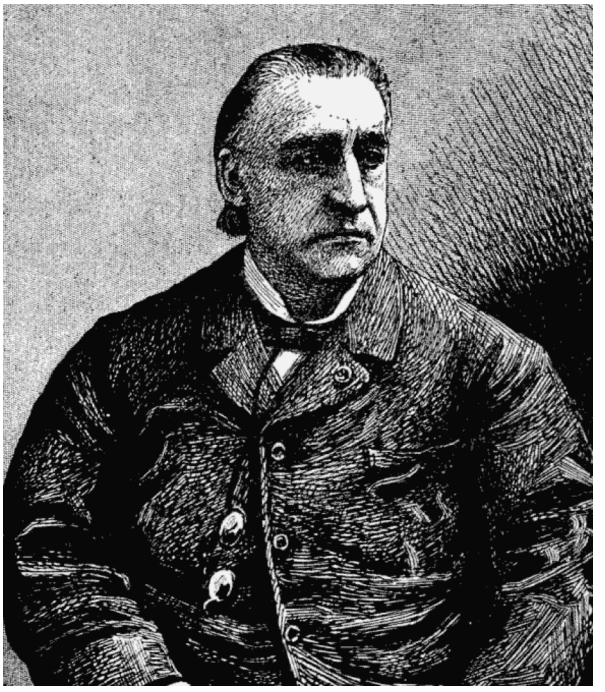

Fig. 1.1. Jean-Martin Charcot 1825–1893.

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scanning or staccato speech, Charcot's triad, is sometimes but not always associated with multiple sclerosis] (Charcot, 1879). He attributed progressive and acute muscular atrophy to lesions of the anterior horns of the spinal cord and locomotor ataxia to the posterior horn and spinal root. He gathered together the data leading to the description of amyotrophic lateral sclerosis as is discussed below.

In 1873 he replaced Dr Alfred Vulpian (see Fig. 1.3) as the Chair of Pathological Anatomy which he held for a decade. He added histology to macroscopic anatomy and undertook the exploration of the enormous resources in pathology at Salpêtrière (Bonduelle, 1994, 1997). In the study of 'Localizations of diseases of the spinal cord (1873–74)' he specified the anatomy and physiology of the cord and subsequently cerebral localizations of motor activities, both integral to his and our understanding of amyotrophic lateral sclerosis (known as Charcot's disease before it popularly became Lou Gehrig's disease) (Bonduelle, 1994, 1997; Goetz, 1994, 2000).

An eminent scientist, Charcot was recognized as one of the world's most prominent professors of neurology. In his time, he was both highly respected and chastized as a third-rate show-off. His scientific career was a continuous mixture of rigorous clinical neurology (including detailed descriptions of amyotrophic lateral sclerosis, Parkinson's disease, brain anatomy, etc.) and uncontrolled, controversial and sometimes even theatrical experiments in the field of hysteria. Charcot's fame was as much the result of the unquestionable quality of his scientific work as that of his theatrical presentations. In the arts as in politics, he was more of a conservative than an opportunist; authoritarian, shy, and brusque, gloomy and taciturn, he nonetheless had a remarkable power to attract.

Charcot's understanding of ALS evolved over a decade and was based on amazingly few patients (Goetz, 2000; Pearce, 2002). At the time of Charcot's descriptions of ALS, primarily 1850 to 1874, clinical diagnosis was rudimentary and the distinction between upper and lower motor neurons had not yet been made, and there was no understanding of the role of the corticospinal tract in connecting them (Goetz et al., 1995). The earliest description of ALS (1865) was that of a young woman whose deficit was restricted to the upper motor

Fig. 1.2. Saltpêtrière in 1882, the year that Charcot turned his thoughts to neurology.

Fig. 1.3. Alfred Vulpian who preceded Charcot as Professor of Anatomy at Saltpêtrière.

neurons (in fact more likely primary lateral sclerosis). She had been thought to be suffering from hysteria. Autopsy showed 'sclerotic changes limited to the lateral columns of the spinal cord' (Charcot, 1865). Four years later (1869), in a series of papers written together with Joffroy, Charcot reported cases of infantile and juvenile spinal muscular atrophy in whom the lesions were restricted to the anterior horn cells (Charcot and Joffroy, 1869a,b,c). Further clinical studies revealed a combination of upper and lower motor neuron signs which led Charcot to coin the term 'amyotrophic lateral sclerosis' (Charcot, 1874, 1880). 'We encountered several patients with the following conditions: paralysis with spasms of the arms and principally the legs (without any loss of sensation), together with progressive amyotrophy, which was confined mostly to the upper limbs and trunk' (Charcot and Joffroy, 1869c).

He thought the anterior horn pathology followed and was caused by disease of the lateral columns and drew a parallel with anterior horn cell pathology in multiple sclerosis, a concept not now in favor. Gowers (1886) strongly contested Charcot's notion that ALS commenced in the descending motor tracts and argued that the upper and lower motor neuron lesions occurred independently of each other, which is the general consensus. Eisen and Krieger (1998), however, have adduced physiologic evidence that reinforced Charcot's ideas about the significance of upper and lower motor neuron pathology.

His clinico-pathological observations led Charcot to believe there was a two-part motor system organization. Anterior horn cell disease resulted in weakness with atrophy, and sclerosis of the lateral columns produced spasticity with contractures (Charcot, 1880). Charcot was not the first to describe cases of ALS, but did coin the term amyotrophic lateral sclerosis (Rowland, 2001). Charles Bell and others reported cases as early as 1824. Having distinguished the motor functions of anterior spinal nerve roots and the sensory functions of the posterior roots, Bell was interested in finding patients with purely motor disorders (Goldblatt, 1968). By mid-century there were fiery debates among famous neurologists. Among the syndromes characterized by limb weakness and muscle atrophy, they ultimately came to separate neurogenic and myopathic diseases. It was not clear whether some syndromes were variants of the same condition or totally different disorders; this puzzle included progressive muscular atrophy, progressive bulbar palsy, primary lateral sclerosis and ALS.

Fortunately Charcot's thoughts were also recorded in English translations of the *Tuesday Lectures* at the Hôpital de la Salpêtrière (references cited by Rowland (2001)) and in translation by George Sigerson, who included the essential concepts of Charcot's ALS lectures in English and Goetz has brought the translations up-to-date (Goetz, 2000). The *Tuesday Lectures* also exemplified Charcot's zest for theatrical performance. For example, during one lecture of 1888, Charcot said: '*(To the patient)*: Give me your left arm. (Using a pin, M. CHARCOT pricks at different points the arm and the hand...).' Charcot followed this performance with another test, explaining to the audience as he did so that: 'You see that I am pulling the patient's finger, even a little brutally perhaps, without her suffering at all [*sans qu'elle éprouve rien*]... .' Turning to his subject, he asked: 'What am I doing to you?' She replied: 'I feel nothing.' The reality and authority of Charcot's lecture demonstrations was largely guaranteed by the fact that they unfolded in real time before the audience (Goetz, 1987).

Even though Charcot is credited as describing the pathology of ALS, Cruveilhier (1853a,b) made an essential contribution earlier, when he noted atrophy of the anterior roots and suspected malfunction of the anterior horn cells. Charcot knew of that work and compared it with his own observations of anterior horn cell pathology in infantile spinal muscular atrophy, poliomyelitis and other disorders characterized by muscular atrophy.

The terminology of these cases was not clarified for decades. Gowers (1886) is sometimes credited for introducing the term 'motor neuron disease' in 1886–1888. However, that term must have come later because Gowers used only the terms chronic spinal muscular atrophy, ALS or chronic poliomyelitis. Brain (1933) may have been the first to use 'motor neuron disease'; in the first edition of his textbook, published in 1933. He gave 'motor neuron disease' as a synonym for ALS (without mentioning why he used the new name).

It was 5 years after Charcot's initial case report that he first used the term 'amyotrophic lateral sclerosis,' which appeared in the title of the paper (http://clearx.library.ubc.ca:2796/cgi/content/full/58/3/512-REF-NHN7430-1; Charcot, 1874). In part IV of that series, he recorded more observations that have become standard teachings: Amyotrophic paralysis starts in the upper limbs as a cervical paraplegia. After 4, 5, 6 months or more, the emaciation spreads and there is protopathic muscular atrophy, which advances for 2 or 3 years. After a delay of 6 or 9 months, the legs are affected…but the muscles are conserved and contrast singularly with the state of the upper limbs.

There is no paralysis of the bladder. The patient has more difficulty walking and then cannot stand... After some time, the patient has noticed that, in bed or sitting, the legs sometimes extend or flex until a position is produced involuntarily…and the legs come to resemble a rigid bar. The rigidity is exaggerated when the patient is held up by assistants who want to walk him. The feet take on a posture of equinus varus. This rigidity, often extreme, affects all joints by a spasmodic action of the muscle. The tremor interferes with standing and walking.

He summarized the features of amyotrophic lateral sclerosis:

(1) Paralysis without loss of sensation of the upper limbs, accompanied by rapid emaciation of the muscles... At a certain time, spasmodic rigidity always takes over with the paralyzed and atrophic muscles, resulting in permanent deformation by contracture.
(2) The legs are affected in turn. Shortly, standing and walking are impossible. Spasms of rigidity are first intermittent, then permanent and complicated at times by tonic spinal epilepsy. The muscles of the paralyzed limb do not atrophy to the same degree as the arms and hands. The bladder and rectum are not affected. There is no tendency to the formation of bedsores.
(3) In the third period, the preceding symptoms worsen and bulbar symptoms appear. These three phases happen in rapid succession – 6 months to 1 year after the onset, all the symptoms have appeared and become worse. Death follows in 2 or 3 years, on average, from the onset of bulbar symptoms. This is the rule but there are a few anomalies. Symptoms may start in the legs or be limited to one side of the body, a form of hemiplegia. In two cases, it started with bulbar symptoms.

At present, the prognosis is grave. As far as I know, there is no case in which all the symptoms occurred and a cure followed. Is this an absolute block? Only the future will tell. Charcot, therefore, gave a complete picture of ALS, emphasizing lower motor neuron signs in the arms and upper motor neuron signs in the legs. His description of the natural history, lamentably, has not changed much in ensuing years. He described the bulbar syndrome in detail. He described clonus and he may have been the first to use the term 'primary lateral sclerosis.'

Charcot's own assessment of ALS was clearly stated: 'I do not think that elsewhere in medicine, in pulmonary or cardiac pathology, greater precision can be achieved. The diagnosis as well as the anatomy and physiology of the condition "amyotrophic lateral sclerosis" is one of the most completely understood conditions in the realm of clinical neurology' (Charcot, 1874). Charcot died in 1893 in Morvan, France.

1.2. Notable names with ALS

Because ALS is rare (an incidence of < 2 per 100,000) the list of famous or household names of people that have or had the disease is rather short. Amongst these is David Niven, the English actor, Dimitri Shostakovich, the Russian composer and Mao Tse Tung, the revolutionary leader of China. Nelson Butters was one of America's most distinguished neuropsychologists of the last 25 years. He died from ALS in 1995 at age 58. Like Stephen Hawking (see below), Dr Butters, toward the end made use of computers to communicate and work. This permitted him to edit a major journal in neuropsychology, even when he could move only one finger and then only one toe. With these small movements he used Email to write to colleagues everywhere – usually on professional matters, but also to transmit amusing academic gossip. However, the two names that have had the most impact are Lou Gehrig (Figs. 1.4 and 1.5) and Stephen Hawking (Fig. 1.6).

Of all the players in baseball history, none possessed as much talent and humility as Lou (Henry Louis) Gehrig. It seems that Lou Gehrig demonstrated the characteristic 'nice personality' of so many patients with ALS. His accomplishments on the field made him an authentic American hero, and his tragic early death

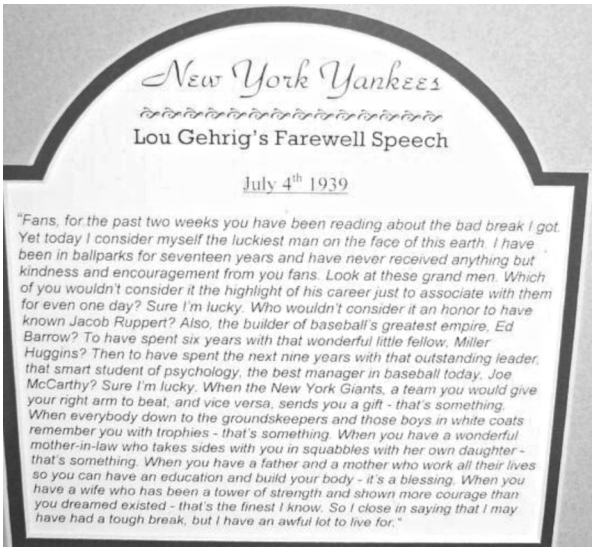

Fig. 1.4. Lou Gehrig's farewell speech.

made him a legend. Gehrig's later glory came from humble beginnings. He was born on June 19, 1903 in New York City. The son of German immigrants, Gehrig was the only one of four children to survive. Is it therefore possible that Lou Gehrig had hereditary ALS, but that his siblings never survived long enough to develop the disease? His mother, Christina, worked tirelessly, cooking, cleaning houses and taking in laundry to make ends meet. His father, Heinrich, often had trouble finding work and had poor health.

Gehrig's consecutive game streak of 2,130 games (a record that stood until Cal Ripken, Jr. broke it in 1995) did not come easily. He played well every day despite a broken thumb, a broken toe and back spasms. Later in his career Gehrig's hands were X-rayed and doctors were able to spot 17 different fractures that had 'healed' while Gehrig continued to play. Despite having pain from lumbago one day, he was listed as the shortstop and leadoff hitter. He singled and was promptly replaced but kept the streak intact. His endurance and strength earned him the nickname 'Iron Horse.' In 1938, Gehrig fell below 0.300 average for the first time since 1925 and it was clear that something was wrong. He lacked his usual strength. Teammate, Wes Ferrell noticed that on the golf course, instead of wearing golf cleats, Gehrig was wearing tennis shoes and sliding his feet along the ground. Gehrig played the first eight games of the 1939 season, but he managed only four hits. On a ball hit back to pitcher Johnny Murphy, Gehrig had trouble getting to first in time for the throw. On June 2, 1941, Lou Gehrig succumbed to ALS and the country mourned. Eleanor, his wife, received over 1,500 notes and telegrams of condolence at their home in Riverdale, New York. President Franklin Delano Roosevelt even sent her flowers. Gehrig was cremated and his ashes were buried at Kensico Cemetery in Valhalla, New York.

Stephen Hawking was born on the 300th anniversary of Galileo's death. He has come to be thought of as the greatest mind in physics since Albert Einstein. With similar interests – discovering the deepest workings of

Fig. 1.5. Lou Gehrig.

Fig. 1.6. Stephen Hawking.

the universe – he has been able to communicate arcane matters not just to other physicists but to the general public.

He grew up outside London in an intellectual family. His father was a physician and specialist in tropical diseases; his mother was active in the Liberal Party. He was an awkward schoolboy, but knew from an early age that he wanted to study science. He became increasingly skilled in mathematics and in 1958 he and some friends built a primitive computer that actually worked. In 1959 he won a scholarship to Oxford University and in 1962 he got his degree with honors and went to Cambridge University to pursue a PhD in cosmology. There he became intrigued with black holes (first proposed by Robert Oppenheimer) and 'space-time singularities' or events in which the laws of physics seem to break down. After receiving his PhD, he stayed at Cambridge, becoming known even in his 20s for his pioneering ideas and use of Einstein's formulae, as well as his questioning of older, established physicists. In 1968 he joined the staff of the Institute of Astronomy in Cambridge and began to apply the laws of thermodynamics to black holes by means of very complicated mathematics.

At the remarkably young age of 32, he was named a fellow of the Royal Society. He received the Albert Einstein Award, the most prestigious in theoretical physics. And in 1979, he was appointed Lucasian Professor of Mathematics at Cambridge, the same post held by Sir Isaac Newton 300 years earlier. In 1988 Hawking wrote *A Brief History of Time: From the Big Bang to Black Holes*, explaining the evolution of his thinking about the cosmos for a general audience. It became a best-seller of long standing and established his reputation as an accessible genius.

He remains extremely busy, his work hardly slowed by amyotrophic lateral sclerosis. "My goal is simple. It is complete understanding of the universe, why it is as it is and why it exists at all."

It is worthy and appropriate to mention one other name, that of Professor Richard Olney, who, at the time of writing, is in the terminal stages of ALS. It is impossible to imagine the nightmare of a neurologist, dedicated to ALS developing the disease that has occupied his career. Richard, a personal friend of mine and many of the contributors of this volume, started the ALS Clinic at the University of California (San Francisco) in 1993. He was dedicated to the disease and care of patients suffering from it. He contributed considerably to the advancement of understanding ALS, especially physiological aspects. He self-diagnosed the disease about 2 years ago when on vacation he began stumbling. There is no other recorded precedent of an ALS specialist developing the disease.

1.3. The first ALS gene

Charcot claimed that ALS was never hereditary. He clearly overlooked Aran's (1850) cases published 20 years earlier. As highlighted by Andersen (2003), amongst Aran's patients was a 43-year-old sea captain presenting with cramps in the upper limb muscles and subsequent wasting and weakness. He died within 2 years of onset of his disease and most likely had ALS. Aran reports that one of the patient's three sisters and two maternal uncles had died of a similar disease. It seems that this was the first hereditary case of ALS. It took another 143 years before the superoxide dismutase gene (SOD1) was discovered to be associated with familial ALS (Rosen et al., 1993). Eleven missence mutations were found in 13 of 18 familial ALS (FALS) pedigrees.

1.4. Western Pacific ALS

It is not the role of this chapter to discuss similarities and differences between Western Pacific ALS and the disease elsewhere. However, the differences may be more apparent than real. Evidence indicates that ALS was prevalent on the island of Guam at least since 1815, some 50 years before Charcot's first descriptions (Lavine et al., 1991). Had Charcot been able to visit Guam one wonders what he would have made of the disease. Although he described the pathology and clinical picture so accurately it seems strange that there was little reference if any as to its possible cause. During the early years of American occupation of Guam (1898–1920) death certificates were written in Spanish and there were frequent deaths attributed to "paralytico" or "lytico" terms the Chamorro used for ALS. The term 'rayput' or 'bodig' (slowness or laziness) was used for Parkinsonism-dementia. The Western Pacific form of ALS has been of interest for over 50 years because its incidence, prevalence and mortality rates were initially 50 to 100 times those of ALS elsewhere. The male:female ratio approximated 2:1, the median age at onset was 44 years, familial aggregation was recognized and ALS was associated frequently with a Parkinsonism/dementia complex (PDC) (Armon, 2003). Recently, the frequency of Western Pacific ALS has declined, implying a temporary exposure to an environmental risk factor, possibly in a genetically susceptible population. This has fueled decades of research and speculation.

Marjorie Whiting, a nutritionist who lived with the Chamorros in Guam, became convinced that the disease resulted from ingestion of the cycad nut used to prepare flour (Whiting, 1963). During the Japanese occupation of Guam during World War II, many Chamorros fled into the forests and may have eaten more cycad flour

than usual. However, there is at least one well recorded case of chronic cycad intake, without apparent harm, in Sergeant Soichi Yokoi of the Japanese Imperial Army. He was captured after 28 years as a fugitive in the jungles of Guam and was wearing clothes that he had made himself from fibers he had peeled from the bark of a Pago tree. Such was the astonishing level of his self-sufficiency that he was met with total disbelief until he explained to his captors how he was able to survive for over a quarter of a century by living off the natural resources of the land. A principal part of his diet was fadang. Remarkably, Sergeant Yokoi not only discovered that fadang was edible but, astonishingly, devised a way to prepare the nuts properly before cooking. He lived to be 82, dying in 1997.

The cycad hypothesis was abandoned because two similar clusters of neurodegenerative disease were found in remote indigenous populations in Japan and Papua New Guinea, neither of whom seemed to eat cycad nuts. Also a good animal model never really evolved (see Chapter 18). However, the cycad story may have come back to life. It has now been suggested that the answer may lie in the Chamorro's favorite entree: flying fox bats boiled in coconut cream (Cox and Sacks, 2002). The bats have been especially desirable food items to the Chamorro, possibly because the tradition is one of few retained from older times before four centuries of upheaval and cultural oppression which began with Spanish colonial rule in 1565. They were served at weddings, fiestas, birthdays and the like. The etiquette of bat-eating and preparation involves rinsing off the outside of the animal like you would a cucumber and tossing it in boiling water. The animals are then served whole in coconut milk and are consumed in their entirety. Meat, internal organs, fur, eyes and wing membranes are all eaten.

So why the dramatic decrease in incidence of ALS on Guam? Flying foxes are slow breeders, with females needing to be 3 years old before they can successfully give birth and rear babies. Then they rear only one youngster each year. Add this to the high death rate that is common in any young wild animal. In fact, numbers of flying foxes has dropped alarmingly towards extinction.

1.5. Spinal muscular atrophies

The clarity with which Charcot was able to describe ALS was not matched by early descriptions of diseases that appeared to be restricted to the lower motor neuron, manifesting primarily by limb weakness. This is hardly surprising when one considers that as recently as 2003 classification of lower motor neuron syndromes (including diffuse, proximal, distal and monomelic) is still very much under discussion (Van den Berg-Vos et al., 2003). The issue is further complicated by early descriptions of primary muscle disease, especially Duchenne muscular dystrophy, which were being published about the same time as the first descriptions of spinal muscular atrophy. Tyler (2003) has recently reviewed the historical roots of Duchenne muscular dystrophy in the 19th century, citing early papers by Conte, Bell, Partridge and Meryon through to the classic monographs of Duchenne and Gowers. It is clear that a number of these cases turned out to be anterior horn cell disease and not primary muscle disease.

In 1850, Francois-Amilcar Aran described cases using the name "progressive spinal muscular atrophy" (Aran, 1850). However, there had not been an autopsy study of these patients and there was no clinical distinction between neurogenic and myopathic diseases, a notion that was yet to come. Aran was born in Bordeaux, where he commenced his medical studies but graduated in Paris. He published his first paper even before he had become MD, for which honor he delivered an inaugural thesis entitled Des palpitations du coeur, considérées principalement dans leur nature et leur traitement (Aran, 1843).

He was active in the publishing of several journals, among them *Archives générales de médecine* and the *Union médicale*, to which he was one of the most prolific contributors, publishing both his own papers as well as analyses of English works. As professor agrégé, he held private courses of therapy at the École pratique. At the Hôtel-Dieu, as deputy to Léon Louis Rostand (1790–1866), Aran's clinical lectures were tremendously successful. In the final years Aran preferably concerned himself with studies of materia medica, while still a prolific writer. One of his papers was on acute rheumatism, from which he himself had suffered repeatedly, and which caused his premature death on February 22, 1861, at the age of only 44 years. He left a large number of unfinished works, one of them a Dictionnaire de thérapeutique, of which only the first letters had been put on paper.

Duchenne (Fig. 1.7) claimed equal priority to describing spinal muscular atrophy. He had studied all of Aran's patients with electrical stimulation (Duchenne de Boulogne, 1851). However, it is not clear whether Aran described Duchenne's patients or vice versa. Duchenne's bid for priority was based on a notice of 50 words, not a scientific paper. The announcement stated that, at a weekly meeting of the Academy (French Academy of Science), he presented a collection of papers, which he called 'Recherches Electro-Physiologiques' and which he intended to be used as evidence by future commission of authorities that never left a record, if it ever existed. The ultimate compromise

Fig. 1.7. Duchenne de Boulogne.

was the eponym 'Aran-Duchenne' syndrome for what we now regard as the broader categories of spinal muscular atrophies.

Guillaume Benjamin Amand Duchenne descended from a family of fishermen, traders and sea captains who had resided in the harbor city Boulogne-sur-Mer in Northern France since the first half of the 18th century. He was predestined for a career at sea, as his father was the commander Jean Duchenne who had been a ship's captain during the Napoleonic wars and expected his son to follow in his keel waters.

Despite his father's efforts to induce him to follow the family seafaring tradition, his love of science prevailed. Duchenne went to a local college at Douai, where he received his baccalauréat at the age of 19. From 1827, aged 21, he studied medicine under teachers like René-Théophile-Hyacinthe Laënnec (1781–1826), Baron Guillaume Dupuytren (1777–1835), François Magendie (1783–1855) and Léon Cruveilhier (1791–1874). He graduated in medicine in Paris in 1831 and, probably influenced by Dupuytren, presented his Thèse de médecine, a monograph on burns.

However, Duchenne's early years in medicine were undistinguished. His interest in "electropuncture," recently invented by Magendie and Jean-Baptiste Sarlandière (1787–1838), enticed him back to Paris where he was met with a rather cool reception, being ridiculed for his provincial accent and his coarse manners. Duchenne was never offered, and never applied for, an appointment at a Paris teaching hospital or at the university. He was known under the name of Duchenne de Boulogne to avoid confusion with Édouard Adolphe Duchesne (1894–1869), a fashionable society physician. Nevertheless, Duchenne was a diligent investigator and meticulous at recording clinical histories. When necessary he would follow his patients from hospital to hospital to complete his studies. In this way he achieved an exceptionally rich and exquisite research material.

Toward the end of his life Duchenne became established and popular, paradoxically Jean-Martin Charcot was amongst his friends, and they held each other in considerable esteem. His clinical ability was such that the great Charcot dubbed him 'The Master.' At this stage of his career he had become an international celebrity. Every month he gave several dinner parties for his colleagues (Charcot, Pierre Paul Broca, Auguste Nélaton and Edmé Félix Alfred Vulpian). During these get-togethers histological slides were projected and discussed – mixed with funny pictures to please Duchenne's grandchild. These were the first attempts at muscle pathology. Duchenne was probably the first person to use biopsy procedure to obtain tissue from a living patient for microscopic examination. This aroused a deal of controversial discussion in the lay press concerning the morality of examining living tissues. In order to perform histopathological diagnostics Duchenne constructed a biopsy needle, which made possible percutaneous muscle biopsies without anesthesia.

1.6. Spino-bulbar muscular atrophy (SBMA) – Kennedy's disease

[I am most grateful to my friend and colleague, Professor William Kennedy for much of what is transcribed verbatim from his records.]

In 1966 William Kennedy (Fig. 1.8) and co-workers described an anterior horn cell disease characterized by X-linked inheritance, onset in the 4th and 5th decades and with slow progression with predominantly proximal spinal and bulbar muscle involvement and tongue muscle furrowing. They commented on the associated features of gynecomastia, diabetes and absence of long tract signs (Kennedy et al., 1966).

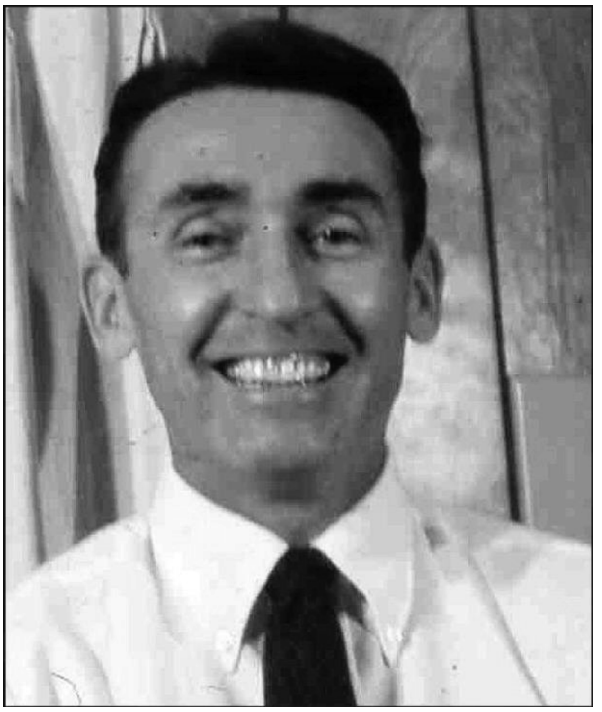

Fig. 1.8. Dr William Kennedy the year he described spinobulbar muscular atrophy.

"In July 1964, George B., age 57, entered my (Dr Kennedy's) office." He was of French-Indian descent from a large family that lived on Grey Cloud island in the Mississippi river. George complained of increasing generalized weakness and pain, mainly in the neck and shoulders. As a youth he could run and work as well as others. Since about age 35 he hadn't felt strong. Muscle cramps began in his chest, abdomen and calf and there was twitching in his chin and shaking with his arms outstretched. Later he had definite weakness noticeable when lifting objects over his head. Distal strength in his hands remained good. When George was 37, a neuropsychiatrist diagnosed primary muscular atrophy and commented on the grooves in George's tongue. Yet, from age 38 to 43 he worked in a slaughterhouse where he split pigs down the back with a 16 lb cleaver. For about 20 years cold weather had hampered fine motions such as buttoning his shirt. At about age 54 he began to aid chewing by holding his chin up with his hands. At the same time his voice changed pitch and began to be slurred. By 1964 walking required great effort.

At examination muscle weakness was generalized, but more severe proximally. The gait was waddling. He could not walk on his toes, hop, squat or rise. Reaching overhead and heelwalking were moderately weak. The biceps tendon reflex was depressed; all others were absent. Large fasciculations were visible in the chin muscles. The tongue was grooved and atrophic. Facial weakness was marked and the lips protruded, but smiling was possible. The voice was low pitched and gravelly. Word pronunciation was poor. Sensation seemed normal. Conduction hearing was decreased. There was bilateral gynecomastia. The patient had been diagnosed with diabetes at age 59.

Motor nerve conduction velocity was normal. EMG showed scattered fibrillation potentials and giant motor unit action potentials (MUAPs) in several muscles. Muscle histology showed groups of atrophic muscle fibers with small prominent groups of very large hypertrophic fibers with central nuclei and some basophilia. He died of pneumonia at home at age 60. There was no autopsy.

George's father, Victor B, died at age 57. He had a marked tremor at age 30. He was a farmhand until age 40 when he became too weak. He could ride a tractor but could not mount or dismount alone. He needed a railing to climb stairs. Rising from a chair required use of his arms. George thought his father had had fasciculations and a shrunken tongue. He was never hospitalized. There are no medical records. His possible involvement initially caused us much confusion.

On August 7, 1964, Robert G, age 68, of German and Swiss descent, was referred for anterior horn cell disease. Robert had generalized weakness, areflexia and the now familiar facies with fasciculations. NCV was normal. EMG showed very large MUAPs. Median nerve sensory responses were absent. The patient and brother Alfred had been previously diagnosed by several neurologists with primary muscular atrophy in 1951 and again in 1957. Alfred died in a university hospital without autopsy. Brother William, with the same disability, died of pneumonia in 1957. Robert died in 1967. Robert's wife requested that autopsy material be sent to me. There was marked reduction of anterior horn cells at all levels, but the cells of Clarke's column and of the posterior horn were preserved. The anterior spinal nerve roots contained fewer myelinated nerve fibers than expected as compared with the posterior spinal nerve roots. Muscle biopsy was identical to that of George B. Similar cases had been described earlier, but not fully appreciated (Kurland, 1957; Gross, 1966). It was not until the 1980s that the association of depressed or absent reflexes and small or absent sensory potentials was described (Barkhaus et al., 1892; Harding et al., 1982). In 1991 that genetic cause of SBMA was identified by Albert La Spada and Kenneth Fischbeck as the expansion of a polymorphic CAG repeat sequence in the first exon of the gene encoding the androgen receptor (La Spada et al., 1991).

Dr Kennedy was unaware that the disease he had discovered was given his name until it appeared as such in a paper by Schoenen et al. (1979).

1.7. Upper neuron syndromes

About a decade following Charcot's (1865) original description of amyotrophic lateral sclerosis (ALS), Erb (1875) described a disorder characterized by exclusive involvement of the corticospinal tract which he named "spastic spinal paralysis." Several cases given the name of 'lateral sclerosis' were described even earlier, and four of these were familial. In retrospect they most likely represented some form of hereditary spastic paraparesis, or one of the recently described infantile ALS syndromes (Lerman-Sagie et al., 1996; Devon et al., 2003). It appears that Charcot's first case of ALS was in fact a case of PLS.

Konzo was first identified in 1936 by Tessitore (Trolli, 1938), a district medical officer in the Kahemba District in the south-eastern part of the Bandundu Province of the Democratic Republic of Congo (DRC). There, konzo is known to be endemic with a prevalence as high as 5% in certain villages. However, amongst 146 identified cases there were reports of some cases being affected 30 to 40 years prior to 1937 when Tessitore identified 140 new cases of konzo in the same area. Konzo was brought to scientific attention by two epidemic outbreaks, each numbering more than 1,000 cases. The first was in Bandundu Region in present-day Zaire in 1936–37 and the second in Nampula Province of Northern Mozambique in 1981. Smaller outbreaks in rural areas have subsequently been reported from Zaire, Mozambique, Tanzania and the Central African Republic. Sporadic cases of konzo also occur in affected areas, years after an extensive outbreak.

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Chapter 2

Comparative anatomy and physiology of the corticospinal system

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2.1. Introduction

In 1905, Campbell compiled his histologic studies of the cerebral cortex in normal apes and humans, in a number of human amputees and in two patients with amyotrophic lateral sclerosis (ALS). He noted that the giant Betz cells of the precentral gyrus: (1) occupied the cortical territory where Grunbaum and Sherrington had elicited contralateral movement with electrical stimulation in the same individual apes, (2) underwent retrograde transneuronal degeneration after amputation, and (3) degenerated in ALS (Campbell, 1905). Synthesizing these observations, Campbell recognized that the cortex containing Betz cells provided the most direct connections from the cerebral cortex to spinal motoneurons, a corticospinal projection.

Since Campbell's time, anatomical and physiological studies in both humans and animals have revealed that the corticospinal system is more complex than a single pathway directly connecting Betz cells in one hemisphere to motoneurons in the contralateral spinal cord. Much of what we know about the corticospinal system in man, however, is based on extrapolation from phylogenetic trends identified in the more precise and detailed studies that can be performed in experimental animals. Care must be taken in extrapolating this information to humans, as species differences clearly exist. For example, the number of axons in the pyramidal tract increases along the phylogenetic scale as follows: rat 73,000; cat 186,000; monkey 554,000; chimpanzee 800,000; human 1,100,000 (Lassek and Rasmussen, 1939; Lassek, 1941; Lassek and Wheatley, 1945). In addition to the greatest number of descending axons, humans probably have more direct corticomotoneuronal connections than any other species, and humans therefore are more dependent on their corticospinal tract for normal movement.

Nevertheless, a comparative approach offers the most detailed understanding possible of the corticospinal tract, which has been studied in numerous mammalian species (Heffner and Masterton, 1975; Armand, 1982). For comparison with humans, we will focus here on the most intensively studied non-human species, the domestic cat (*Felix domesiticus*), and old world, macaque monkeys (*Macaca* species). Other species – rodents, new world monkeys, baboons, apes, etc. – will be mentioned only to develop specific points. For detailed and extensive information, the interested reader is referred to a number of comprehensive monographs (Lassek, 1954; Phillips and Porter, 1977; Porter and Lemon, 1993).

2.2. The corticospinal tract

The general course of the corticospinal tract is well-known, descending from the motor cortex to the medullary pyramid and then decussating to the dorsolateral funiculus of the contralateral spinal cord. Weakness, therefore, is contralateral to lesions of this pathway in the brain. A more detailed consideration of the corticospinal tract reveals additional complexities, however, that may account for a wider variety of phenomena observed clinically.

In all species, the corticospinal tract arises by and large from Brodmann's area 4, which is considered to be the primary motor cortex (M1). In cats, area M1 lies within the lateral aspect of the cruciate sulcus and extends on to the surrounding hemispheric surface. In macaque monkeys, M1 lies in the anterior bank of the

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central sulcus and extends onto the posterior half of the surface of the precentral gyrus. In humans, M1 lies largely within the anterior bank of the central sulcus, extending on to the surface of the precentral gyrus primarily in the medial, leg representation (see Fig. 2.8(A,B)) (Campbell, 1905; Zilles et al., 1995).

The pyramidal somata of corticospinal tract neurons are located in the deepest part of cortical layer V. Although the giant Betz cells often are assumed to be the only neurons from which the corticospinal projection originates, many other large and moderate sized pyramid-shaped somata in layer V contribute axons to the corticospinal tract. This can be illustrated by comparing the number of Betz cells in the human M1, 34,000, to the number of axons in human pyramid, 1,100,000 (Lassek and Wheatley, 1945). Only 3% of the tract arises from Betz cells, while the remaining 97% arises from smaller neurons.

As axons descend from layer V toward the white matter, some give off collaterals that travel horizontally within the cortical gray up to several millimeters, providing interconnections within the major body part representations of M1 (Ghosh and Porter, 1988; Huntley and Jones, 1991). Descending through the centrum semiovale, corticospinal axons from M1 converge in the middle third of the posterior limb of the internal capsule. Descending to the level of the midbrain, corticospinal fibers lie in the middle third of the cerebral peduncle, with corticobulbar fibers from more anterior regions of the frontal lobe in the medial third and those from the parietal and temporal lobes in the lateral third of the peduncle. As they enter the base of the pons, the descending fibers of the cerebral peduncle become intermingled with the somata and crossing axons of the neurons of the pontine nuclei. The bulk of corticofugal fibers that enter the pons from the cerebral peduncle terminate here in the pontine nuclei. Fibers destined for the spinal cord emerge on the ventral aspect of the medulla as the pyramid.

In the pyramid of macaque monkeys, axons that have descended from the face representation tend to lie dorsally, while those that have descended from the upper extremity and lower extremity representations are intermingled throughout the cross-sectional area (Coxe and Landau, 1970). As they approach the cervicomedullary junction, fibers from the face representation turn dorsally to enter the medullary tegmentum, the majority decussating to the opposite side to innervate the pontomedullary reticular formation and bulbar nuclei. In monkeys, cortical innervation of the facial nucleus is directed primarily to the lateral motoneurons that innervate lower facial muscles, whereas the dorsal motoneurons that innervate upper facial muscles receive relatively little cortical innervation (Jenny and Saper, 1987). In humans, this difference may account in part for the relative sparing of upper facial strength after unilateral cortical lesions.

As fibers from the upper and lower extremity representations reach the cervicomedullary junction, the majority likewise leave their position on the ventral aspect of the neuraxis, turn dorsally and decussate, entering the lateral column of the spinal cord, where they concentrate in the dorsolateral funiculus (Fig. 2.1). (In rodents, however, the crossed corticospinal fibers descend in the ventral-most base of the dorsal column (Brown, 1971; Wise and Donoghue, 1986).) A minority (~10%) of corticospinal fibers remain in their ventral location, uncrossed, in the anterior column of the cord adjacent to the anterior fissure. In approximately 75% of human cases, the lateral and anterior corticospinal tracts are asymmetric, with the right side larger than the left (Nathan et al., 1990). Such asymmetry has not been reported in monkeys and may be one anatomical feature related to human handedness.

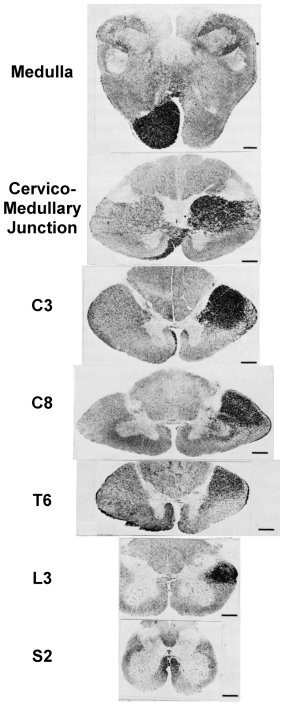

Fig. 2.1. The human corticospinal tracts. Sections of the medulla and spinal cord stained with the Marchi method for degenerating fibers are shown from a 69-year-old man who sustained an extensive infarct in the territory of the right middle cerebral artery 17 days before death. The right medullary pyramid and left dorsolateral funiculus show numerous degenerating fibers. In the C3 section, the anterior corticospinal tract can be seen as a crescent of degenerating fibers in the right anterior column adjacent to the central fissure. Calibration bars represent 1 mm. Modified from Nathan et al. (1990).

The lateral and anterior corticospinal tracts typically are assumed to be crossed versus uncrossed, respectively. In monkeys, however, a small number of uncrossed axons can be observed in the dorsolateral funiculus (Liu and Chambers, 1964). These uncrossed axons in the lateral column when stimulated are sufficient to excite motoneurons ipsilateral to the hemisphere of origin (Bernhard et al., 1953). Other corticospinal axons that have decussated at the cervicomedullary junction and descend in the dorsolateral funiculus cross back through the gray matter commissure of the spinal cord, terminating ipsilateral to the hemisphere of origin (Chambers and Liu, 1957; Liu and Chambers, 1964; Galea and Darian-Smith, 1997a). Similarly, a small number of corticospinal axons that have decussated at the cervicomedullary junction descend close to the medial aspect of the ventral horn. Some of these decussated fibers eventually cross back in the anterior white matter commissure, terminating ipsilateral to the hemisphere of origin (Chambers and Liu, 1957; Liu and Chambers, 1964; Nathan et al., 1990). Both uncrossed and doubly decussating fibers may contribute to observations in human patients that, although only weakness contralateral to a lesion above the pyramidal decussation may be appreciated clinically, ipsilateral weakness can be measured objectively as well (Colebatch and Gandevia, 1989; Adams et al., 1990).

Nevertheless, the bulk of the lateral corticospinal tract is crossed and descends in a position close to the dorsolateral aspect of the ventral horn, where the motor nuclei of distal limb musculature are located (Kuypers, 1982; Dum and Strick, 1996). In contrast, the bulk of the anterior corticospinal tract is uncrossed and descends in a position close to the anteromedial aspect of the ventral horn, where the motor nuclei of proximal limb musculature are located. In general the lateral corticospinal tract exerts greater control over distal limb than axial musculature, whereas the anterior corticospinal tract exerts more control over axial and proximal limb than distal limb musculature.

Fibers descending from the hand and foot representations in the motor cortex are intermingled in the lateral and ventral tracts. The majority of fibers originating in the upper extremity representation terminate in the gray matter of the cervical enlargement, while the majority of fibers from the leg representation terminate in the lumbosacral enlargement. Some fibers from the motor cortex forelimb representation, however, send collaterals to terminate at cervical levels, and then continue to descend in the dorsolateral funiculus down to lumbosacral levels, where they terminate in the intermediate zone of the spinal gray (Kuypers, 1960; Liu and Chambers, 1964; Shinoda et al., 1976). Some fibers from the hindlimb representation conversely send collaterals to terminate in the cervical enlargement as they descend to end at lumbosacral levels. These corticospinal axons that terminate at somatotopically inappropriate spinal levels may play a role in coordinating posture and movement of the upper and lower extremities.

2.3. Terminations in the spinal gray matter

As they reach the appropriate spinal levels, descending corticospinal axons enter the spinal gray matter. Ascending the phylogenetic scale from rat to cat through monkey and chimpanzee to the human, corticospinal terminations shift progressively more ventrally in the spinal gray (Fig. 2.2). While maintaining some contact with the dorsal horn, the corticospinal terminations overall achieve progressively closer interneuronal access to motor output and ultimately increasingly numerous, direct synaptic contacts to the spinal motoneurons themselves (Kuypers, 1958, 1960, 1982). A parallel trend from sensory to increasingly direct motor innervation is found comparing the corticobulbar projection in different species.

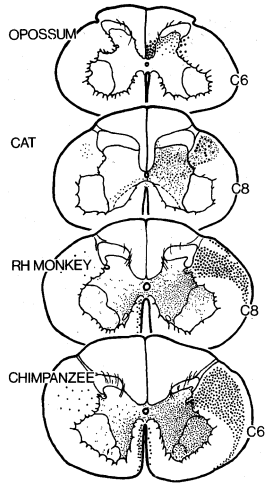

Fig. 2.2. Comparative trends in the corticospinal tract and its terminations. The position of descending corticospinal fibers and their terminations in the gray matter of the brachial enlargement is illustrated schematically for four species: opossum, cat, Rhesus (macaque) monkey and chimpanzee. In rodents and marsupials the corticospinal tract descends primarily in the contralateral dorsal column and terminates largely in the dorsal horn. In higher mammals the tract descends largely in the contralateral dorsolateral funiculus, although some uncrossed fibers are found in the ipsilateral dorsolateral column and others in the anterior column, particularly in primates. Terminations in these species are largely in the intermediate zone, though in monkeys and chimpanzees increasingly numerous terminations are found among the motoneuron cell columns (lamina IX). Reproduced from Kuypers (1982) with permission from Elsevier.

Within the gray matter, corticospinal axons ramify and synapse extensively on interneurons. In all species that have been studied, the greatest number of corticospinal terminations are found in Rexed's laminae V and VI (Heffner and Masterton, 1975). In cats, the bulk of terminations are found in the base of the dorsal horn and intermediate zone (Chambers and Liu, 1957). Some terminations extend as well into lamina VII, but do not reach lamina IX (Futami et al., 1979; Shinoda et al., 1986). Although motoneuron dendrites extend into the intermediate zone of the spinal gray matter and some light microscopy studies have visualized corticospinal boutons on motoneurons (Liang et al., 1991), in rats and cats corticospinal axons do not make physiologically evident synaptic contact with motoneurons (Lloyd, 1941; Hern et al., 1962; Alstermark et al., 2004).

Instead, feline corticospinal axons synapse on interneurons in the intermediate zone. Many of these interneurons have excitatory effects on motoneurons. Through excitatory interneurons, trains of pyramidal tract stimulation can facilitate the mono-synaptic reflex as well as oligo-synaptic reflexes (Lloyd, 1941) and evoke movement (Adrian et al., 1939; Landau, 1952). Pyramidal volleys can facilitate cutaneous reflexes as well (Sasaki et al., 1996). Especially well studied in cats are certain classes of inhibitory interneurons. Ia inhibitory interneurons receive synaptic inputs from primary muscle spindle afferents and deliver synaptic inhibition to heteronymous motoneurons. Ib inhibitory interneurons receive synaptic input from Golgi tendon organs and deliver synaptic inhibition to homonymous motoneurons. These Ia and Ib inhibitory interneurons receive additional synaptic inputs from numerous other segmental and descending sources, including corticospinal neurons (Lundberg, 1979). Via these synaptic connections to spinal interneurons, the corticospinal system can influence basic reflexes. In monkeys as well, corticospinal neurons inhibit motoneurons via spinal inhibitory interneurons (Preston and Whitlock, 1960, 1961). Though studied less directly in humans, the organization of these reflex interneurons and their control by the corticospinal system appear to be generally similar to that in the cat (Jankowska and Hammar, 2002; Petersen et al., 2003).

The loss of corticospinal control may account in part for reflex changes associated with corticospinal lesions. Reduced corticospinal input to Ia and Ib inhibitory interneurons may contribute to hyperreflexia. Also following corticospinal lesions, tendon jerks that normally elicit reflex contraction only in the stretched muscle may elicit contraction in additional muscles. Stretch of the flexor digitorum profundus tendons, for example, may elicit an abnormal reflex contraction of the flexor pollicis longus (Hoffmann's sign). In cats, muscle afferents normally facilitate many heteronymous motoneurons in addition to homonymous motoneurons (Fritz et al., 1989; Wilmink and Nichols, 2003). Transmission through these heteronymous reflex pathways normally may be checked by corticospinal influence on inhibitory interneurons in the spinal cord. Loss of this influence then may lead to the abnormal spread of reflexes observed after corticospinal lesions in humans.

In addition to modulating spinal reflex pathways, the corticospinal system has influence over neuronal circuits in the spinal cord that constitute the central pattern generators (CPGs) for cyclical motor behaviors such as walking. In rats, repetitive discharge of a single motor cortex neuron (driven by intracellular depolarization) can be sufficient to activate the CPG that drives whisking movements of the vibrissae (Brecht et al., 2004). In cats, although the corticospinal tract is not essential for initiation of locomotion or for ambulation on a flat surface (Drew et al., 2002), pyramidal tract neurons discharge intensely in lifting the foot over an obstacle or during complex locomotion on the rungs of a horizontal ladder (Beloozerova and Sirota, 1993a; Drew, 1993; Widajewicz et al., 1994). The primary role of the corticospinal system in feline locomotion thus may lie in modifying the basic rhythmic pattern to adapt to complex circumstances.

Corticospinal neurons also contact a special class of long propriospinal neurons at cervical levels just above the brachial enlargement, C3–C4. In cats, these propriospinal neurons help mediate visually guided reaching. Collaterals of descending corticospinal, rubrospinal and tectospinal inputs converge on these C3–C4 propriospinal neurons, which in turn send their axons in the ventrolateral funiculus down to forelimb motoneurons in the lower cervical segments (Illert et al., 1978; Alstermark et al., 1991). Stimulation of the pyramidal tract produces disynaptic EPSPs in forelimb motoneurons, which persist after a lesion of the lateral corticospinal tract at C5, but are abolished by an additional lesion of the ventrolateral funiculus at C5. These observations indicate that the lateral corticospinal tract excites the C3–C4 propriospinal neurons, which in turn excite distal forelimb motoneurons in the C6–T1 spinal segments. Lesions of the corticospinal tract at C2, or of the ventrolateral funiculus at C5, result in inaccurate reaching, indicating that the information transmitted by the descending corticospinal and rubrospinal systems to the C3–C4 propriospinal neurons and thence to distal forelimb motoneurons, plays an important role in visually guided reaching (Alstermark et al., 1981).

In primates, the disynaptic EPSPs in forelimb motoneurons characteristic of the C3–C4 propriospinal neurons are weaker and less common than in cats (Maier et al., 1998). Administration of strychnine,

however, reveals disynaptic EPSPs that are abolished by a lesion in the dorsolateral funiculus at C2, but not by a similar lesion at C5 (Alstermark et al., 1999). These observations suggest that C3–C4 propriospinal neurons in primates receive more glycinergic inhibition than do the homologous neurons in cats. Alternatively, the strength of C3–C4 propriospinal input to forelimb motoneurons may decrease from cats through different species of primates to humans, as the strength of direct corticomotoneuronal projections increase (Nakajima et al., 2000). Nevertheless, in macaque monkeys the C3–C4 propriospinal system still appears to contribute to accurate control of dexterous finger movements (Sasaki et al., 2004).

In humans, the presence of similar propriospinal neurons is indicated by the facilitation of H-reflexes resulting from stimulation of cutaneous or mixed nerve afferents. The central latency of such facilitation (typically 3–6 milliseconds) is too long to be attributed to segmental interneurons, but too short to be mediated by supraspinal loops, suggesting that the afferent impulses act via neurons a few spinal segments away from the motoneurons probed by the H-reflex (Burke et al., 1992; Gracies et al., 1994; Mazevet et al., 1996). Additional facilitation appears during weak voluntary contraction of the muscle, suggesting that descending and afferent inputs converge on human propriospinal interneurons.

2.4. Direct cortico-motoneuronal connections

Although the bulk of corticospinal terminations still are found in the intermediate zone of the spinal gray, in macaque monkeys many corticospinal axons extend ventrally into lamina IX of the spinal gray matter (Figs 2.2 and 2.9(A)) (Liu and Chambers, 1964; Kuypers, 1982; Dum and Strick, 1996). Here they ramify and make direct synaptic contact with motoneurons (Hoff and Hoff, 1934; Lawrence et al., 1985). The ramifications and terminations of corticospinal axons in lamina IX are denser still in chimpanzees (Kuypers, 1982) and humans (Schoen, 1969). These corticospinal connections with motoneurons may be particularly associated with relatively fine, independent digit movements, which are more highly developed as one progresses from monkeys to apes to humans. Comparing two species of new world monkeys, for example, revealed that the more dexterous cebus monkey has more corticospinal terminations in lamina IX than the less dexterous squirrel monkey (Bortoff and Strick, 1993). Certain dexterous carnivores, including the raccoon and the kinkajou, also have corticospinal terminations in lamina IX (Heffner and Masterton, 1975).

Experimental demonstration of physiologically active synapses made directly on motoneurons by corticospinal axons has been obtained in monkeys and baboons. The delay from arrival of an electrically evoked descending corticospinal volley at a given spinal segment to the appearance of an evoked volley in the ventral roots (Bernhard et al., 1953), to facilitation of monosynaptic reflexes (Preston and Whitlock, 1960) or to the onset of EPSPs in motoneurons (Preston and Whitlock, 1961; Clough et al., 1968; Jankowska et al., 1975), all are consistent with monosynaptic transmission. Based on the phylogenetic trend from monkeys to apes to humans, these direct cortico-motoneuronal (CM) synaptic connections generally are inferred to be even more important for normal function in humans than in monkeys.

Individually, these corticomotoneuronal connections are not necessarily the strongest synaptic inputs received by motoneurons. In macaque monkeys, a single corticospinal axon makes only 1–2 synaptic boutons on the proximal dendrites of a given cervical motoneuron (Fig. 2.3(A,B)) (Lawrence et al., 1985). Minimal cortically evoked monosynaptic EPSPs in lumbar motoneurons are smaller than minimal Ia EPSPs (Porter and Hore, 1969). The time constants of corticomotoneuronal EPSPs also are longer than those of Ia EPSPs, indicating that the CM synapses are situated more peripherally on the motoneuron dendrites. Nevertheless, the maximal CM EPSPs in baboon cervical motoneurons evoked by stimulation of the cortical surface are larger than the maximal homonymous Ia EPSPs evoked by stimulation of the muscle nerve, suggesting a greater total input to the motoneurons from CM cells than from Ia afferents (Clough et al., 1968). In humans, CM synaptic boutons may be located in part on the motoneuron somata (Schoen, 1969), which suggests a stronger synaptic effect than in monkeys.

The effectiveness of primate CM synapses is enhanced further by facilitation at higher frequencies (Fig. 2.3(C)). When the cortex is stimulated with short, high frequency bursts (e.g. above 50 Hz), the sequential CM EPSPs within a burst become progressively larger, beyond simple temporal summation (Landgren et al., 1962b) and this facilitation becomes more prominent as stimulation frequency increases (Muir and Porter, 1973). The corticospinal volley recorded in the lateral column does not facilitate during such bursts, and facilitation also is seen when the pyramidal tract is stimulated, suggesting that this facilitation involves a mechanism within the spinal cord (Phillips and Porter, 1964). Such facilitation is not seen when stimulation of the peripheral nerve produces Ia EPSP volleys in the same temporal pattern, and thus the facilitating EPSPs appear to be a property specific to CM synapses. Facilitation at higher frequencies also has been shown for the projection of single CM cells on a motoneuron

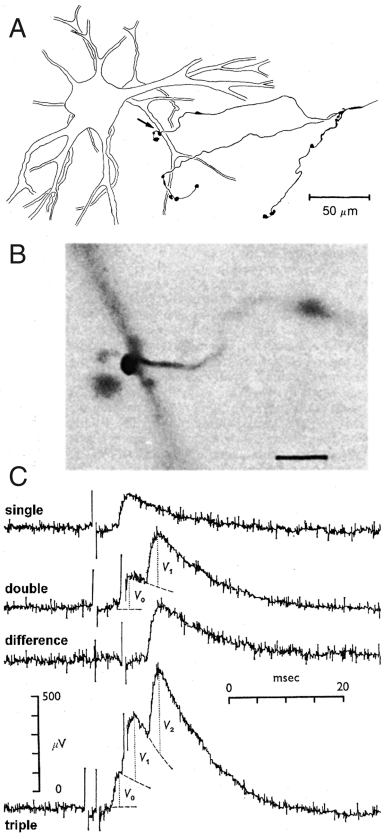

Fig. 2.3. The corticomotoneuronal synapse. **(A)** Camera lucida drawing of a corticospinal axon ramifying in lamina IX and contacting a proximal dendrite of a motoneuron with a single bouton (arrow). **(B)** Light micrograph of the same synapse indicated by the arrow in **A**. Calibration bar represents 10 μ. **(C)** Facilitation of corticomotoneuronal EPSPs. Each trace shows the intracellular voltage recorded from a motoneuron averaged across 256 repetitions of the same stimulus. In the top trace, a single cortical shock produced an EPSP. In the next trace, double cortical shocks produced two temporally summated EPSPs, with the second (V_1) larger than the first (V_0) or the single EPSP. The difference trace (double minus single) emphasizes the larger amplitude of the second EPSP. In the bottom trace, triple cortical shocks evoked progressively larger EPSPs, V_0, V_1 and V_2. Calibrations apply to all traces. **A** and **B** are reproduced with permission from Lawrence et al. (1985), **C** from Muir and Porter (1973).

pool in awake behaving monkeys (Lemon and Mantel, 1989). Thought to result from a mechanism in the presynaptic terminals, this facilitation makes CM EPSPs more effective at discharging motoneurons when CM cells discharge high frequency bursts. After lesions of the corticospinal system in humans, loss of this potent excitation of motoneurons may result in a reduced ability to recruit motoneurons and to drive them at high frequencies, with consequent weakness and slowness of voluntary movement.

Thus, as one ascends the scale from cats to monkeys to chimpanzees, a trend becomes apparent for increased numbers of direct corticomotoneuronal connections. Although histologic evidence of such CM synapses in humans is limited (Schoen, 1969), the trend generally is used to project that in humans direct CM connections are more numerous than in any other species. Physiologically, the presence of CM connections in humans has been inferred from the features of post-stimulus time histogram peaks in the discharge times of single motor units aligned on the delivery of transcranial magnetic stimulation pulses to the motor cortex (Palmer and Ashby, 1992; Petersen et al., 2003). The extent of direct corticomotoneuronal connections in humans, however, has yet to be fully explored.

2.5. Divergence and convergence in the corticomotoneuronal projection

The common clinical appellation, 'upper motor neuron,' has fostered the assumption that individual corticospinal neurons contact only spinal motoneurons innervating a single muscle. However, evidence accumulated over the last three decades shows that this is not the case. Single pyramidal tract neurons initially were shown to be antidromically activated by electrical microstimulation in the motor nuclei of more than one hindlimb muscle in the monkey lumbar enlargement (Asanuma et al., 1979) and at more than one segmental level in the cervical enlargement (Shinoda et al., 1979). Filling with HRP then revealed that single corticospinal axons indeed give off multiple collaterals that enter the spinal gray at different segmental levels and ramify in the motor nuclei of multiple muscles (Fig. 2.4(A)) (Shinoda et al., 1981).

That such terminal ramifications indeed provide physiological innervation of multiple motoneuron pools from single CM cells has been shown by spike-triggered averaging of EMG activity in awake behaving monkeys. Averages of rectified EMG triggered from the spikes discharged by a single neuron in the primary motor cortex sometimes show post-spike facilitatory peaks. Such peaks indicate that excitatory input arrived in the motoneuron pool at a fixed latency consistent with a monosynaptic connection from the recorded M1 neuron to spinal motoneurons contributing to the EMG. Such post-spike facilitation has been observed in the EMG from multiple muscles recorded simultaneously with the spike discharge of a single monkey M1 neuron (Fig. 2.4(B)). Shown first in forearm muscles acting on the wrist and fingers (Fetz and Cheney, 1980), multiple intrinsic muscles of the hand also may receive input from a single M1 neuron (Buys et al., 1986). Post-spike facilitation from CM cells is more prevalent in intrinsic

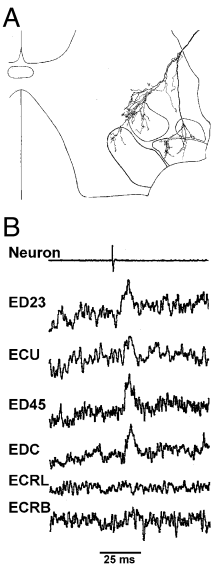

Fig. 2.4. Divergent projections of single corticospinal neurons. **(A)** A corticospinal axon reconstructed in the transverse plane of the ventral horn of the monkey spinal cord entered the spinal gray matter from the lateral column and then branched to supply terminal ramifications in the outlined motoneuron pools of four different muscles. (Reproduced from Shinoda et al. (1981).) **(B)** Averages of rectified EMG from six muscles – extensor digitorum secundi et tertii (ED23), extensor carpi ulnaris (ECU), extensor digitorum quarti et quinti (ED45), extensor digitorum communis (EDC), extensor carpi radialis longus (ECRL) and extensor carpi radialis brevis (ECRB) – that act on the wrist and/or fingers in macaque monkeys, each was triggered from several thousand spikes discharged by the simultaneously recorded M1 neuron whose averaged action potential is shown in the top trace. The brief (~10 ms) peaks that begin shortly after the neuron spike in each of the top four EMG averages indicate that motoneurons innervating these four muscles received synaptic excitation at a short and fixed latency following the spikes of the M1 neuron. Modified from Fetz and Cheney (1980); composite Figure reproduced from Schieber (2001).

hand muscles than in forearm muscles, including the extrinsic finger muscles (Fig. 2.6). Nevertheless, single M1 neurons also have been observed to produce post-spike effects in muscles at multiple proximodistal levels, acting on the fingers, wrist, elbow and shoulder (McKiernan et al., 1998). Both anatomical and physiological studies in monkeys thus have shown that single CM cells may have projections that diverge to innervate multiple motoneuron pools.

Although spike-triggered averaging of EMG has not been applied to human M1 neurons, evidence of divergent projections from human CM cells has been obtained through studies of short-term synchronization between motor units. Cross-correlation histograms of the spike trains discharged by pairs of motor units recorded simultaneously in the same or in different muscles sometimes reveal a tendency (beyond what can be attributed to chance alone) for action potentials to be discharged synchronously (within a few milliseconds) by the two motor units (Datta and Stephens, 1990; Bremner et al., 1991). Such short-term synchronization implies that the two motor units both receive synaptic input from branches of the same axon. Furthermore, short-term synchronization is reduced or abolished in humans with corticospinal lesions (Datta et al., 1991; Farmer et al., 1993). Hence, the observation that short-term synchronization can be seen in motor units recorded from different muscles indicates that in humans, as in monkeys, CM cell axons diverge to innervate multiple motoneuron pools. However, because such studies typically have been performed with only two simultaneous motor unit recordings, the extent of such divergence in humans has yet to be assessed fully.

Divergence of the output from single CM cells to multiple motoneuron pools indicates that different muscles acting on closely related parts of a limb are not represented in spatially separate regions of the primary motor cortex (M1). Conversely, the cortical territory that provides corticospinal input to a given motoneuron pool has a considerable spatial extent in M1, and overlaps extensively with the territory providing input to other nearby muscles. Although somatotopic segregation of within-limb representation appears to have increased along the phylogenetic scale, even in humans considerable evidence indicates overlapping territories controlling different movements and muscles (Schieber, 2001).

Some of the earliest evidence of this overlap came from studies that mapped the movements evoked by electrical stimulation at different points in M1. Electrical stimulation of the cortical surface in monkeys (Woolsey et al., 1952), apes (Leyton and Sherrington, 1917) and humans (Penfield and Boldrey, 1937) demonstrated distinct M1 representations of the face, upper extremity and lower extremity. Within any of these major representations, however, overlap of the representations of nearby body parts was found. Movement of a single finger, for example, rarely was evoked by stimulation at any point in the upper extremity representation. Rather, multiple fingers typically were moved. Movement of a given finger was evoked by stimulation at several different loci, and the territory from which movement of a given finger was evoked overlapped with the territory from which movement of any other finger, or the wrist, was evoked.

More recent studies using more focal, intracortical microstimulation (ICMS) have produced similar observations in new world monkeys (with a comparatively lissencephalic cortex) (Gould et al., 1986), in old world

macaques (Kwan et al., 1978) and in baboons (Waters et al., 1990). Even with ICMS, movements of a given joint or body part typically can be evoked by threshold stimulation at many foci within a major body part representation. Although some of these foci are contiguous, others are scattered through the major representation. This gives the impression of a complex mosaic of within limb representation, with intermingling of the cortex controlling different parts of the limb. When stimulating current is increased beyond the threshold for the slightest movement, not only does the initially observed movement become more intense, but movements of additional body parts in the same major representation are evoked.

Other studies have examined the activation of a number of muscles simultaneously – either by recording evoked EMG or by measuring tendon tensions – while stimulating different foci in M1 (Chang et al., 1947; Donoghue et al., 1992; Park et al., 2001). Like studies showing that movements of different body parts can be evoked by stimulation at a given location, these studies show that multiple muscles are activated during stimulation of any given point. In baboons, the cortical territories from which outputs converge on a single upper extremity motoneuron pool can be on the order of 20 mm^2 (Landgren et al., 1962a) – a large fraction of the ~50 mm^2 upper extremity representation (Waters et al., 1990). Even when motor units were recorded simultaneously from the thenar eminence, the first dorsal interosseous and the extensor digitorum communis of a baboon, the three territories from which ICMS evoked responses of motor units in the three different muscles all overlapped (Fig. 2.5) (Andersen et al., 1975). Similarly in the hindlimb representation of macaques, which covers approximately

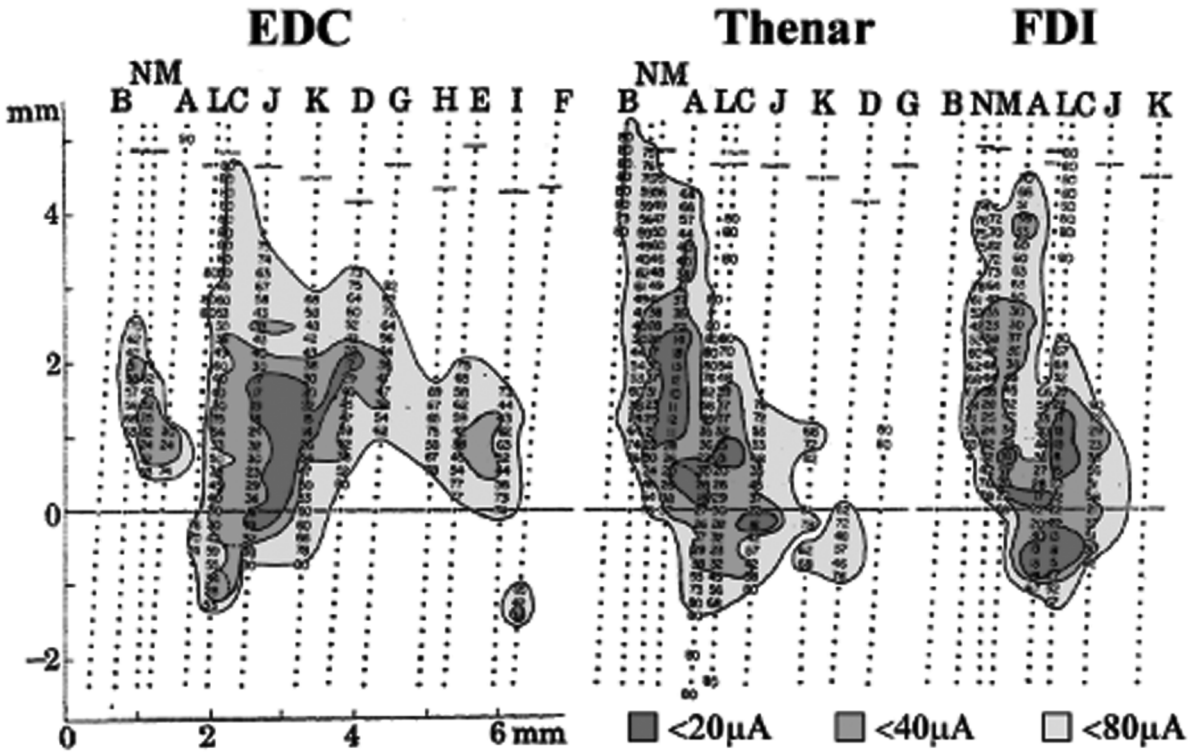

Fig. 2.5. For full color figure, see plate section. Cortical territories from which inputs converge on motor units in three different muscles. Maps are shown of points stimulated using intracortical microstimulation of up to 80 μA in 12 microelectrode penetrations (denoted A through N) down the anterior wall of a baboon's central sulcus. Single motor units were recorded simultaneously from three different muscles that acted on different digits and were served by different peripheral nerves: extensor digitorum communis (EDC, which extends all four fingers, radial nerve innervation); the thenar eminence (Thenar, which act only on the thumb, median nerve innervation); and the first dorsal interosseous (FDI, which acts on the index finger, ulnar nerve innervation). Black dots indicate locations where stimulation was ineffective for evoking motor unit discharges, whereas numbers indicate threshold current (μA). Lateral is to the viewer's left and medial to the right. With currents up to 20 μA (red), multiple small zones are revealed from which the motor units in each muscle could be discharged. Though largely intermingled, on close inspection these small zones also overlapped to some extent. At higher currents (up to 40 (orange) or 80 μA (yellow)) the zones for each motor unit expanded and coalesced into large cortical territories, with increased mutual overlap. Current spread could not account for these observations. Modified from Andersen et al. (1975); colorized figure reproduced from Schieber (2001).

30 mm^2, single spinal motoneurons may receive EPSPs from cortical territories of 1–3 mm^2, and the total territory from which a single motoneuron pool receives EPSPs may cover 20 mm^2 (Jankowska et al., 1975). TMS mapping in humans is consistent with extensive overlap of different upper extremity muscle representations as well (Wassermann et al., 1992).

Because of the divergence and convergence in the corticospinal projection, corticospinal lesions affect functionally related muscles in parallel. Whereas a radial nerve lesion will paralyse the brachioradialis muscle while leaving the biceps brachii strong, a corticospinal lesion will weaken the elbow flexors all to a similar degree. Likewise, even small lesions affect many muscles, multiple body parts and several joints concurrently (Schieber, 1999).

Paradoxically perhaps, the same divergence and convergence may underlie the normal ability to make fine, relatively independent movements, such as the finger movements used in buttoning or typing. Because of the complex mechanics of the musculoskeletal system, controlling such movements requires not only activity in particular muscles to produce the intended movement, but also activity in other muscles to check unintended motion (Beevor, 1903; Schieber, 1995). In flexing the index finger, for example, the contractions of the flexor digitorum superficialis and profundus would flex the wrist too, if the wrist were not stabilized by concurrent activity in extensor muscles.

When this aspect of normal corticospinal function is lost, one body part cannot be moved without an abnormal degree of motion in adjacent body parts. While particularly evident in the impairment of individuated finger movements (Lang and Schieber, 2003), the same phenomenon is present in movements of the entire upper extremity (Zackowski et al., 2004) and can affect the face and lower extremity as well. This loss of individuation reflects not only a loss of stabilizing contractions, but also contraction of inappropriate muscles. When a patient with pure motor hemiplegia attempts to move a given finger, for example, contraction occurs in intrinsic muscles of the hand that normally would remain inactive (Lang and Schieber, 2004). Remaining movements of the arm tend to be limited to a few stereotyped patterns of synergistic contraction in multiple muscles, which presumably are mediated via non-corticospinal descending pathways (Brunstrom, 1970; Dewald et al., 1995; Beer et al., 2004). Beyond weakness, corticospinal lesions impair the ability to generate stabilizing muscle contractions and volitional effort activates additional, inappropriate muscle contractions. The result is an inability to generate the fine, relatively independent motion of discrete body parts that normally characterizes human movement.

Normal corticospinal output is not distributed evenly to all motoneuron pools. The compound monosynaptic EPSPs evoked in single motoneurons by stimulating the baboon cortex is stronger in the motoneurons of distal muscles than in those of proximal muscles (Phillips and Porter, 1964) and stronger still for intrinsic muscles of the hand and the extrinsic extensor digitorum communis than for other forearm muscles (Clough et al., 1968). Cortically evoked EPSPs are also more common and larger in the motoneurons of distal than proximal macaque hindlimb muscles (Jankowska et al., 1975). Similar findings have been obtained using spike-triggered averaging of EMG activity in macaque monkeys (Fig. 2.6). Post-spike effects are more common in wrist and digit muscles than in shoulder and elbow muscles (McKiernan et al., 1998) and more common in intrinsic than extrinsic finger muscles (Buys et al., 1986). In humans, TMS indicates greater distal than proximal representation in the corticospinal output to the upper extremity, although some exceptions may be found in the lower extremity (Petersen et al., 2003).

These observations on the distribution of corticospinal output correlate with the distribution of weakness typically observed following corticospinal lesions in humans,

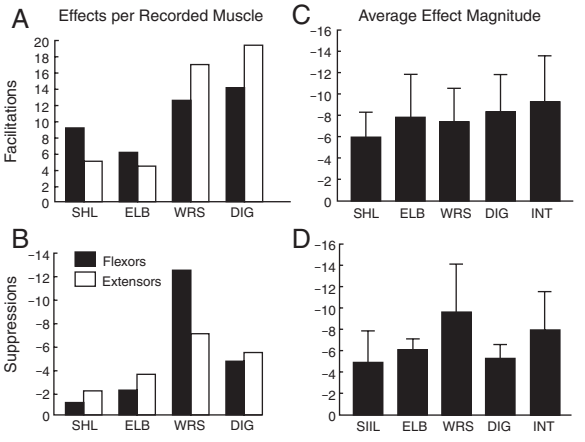

Fig. 2.6. Distribution of corticomotoneuronal input to upper extremity muscles as quantified with spike-triggered averaging in the macaque monkey. In the left column, the average number of facilitatory **(A)** and suppressive **(B)** post-spike effects is shown separately for flexor (filled) and extensor (open) muscles acting about the different parts of the Rhesus monkey upper extremity: shoulder (SHL), elbow (ELB), wrist (WRS) and digits (DIG). In the right column the average peak percent increase of facilitatory **(C)** or peak percent decrease of suppressive **(D)** post-spike effects is shown, including effects in the intrinsic muscles of the hand (INT). Overall, corticomotoneuronal inputs are more frequent in wrist and digit muscles than in shoulder and elbow muscles and are slightly stronger in the more distal muscles as well. Modified with permission from McKiernan et al. (1998).

in which the distal musculature typically is affected more profoundly than proximal musculature (Colebatch and Gandevia, 1989; Adams et al., 1990). Clinically assessed weakness typically is greater in the extensors than the flexors of the upper extremity, particularly for the wrist and fingers. Though in part this may reflect the fact that the extensors at these joints normally are less powerful than the flexors (Colebatch and Gandevia, 1989), in both monkeys (Fig. 2.6) and baboons, corticomotoneuronal facilitation of the wrist and finger extensors is somewhat greater than that of the flexors.

2.6. Natural activity in the corticospinal system

Although normal ambulation in humans appears to depend substantially on the corticospinal system, the activity of corticospinal neurons has yet to be examined directly during ambulation in primates. In cats, corticospinal neurons in both the forelimb and hindlimb representations normally show rhythmic modulation of discharge frequency related to the step cycles of the appropriate contralateral extremity (Armstrong and Drew, 1984; Beloozerova and Sirota, 1993b). Identified pyramidal tract neurons (PTNs) show relatively little change in this modulation as the walking cat turns or ascends an incline.

When the cat steps over obstacles, however, many PTNs show a marked increase in discharge frequency, as well as changes in the timing of discharge (Beloozerova and Sirota, 1993a; Drew, 1993; Widajewicz et al., 1994). Although in cats the activity of any particular PTN cannot be directly related to the activity of a particular muscle (as cats lack direct corticomotoneuronal synapses), these changes in PTN activity appear consistent with a role of the PTNs in controlling the altered steps needed to avoid obstacles. That PTN modulation is more prominent when the contralateral limb crosses the obstacle first rather than second suggests that the PTNs produce the altered steps based on visual information about the approaching obstacle.

Although less dexterous than monkeys, cats use the forelimb and paw to reach out and manipulate objects such as food morsels. Neurons in the cat primary motor cortex (presumably including corticospinal neurons) discharge in relation to these reaching movements (Vicario et al., 1983; Martin and Ghez, 1985). In macaque monkeys, CM cells have been identified using spike-triggered averaging during reach and prehension movements (McKiernan et al., 1998, 2000). During these movements, the spike frequency of a CM cell tends to correlate with the temporal modulation of EMG activity in those muscles that receive post-spike effects from the CM cell. Positive correlations are found most often in muscles that received post-spike facilitation and negative correlations in muscles that received post-spike suppression. In monkeys, CM cells thus appear to drive motoneurons during reach and prehension movements. Consistent with these observations in monkeys, TMS in humans indicates increased excitability of the corticospinal output to particular muscles during those phases of reach and prehension movements when each muscle becomes active (Lemon et al., 1996).

In studies employing more restricted movements, such as isotonic or isometric wrist movements, the discharge rates of identified monkey pyramidal tract neurons and CM cells have been shown to vary in relation to joint position, movement direction, force exerted and even rate of change of force (Fig. 2.7) (Evarts,

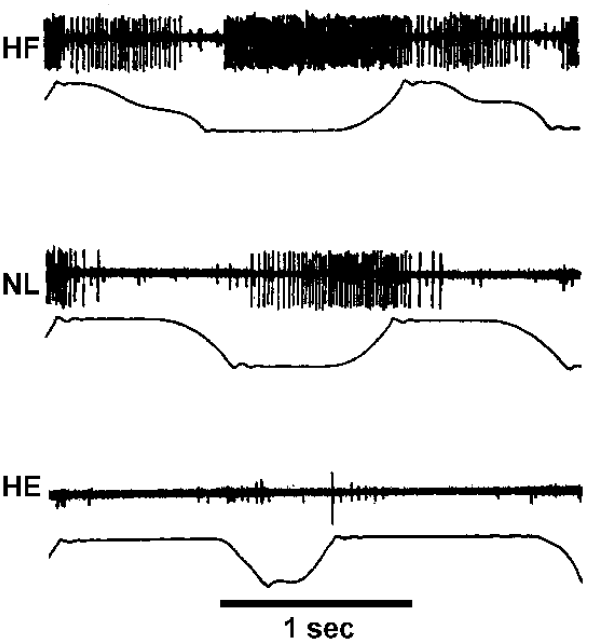

Fig. 2.7. Discharge of a pyramidal tract neuron. The train of action potentials discharged by a neuron in the primary motor cortex was recorded extracellularly (upper traces) as an awake monkey actively flexed and extended its contralateral wrist (lower traces, flexion upward) against no load (NL, center), a high flexor load (HF, top) or a high extensor load (HE, bottom). The neuron was identified as a pyramidal tract neuron by observing its discharge of antidromic action potentials in response to stimulation of the medullary pyramid. Although this is one of the earliest recordings of natural activity in an identified corticospinal neuron, it illustrates several features. In the no load condition, the neuron begins to discharge several hundred milliseconds before the onset of wrist flexion, but discharge decreases to nil with extension; hence its discharge was related to movement direction. Discharge was greater when the monkey worked against a flexor load, and less when the monkey worked against an extensor load; hence discharge frequency was related to the force exerted at the wrist. Modified with permission from Evarts (1968).

1968, 1969; Cheney and Fetz, 1980). Unidentified motor cortex neurons, which may or may not have corticospinal axons, also discharge in relation to movements. The discharge frequency of these neurons varies in relation to the force exerted, the direction and speed of movement and other movement parameters as well (Ashe and Georgopoulos, 1994; Schwartz and Moran, 2000; Reina et al., 2001). Although the spike frequency of individual neurons may correlate only partially with any particular parameter (Fu et al., 1995), more precise information on each parameter can be decoded from a large population of neurons (Georgopoulos et al., 1986; Moran and Schwartz, 1999). While these findings may be viewed as indicative of an abstract representation of kinematic and dynamic movement parameters in the motor cortex, the activation of muscles will show similar relationships due to the familiar length-tension and force-velocity properties of muscle contraction. In any case, the loss of corticospinal discharge that increases with voluntary effort contributes to the weakness that results from corticospinal lesions.

In addition to variation of discharge rate in relation to parameters of movement, unidentified neurons in monkey M1 also show discharge rate variations in relation to other features that can be dissociated from the movement per se. For example, the discharge frequencies of an appreciable fraction of M1 neurons vary in relation to the spatial location of a visual stimulus, although different patterns of muscle contraction are used to move the limb toward the stimulus (Thach, 1978; Kakei et al., 1999) or though the movement will be made to a location different from that of the stimulus (Georgopoulos et al., 1989; Alexander and Crutcher, 1990). Unidentified M1 neurons also discharge while a monkey waits for a go signal to make a previously instructed movement (Tanji and Evarts, 1976; Thach, 1978; di Pellegrino and Wise, 1991; Crammond and Kalaska, 2000). Such delay period activity recently has been observed as well in spinal interneurons (Prut and Fetz, 1999; Fetz et al., 2002). The extent to which these more abstract features of motor tasks are transmitted to the spinal cord by corticospinal neurons has yet to be explored.

Many corticospinal neurons in monkeys are particularly active during small precise movements of the forearm and hand. In monkeys making pronation/supination movements of the forearm, for example, many PTNs showed marked discharge modulation related to small changes in force exerted against small external loads (Fromm and Evarts, 1981; Evarts et al., 1983). Many CM cells discharge more intensely during a precision pinch between the thumb and index finger than during a power grip using the whole hand (Muir and Lemon, 1983). This special relationship between the activity of corticospinal neurons and fine, individuated movements probably underlies the clinical observation that such movements are among the first to suffer and the last to recover when lesions damage the corticospinal system.

2.7. Corticospinal projections from additional cortical areas

Although the bulk of the corticospinal tract arises from the primary motor cortex, Brodmann's area 4, other cortical areas near area 4 also contribute axons to the corticospinal tract. Following injection of retrograde tracers in the high cervical spinal cord of monkeys, labeled neurons are found most densely in area 4 (Fig. 2.8(C,D)) (Toyoshima and Sakai, 1982; He et al., 1993, 1995; Galea and Darian-Smith, 1994). However, corticospinal neurons also are found in the caudal subdivisions of area 6 (the supplementary motor area, SMA; and the caudal portions of the dorsal and ventral premotor cortex), and more medially in the caudal cingulate cortex of area 24. The contributions of these non-primary cortical motor areas to normal control of movement are currently the topic of active investigation (Rizzolatti and Luppino, 2001). Although the non-primary cortical motor areas are defined most clearly in macaque monkeys (Fig. 2.8(B)), human homologues can be identified (Fig. 2.8(A)) (Zilles et al., 1995). Still more corticospinal neurons are found more posteriorly in areas 3a, 3b, 1, 2 (the primary somatosensory cortex, SI), area 5 and in the secondary somatosensory area (SII). The same cortical regions that contribute to the corticospinal tract also tend to have appreciable cortico-cortical connections with M1.

Axons descending from these other cortical areas pass through the centrum semiovale and converge in the internal capsule (Fig. 2.8(E)). Fibers from non-primary cortical motor areas located rostral to M1 in the frontal lobe tend to lie more anteriorly in the capsule, extending as far rostrally as the genu (Fries et al., 1993; Morecraft et al., 2002). Corticospinal fibers descending from the parietal lobe lie more posteriorly in the capsule.

Upon reaching the appropriate spinal segments, the corticospinal fibers from areas other than M1 also show somewhat different patterns of termination in the spinal gray matter (Fig. 2.9). Corticospinal projections from the frontal lobe to the cervical enlargement terminate primarily in the intermediate zone (laminae V, VI) and ventral horn (lamina VII, with some terminations in lamina VIII). However, as illustrated in Fig. 2.9(A), M1 provides considerably more terminations in the motor nuclei (lamina IX) than do the cingulate motor areas or the SMA (Dum and Strick, 1996). Consistent with these anatomical observations, M1 provides substantially more monosynaptic excitation of cervical motoneurons

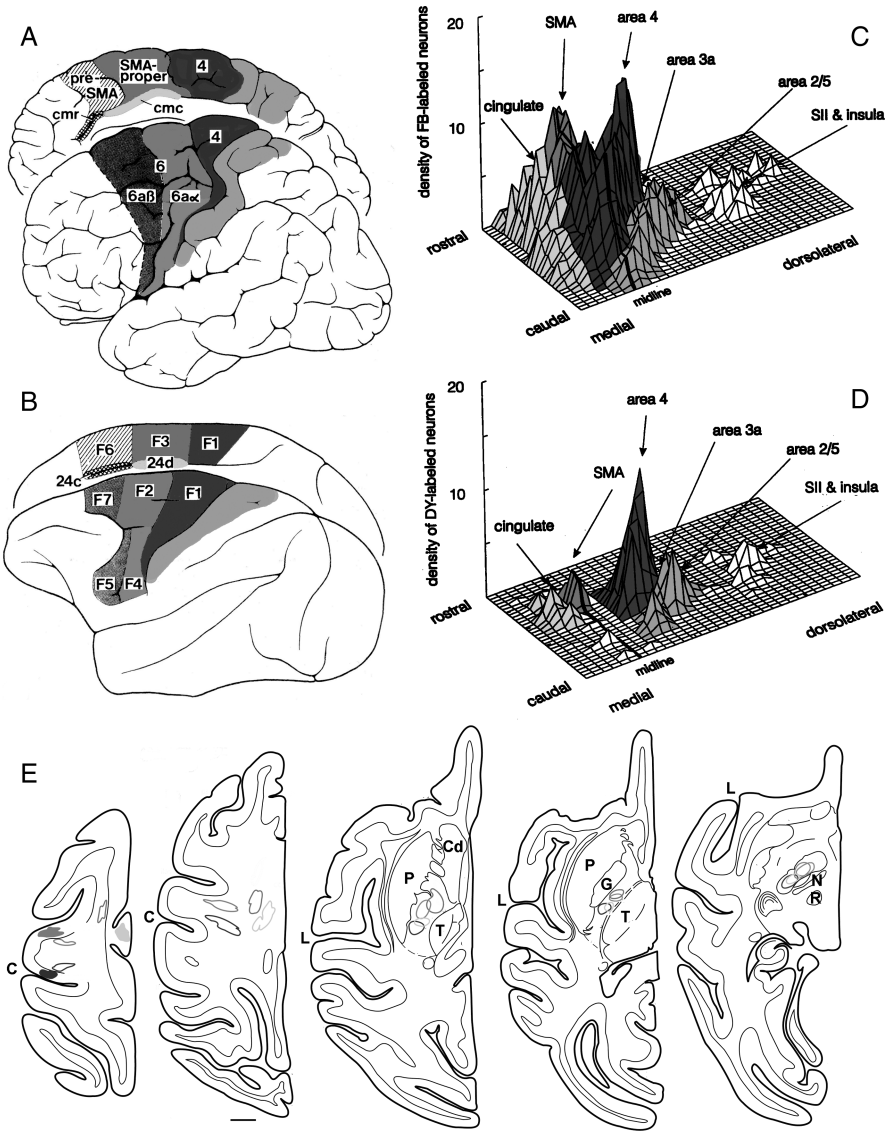

Fig. 2.8. For full color figure, see plate section. Origins of the corticospinal tract. Cortical regions that make major contributions to the corticospinal tract are shown in color in drawings of the human **(A)** and macaque monkey **(B)** brain. The density of neurons labeled retrogradely by injection of different tracers in the dorsolateral funiculus (**C**, all corticospinal axons) and gray matter (**D**, terminations in the cervical enlargement only) of the monkey spinal cord at C5–C7 varies among various cortical regions. Different authors have used different nomenclature for cortical regions. The primary motor cortex (dark blue) is the source of the greatest number of corticospinal axons **(C, D)**. Caudal subdivisions of area 6 – the supplementary motor area (red), the dorsal premotor cortex (green) and the ventral premotor cortex (purple) – also contribute corticospinal axons, as does the caudal cingulate motor area (yellow). The descending projections from these frontal cortical motor areas converge as they approach the posterior limb of the internal capsule, though tending to remain anterior to the fibers descending from area 4, as illustrated by the colored rings drawn on horizontal sections of a macaque brain **(E)**. The more rostral subdivisions of area 6 and area 24 provide relatively few corticospinal axons. In the parietal lobe, areas 3, 1, 2 and 5 (light blue) also contribute to the corticospinal tract. A small number of corticospinal neurons are found as well in the secondary somatosensory area and insula. **A** and **B** are modified from Zilles et al. (1995), **C** and **D** from Galea and Darian-Smith (1994) and **E** from Morecraft et al. (2002).

than does the SMA (Maier et al., 2002). Similarly, electrical stimulation of the rostral ventral premotor cortex (F5) excites cervical motoneurons largely via corticocortical connections to M1 (Shimazu et al., 2004). Corticospinal projections from non-primary cortical motor areas thus provide less direct access to motoneurons than does the projection from M1.

In contrast to these projections from the frontal lobe, corticospinal projections from the parietal lobe (Fig. 2.9(B)) terminate chiefly in the intermediate zone

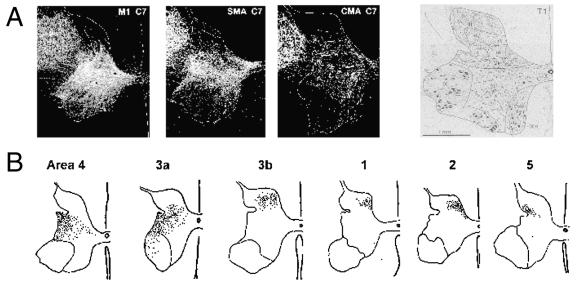

Fig. 2.9. Terminations of corticospinal projections from various cortical areas. **(A)** Three digitized darkfield images show the anterograde labeling in cross sections of the C7 spinal segment following injections of horseradish peroxidase in the primary motor cortex (M1, left), supplementary motor area (SMA, middle) or cingulate motor area (CMA, right). Outlines of the laminae of the spinal gray contralateral to the injections are drawn in white, with the midline at the right of each frame. Labeling of descending fibers in the dorsolateral funiculus is apparent in the upper left of each frame. The bulk of the projection from all three frontal cortical motor areas terminates in the intermediate zone – laminae V, VI and VII. The projection from M1 is heavier than that from the SMA or CMA and also is more extensive in lamina IX. A brightfield micrograph illustrating Rexed's laminae at the T1 level is shown for comparison at the far right. Modified with permission from Dum and Strick (1996). **(B)** Schematic drawings illustrate the region of corticospinal terminations from area 4 and from somatosensory areas in the parietal lobe. Note that the corticospinal projections from the somatosensory cortical areas terminate primarily in the dorsal horn. Modified with permission from Coulter and Jones (1977).

(laminae V and VI) and the dorsal horn (laminae III and IV), though not extending dorsally into the substantia gelatinosa (Kuypers, 1960; Liu and Chambers, 1964; Coulter and Jones, 1977). These corticospinal terminations are thought to regulate inflow of somatosensory afferent information, both to spinal reflexes and interneuron systems and to ascending spinocerebellar and spinothalamic pathways.

2.8. Non-corticospinal descending motor pathways

The corticospinal tract is not the only descending pathway through which the brain accesses the spinal cord to control bodily movement. Other pathways descend from the red nucleus (rubrospinal), from the pontomedullary reticular formation (reticulospinal) and from the vestibular nuclei (vestibulospinal) to the spinal gray matter. These non-corticospinal pathways all descend through the medullary tegmentum dorsal to the pyramid. Hence, the non-corticospinal descending axons all lie outside the pyramid and historically have been referred to collectively as extrapyramidal pathways. A number of neurological disorders once were thought to produce involuntary movement abnormalities acting over these extrapyramidal pathways, and therefore became known as extrapyramidal syndromes. We now know that most of these involuntary movements actually reach the spinal cord via the corticospinal tract. However, the term 'extrapyramidal' has become so closely associated with involuntary movement disorders that here we will use the term non-corticospinal to refer collectively to the rubrospinal, reticulospinal and vestibulospinal tracts.

The non-corticospinal descending pathways do not function independently of the corticospinal system, however. Areas 4 and 6 send corticobulbar projections to the red nucleus and the reticular formation (Kuypers and Lawrence, 1967; Humphrey et al., 1984; Matsuyama and Drew, 1997). Similar corticobulbar projections from the motor cortex to the vestibular nuclei are not known, but the vestibular nuclei may receive cortical input indirectly via the reticular formation (Kuypers, 1982). In addition, descending corticospinal axons also give off axon collaterals that innervate the ipsilateral red nucleus and/or the pontomedullary reticular formation (Keizer and Kuypers, 1984, 1989; Kably and Drew, 1998).

That these non-corticospinal pathways provide an alternate route from the primary motor cortex to the spinal cord has been demonstrated in anesthetized monkeys by cutting the pyramid acutely (Woolsey et al., 1972). Electrical stimulation of the motor cortex ipsilateral to the cut pyramid still evoked somatotopically appropriate movement of the contralateral body, though stronger stimuli were required and distal movements were more difficult to evoke.

Although the red nucleus and the pontomedullary reticular formation have been thought to participate primarily in control of proximal musculature for posture, these structures also contribute to the control of voluntary limb movement. Rubrospinal axons originate primarily from the magnocellular division of the red nucleus, decussate promptly and descend through the brainstem tegmentum to reach the dorsolateral funiculus of the spinal cord. Here rubrospinal axons lie somewhat ventral to, but are largely intermingled with, the corticospinal tract, and finally terminate in the intermediate zone of the spinal gray matter. In cats, neurons in the magnocellular red nucleus are active during voluntary gait modifications as the contralateral extremity steps over an obstacle (Lavoie and Drew, 2002) and during reaching movements made with the forelimb (Ghez and Kubota, 1977; Soechting et al., 1978). In monkeys, neurons of the magnocellular red nucleus are active in relation to many limb movements, including those of the hand and fingers (Gibson et al., 1985a,b; Houk et al., 1988). Like neurons in the motor cortex, the discharge rate of neurons in the red nucleus often correlates well with kinematic and dynamic parameters of limb movement

and with EMG activity (Miller et al., 1993; Miller and Sinkjaer, 1998). Some of these rubrospinal neurons produce facilitatory and/or suppressive effects in spike-triggered averages of EMG activity, indicating that they have relatively direct, though not monosynaptic, connections to spinal motoneurons (Cheney, 1980; Cheney et al., 1988; Mewes and Cheney, 1991, 1994). In humans, the role of the rubrospinal tract is less certain. Anatomically, the rubrospinal tract is comparatively small, and has not been traced caudal to the upper cervical cord (Nathan and Smith, 1982).

Whereas the corticorubral projection is ipsilateral, the corticoreticular projection from areas 4 and 6 is bilateral (Kuypers and Lawrence, 1967). Reticulospinal axons descend in the ventral column of the spinal cord, and terminate in the ventromedial portion of the intermediate zone, closest to the motor nuclei of proximal limb muscles. In humans, the reticulospinal tract terminates primarily at cervical levels, with only a small proportion of the fibers descending to thoracic, lumbar or sacral levels (Nathan et al., 1996).

Neurons in the reticular formation are active during limb movements. In cats, reticulospinal neurons discharge in relation to walking on a level surface and show modulation of this discharge if postural adjustments must be made on an incline or to step over obstacles (Matsuyama and Drew, 2000a,b; Prentice and Drew, 2001). In monkeys, stimulation in the reticular formation excites ipsilateral, proximal upper extremity muscles (Davidson and Buford, 2004) and reticular formation neurons discharge during reaching movements (Stuphorn et al., 1999; Buford and Davidson, 2004). Even in humans, TMS studies have suggested that the reticulospinal system may be able to release planned movements in response to a sudden stimulus faster than the corticospinal system (Valls-Sole et al., 1999). Both the rubrospinal and reticulospinal tracts thus work in parallel with the corticospinal tract in producing voluntary movements.

2.9. Lesions of the corticospinal system

In humans, lesions of the corticospinal tract result in a number of well-known abnormalities, including weakness with its typical distribution; slowness of remaining movements; loss of fine, individuated movements which become replaced by larger and more stereotyped movement synergies; reflex changes; and spasticity. Although variations are found from patient to patient, corticospinal lesions typically are regarded as producing a single syndrome of contralateral spastic paresis regardless of the level at which the lesion occurs, from spinal cord to cortex (Bucy, 1949; Twitchell, 1951; Lassek, 1954; Laplane et al., 1977; Nathan, 1994; Bucy et al., 1995). Yet because M1 and other cortical motor areas also project to subcortical centers, including the basal ganglia, the cerebellum and the brainstem origins of the non-corticospinal descending pathways, certain features of the corticospinal syndrome may vary depending in part on the level of the lesion. Though lesions produced by disease in human patients rarely affect the corticospinal pathway alone, relatively selective lesions can be produced in experimental animals. This selectivity reduces the confounding effects of involvement of adjacent structures, but again requires care in extrapolating the effects of experimental lesions in animals to the human condition. Furthermore, because plastic changes occur in undamaged parts of the nervous system that compensate in part for the deficits resulting from any lesion, the observed effects of experimental lesions of the corticospinal system reflect the residual deficits for which the remaining nervous system was unable to compensate.

Lesions of the dorsolateral funiculus of the spinal cord that damage the lateral corticospinal tract inevitably involve the rubrospinal tract, the fibers of which descend in the cord largely intermingled with the dorsolateral corticospinal fibers. Following such lesions at thoracic levels in cats, basic locomotion may recover, but the ability of the hindlimb to step over obstacles remains impaired (Drew et al., 2002). Even after complete hemisection of the cervical spinal cord, juvenile macaque monkeys over several weeks recover virtually normal motor function, with the exception that finger movements may not be as dexterous or as strong as normal (Galea and Darian-Smith, 1997b). This recovery may be mediated in part by corticospinal fibers that descend in the contralateral spinal cord and decussate within the cervical enlargement (Galea and Darian-Smith, 1997a). Such fibers include axons from the contralateral hemisphere that remain uncrossed at the pyramidal decussation, as well as others from the ipsilateral hemisphere that crossed at the pyramidal decussation and crossed back within the cervical enlargement. Although the crossed rubrospinal tract is destroyed by hemisection of the cord, bilaterally projecting reticulospinal neurons also may participate in recovery from unilateral spinal cord lesions.

In contrast to lesions of the spinal cord, lesions of the medullary pyramid interrupt only corticospinal axons, and total lesions of the pyramid destroy all the collected corticospinal axons from the ipsilateral hemisphere. Following pyramidal lesions, however, the corticofugal projections within the cerebrum and brainstem remain intact, as do the non-corticospinal descending pathways. In cats, initial weakness of the limbs following pyramidotomy recovers rapidly to the degree that ambulation is close to normal (Laursen and

Wiesendanger, 1966; Eidelberg and Yu, 1981). The limbs may be held slightly more extended and may flex somewhat less readily than normal, however. In the rat and the cat, which lack direct corticomotoneuronal synapses, unilateral pyramidal lesions nevertheless impair distal forelimb movement more profoundly than proximal movement (Castro, 1972; Gorska and Sybirska, 1980; Whishaw and Metz, 2002). Proximal and axial movements are more affected by bilateral pyramidal lesions, but still improve more rapidly than distal deficits, which may persist indefinitely. Movements of the paw and claws used to extract food morsels from narrow tubes are affected most profoundly.

In macaque monkeys, unilateral lesions of the medullary pyramid similarly produce contralateral weakness of the distal limb musculature greater than proximal limb or axial musculature (Tower, 1940; Gilman and Marco, 1971). Movements are slower and fatigue more rapidly than normal. The monkey uses the unaffected side when able, and leads with the unaffected side when bilateral movements are needed. Relatively isolated movements are lost, particularly movements of the digits, and attempts at such movements engage more of the extremity than normal. When passive, the affected extremities hang loosely, and the upper extremity does not show the flexed, adducted posture commonly associated with the corticospinal syndrome in humans. Resting tone is diminished (Tower, 1940; Gilman and Marco, 1971; Schwartzman, 1978; Chapman and Wiesendanger, 1982). Although tendon jerk reflexes are full (unchecked by contraction of the antagonist muscle), the velocity dependent response to stretch and clasp-knife phenomenon that characterize spasticity are absent.

In monkeys, relatively stereotyped synergistic movements such as flexion of the elbow with adduction of the shoulder or extension of the elbow with abduction of the shoulder, recover rapidly after pyramidotomy. Speed and accuracy in reaching also show considerable recovery (Lawrence and Kuypers, 1968a; Beck and Chambers, 1970). Even when reaching accurately and grasping with the whole hand, however, monkeys show a persistent deficit in the relatively independent finger movements used for grooming or manipulation of small objects, as in extracting a food morsel from a narrow hole. Similar deficits in making fine adjustments to gross patterns can be observed in more proximal movements as well (Tower, 1940).

Sparing of even a small fraction of the pyramidal fibers results in superior recovery, even recovery of finger movements (Schwartzman, 1978). Substantially better recovery of function also is seen, paradoxically, in monkeys with bilateral pyramidal lesions (Tower, 1940; Lawrence and Kuypers, 1968a). Ambulation improves toward normal, and the monkey also regains the ability to use both the upper and lower extremities to climb deftly, grasping with both the hands and the feet. Use of the upper extremities to reach out to objects also regains more accuracy after bilateral than after unilateral pyramidotomy. The hands can be used effectively to grasp objects, although relatively independent finger movements remain impaired. Although recovery following unilateral pyramidotomy can be attributed in part to the uncrossed fibers of the remaining pyramid, the superior recovery following bilateral pyramidotomy cannot.

The superior recovery following bilateral pyramidotomy therefore indicates that reorganization in monkeys can engage the non-corticospinal descending pathways to compensate for much of the lost function normally achieved by the pyramidal tract. The non-corticospinal pathways thus can mediate a certain repertoire of voluntary movement. Following unilateral pyramidotomy, however, the ability to use the relatively unaffected ipsilateral limbs may provide less incentive for reorganization to engage the non-corticospinal descending pathways.

After pyramidotomy, the corticospinal system rostral to the lesion remains relatively intact, and collateral cortical projections to the red nucleus and the reticular formation therefore may participate in compensatory reorganization if active use of the limb is demanded. Recovery after unilateral pyramidotomy is facilitated by periodically restraining the unaffected arm, and forcing the monkey to use the affected hand (Lawrence and Kuypers, 1968a; Chapman and Wiesendanger, 1982). Participation of the rubrospinal system has been suggested by the addition of lesions in the lateral medulla to disrupt rubrospinal fibers after many months of recovery from bilateral pyramidotomy. These added lesions resulted in the reappearance of profound weakness in the upper greater than lower extremity, with reduced ability to flex the fingers without concurrent flexion of the arm (Lawrence and Kuypers, 1968b). Furthermore, whereas microstimulation in the macaque magnocellular red nucleus normally excites extensor muscles almost exclusively, in a monkey with a chronic pyramidal lesion considerable excitation of flexor muscles was observed, suggesting substantial reorganization of rubrospinal output (Belhaj-Saïf and Cheney, 2000).

The syndrome resulting from lesions confined to the primary motor cortex (area 4) of monkeys and apes is quite similar to that observed following pyramidotomy. The contralateral limbs are weak, with reduced tone, but without hyperreflexia or spasticity (Fulton and Keller, 1932; Fulton and Kennard, 1932). The animal recovers the ability to ambulate and climb, using the hands and feet to grasp. The arm recovers the ability to reach accurately, and the hand can close around a large

object, but the ability to produce the fine, relatively independent finger movements needed to manipulate small objects persists (Travis, 1955; Hamuy, 1956). Such a deficit restricted to fine, individuated finger movements can be produced rapidly and reversibly by focal injections of muscimol (a long acting $GABA_A$ agonist that produces a profound inhibition of neuronal discharge) into the hand representation of the primary motor cortex (Kubota, 1996; Brochier et al., 1999). Movements of the digits are slowed, and attempts to move one finger produce more than the normal degree of motion in other digits (Schieber and Poliakov, 1998).

As with pyramidal lesions, superior recovery can be obtained following focal lesions of the primary motor cortex if the monkey is required to make use of its paretic extremity. In squirrel monkeys, focal lesions of the digit representation of the primary motor cortex are followed by a reduced ability to use the hand effectively to retrieve food morsels from small wells (Nudo and Milliken, 1996). If the animal is permitted to use the relatively unaffected hand ipsilateral to the lesion, little recovery occurs in the affected hand, and the remaining microstimulation-defined cortical hand representation on the side of lesion may diminish in extent; however, if the animal is required to use the affected hand, the ability of that hand improves, and reorganization occurs in the primary motor cortex such that territory where microstimulation previously had evoked more proximal movement now evokes movements of the digits (Nudo et al., 1996). Reorganization of remaining M1 related to recovery of function after destruction of the macaque M1 in infancy has also been observed (Rouiller et al., 1998). If the M1 upper extremity representation is damaged more extensively in the adult macaque, functional recovery of hand use may involve the premotor cortex (Liu and Rouiller, 1999) and/or SMA (Aizawa et al., 1991). Reorganization involving the remaining M1, premotor cortex and SMA presumably may underlie recovery of function promoted by encouraging use of the impaired extremities in humans as well (Mark and Taub, 2004).

Spasticity, hyperreflexia, tonic postures of the upper and lower extremity and forced grasping, all appear in monkeys and apes if the lesion includes more rostral cortex (Fulton and Kennard, 1932; Kennard et al., 1934; Hines, 1936; Denny-Brown and Botterell, 1948). These signs appear if the lesion includes the more rostral portion of area 4 and/or the more caudal portion of area 6, and are most profound and persistent if both areas 4 and 6 are lesioned bilaterally. The corticofugal projections from area 4 and from the opposite area 6 may in part ameliorate the effects of a unilateral lesion in area 6. Even after bilateral lesions of both areas 4 and 6, however, the animal nevertheless recovers the ability to ambulate and climb, though more awkwardly, and to grasp, though less dexterously, than following lesions limited to the primary motor cortex (area 4). This recovery again reflects the capacity of the remaining brain in non-human primates to generate rudimentary voluntary movement via the residual descending pathways.

That spasticity, hyperreflexia and posturing do not follow pyramidotomy in monkeys, though the corticospinal fibers arising from the premotor cortex descend through the pyramid, indicates that they do not result simply from the loss of the corticospinal projection from area 6. The cortical projection to the magnocellular red nucleus in monkeys arises from rostral area 4 and caudal area 6 (Humphrey et al., 1984) and the projection to the pontomedullary reticular formation arises primarily from area 6 (Keizer and Kuypers, 1989). Combined lesions of the reticulospinal and vestibulospinal pathways result in flexion posturing of the extremities, with adduction of the shoulder (Lawrence and Kuypers, 1968b). Therefore, by damaging the corticobulbar projection, especially that to the pontomedullary reticular formation, lesions rostrally in area 4 and in area 6 probably result in spasticity, hyperreflexia and posturing. The situation in humans may be somewhat different, however. In rare human cases of relatively isolated pyramidal infarction, initial flaccid weakness with hyporeflexia typically progresses to hyperreflexia, often with some degree of spastic tone (Ropper et al., 1979; Paulson et al., 1986; Sherman et al., 2000). This suggests that in humans the corticospinal tract has assumed control over spinal circuits that is achieved in monkeys via the non-corticospinal descending pathways.

Finally, we should note that monkeys do not show Babinski's upgoing toe sign, but chimpanzees do (Fulton and Keller, 1932; Fulton and Kennard, 1932). Lesions restricted to area 4 in the chimpanzee result in an upgoing toe without fanning of the toes. The latter appears if the lesion involves area 6 as well.

2.10. Summary

The corticospinal tract provides the most direct pathway over which the cerebral cortex controls movement. In rodents and marsupials this influence is exerted largely upon interneurons in the dorsal horn of the spinal gray matter. However, ascending the phylogenetic scale through carnivores and primates, the number of corticospinal axons grows and corticospinal terminations shift progressively toward the interneurons of the intermediate zone and ventral horn, ultimately forming increasing numbers of synaptic terminations directly on the motoneurons themselves. Based on this phylogenetic trend, humans are believed to have more direct corticomotoneuronal synapses than any other species,

consistent with observations that humans suffer more extensive loss of motility from lesions of the corticospinal tract than do other mammals.

Beyond this phylogenetic trend, studies of the corticospinal system in animals have provided insight into the motor abnormalities that result from corticospinal lesions in humans. Corticospinal lesions impair many functionally related muscles and movements in parallel, both because of the divergent output from single corticomotoneuronal cells to multiple motoneuron pools, and because of the convergent input to different motoneuron pools from large, overlapping cortical territories. Furthermore, the weakness, slowness and inflexible, stereotyped movements that remain after corticospinal lesions reflect the loss of input to spinal interneurons and motoneurons from corticospinal neurons, the discharge frequency of which varies with the force, direction and speed of both gross and fine movements.

That these deficits resulting from corticospinal lesions are more prominent in humans than in animals indicates, moreover, that animals make greater use of additional descending pathways to control movement. Animal studies have shown that although the bulk of the corticospinal tract arises from the primary motor cortex, this projection is not the only route via which the brain controls movement. Adjacent areas in the frontal and parietal lobes also contribute axons to the corticospinal tract, as well as having corticocortical connections with the motor cortex. Furthermore, the motor cortex and premotor cortex both project to the red nucleus and to the pontomedullary reticular formation, from which the rubrospinal and reticulospinal tracts arise. However, given the limitations on experimental studies in humans, comparative animal studies of the distributed descending system through which the brain controls movement continue to provide deeper understanding and insight into the deficits resulting from human corticospinal lesions, whether caused by stroke, tumor, multiple sclerosis, trauma or ALS.

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Chapter 3

Development of the corticospinal system and spinal motor circuits

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3.1. Introduction

The corticospinal (CS) system, which comprises the primary motor cortex, higher order cortical motor areas, and their descending pathway, the CS tract, is the principal motor system for controlling skilled movements in humans. The CS tract is traditionally, and clinically, considered to originate from the upper motoneurons in layer 5 of the cortex. CS axons grow into the spinal cord and terminate within the gray matter during the late prenatal and early postnatal periods; it is the last of the descending pathways to develop. In humans, based on myelin-stained tissue, CS axons begin to grow into the cord during the middle of the second trimester and grow to the caudal cord by term (Altman and Bayer, 2001). Despite these early beginnings, development of the CS system is protracted; in humans especially. While the bulk of CS axon myelination occurs by about 2 years (Yakolev and Lecours, 1967), physiological evidence indicates that it continues well into adolescence (Koh and Eyre, 1988; Eyre et al., 1991). While human babies begin to use their hands to explore the world around them after the time the CS projection to the cord is complete (Hofsten, 1993; Meer et al., 1995), effective hand skills take many years to develop (Porter and Lemon, 1993; Eyre et al., 2000). The CS system, with its projection to the spinal cord present before the individual's motor repertoire develops, is now well-poised to be influenced by, as well as to direct, the child's early behavioral experiences.

Building CS circuits – locally within the cortical motor, between cortex and spinal cord, and within the cord areas – is undoubtedly a complex process; one that recruits genetic, molecular and systems-level mechanisms (Joosten, 1997; Martin, 2005). What are the over-riding principles that drive development of CS motor control circuits? Like other neural systems, development of the CS system depends on a complex interplay between factors that are intrinsic to: (1) CS neurons, (2) the regions through which CS axons grow to reach their spinal gray matter targets and (3) the spinal gray matter itself. These factors guide developing CS axons to their postsynaptic target neurons. And like other neural systems, development of CS circuits also depends on neural activity and behavioral experience (Goodman and Shatz, 1993; Martin, 2005). This leads to formation of the specific patterns of connections that are necessary for behavior. This dual dependence on intrinsic factors and neural activity/experience has profound clinical significance because not only can genetic factors affect the system's development, but also the functional state of the motor systems during critical perinatal periods.

This chapter will review animal and human studies that elucidate principles of development of the CS system. While my focus is on normal development, I show the relationship between principles of development in animals and impairments in CS development in humans. I will review findings that point both to the importance of intrinsic factors in CS system development as well as the key role of experience and neural activity during early postnatal life. Before describing development of this system, I present an overview of the spinal targets of the CS projection, motoneurons and interneurons, and the mechanisms that determine how they are generated from precursor cells. These are important new results that are likely to yield future insights into how neural circuits are formed between

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the cerebral cortex and the spinal cord. Then, I consider development of the descending CS projection to the spinal gray matter and development of the cortical motor map. The motor cortex is a key site for integrating inputs from different subcortical motor centers and the spinal cord, for integrating descending control and somatic sensory inputs. I will consider the spinal projections of the CS system first because they develop before the motor functions of the cortex. Finally, I consider the implication of what we have learned about CS system development for therapeutic intervention to promote normal motor functions after damage to the CS system.

3.2. Spinal targets of CS neurons and their early development

CS neurons in humans and several animal species synapse directly on spinal motoneurons. Despite these direct connections, anatomical and physiological data indicate that the CS system also makes two additional important connections. First, in humans and animals there are connections between corticospinal axon terminals and various classes of spinal interneurons (Baldissera et al., 1981; Pierrot-Deseilligny, 1996) in the intermediate zone of the spinal cord (Kuypers, 1981). Second, there are connections with brainstem motor systems, forming indirect spinal pathways such as a corticoreticulospinal and a corticorubrospinal projection (Kuypers, 1981). The diversity of corticofugal paths to motoneurons is clinically relevant because it affords the motor systems with multiple routes for transmission of motor control signals after brain or spinal injury.

Motoneurons are located in the motor nuclei within the ventral horn. Interneurons are located more diffusely, within the deeper portion of the dorsal horn, intermediate zone, as well as the ventral horn. The dorsoventral location of a spinal cord precursor cell early in development is important in determining what class of interneuron or motoneuron it will become (Fig. 3.1(A–C)) (Jessell, 2000). The graded concentration of the protein sonic hedgehog (Shh), which is

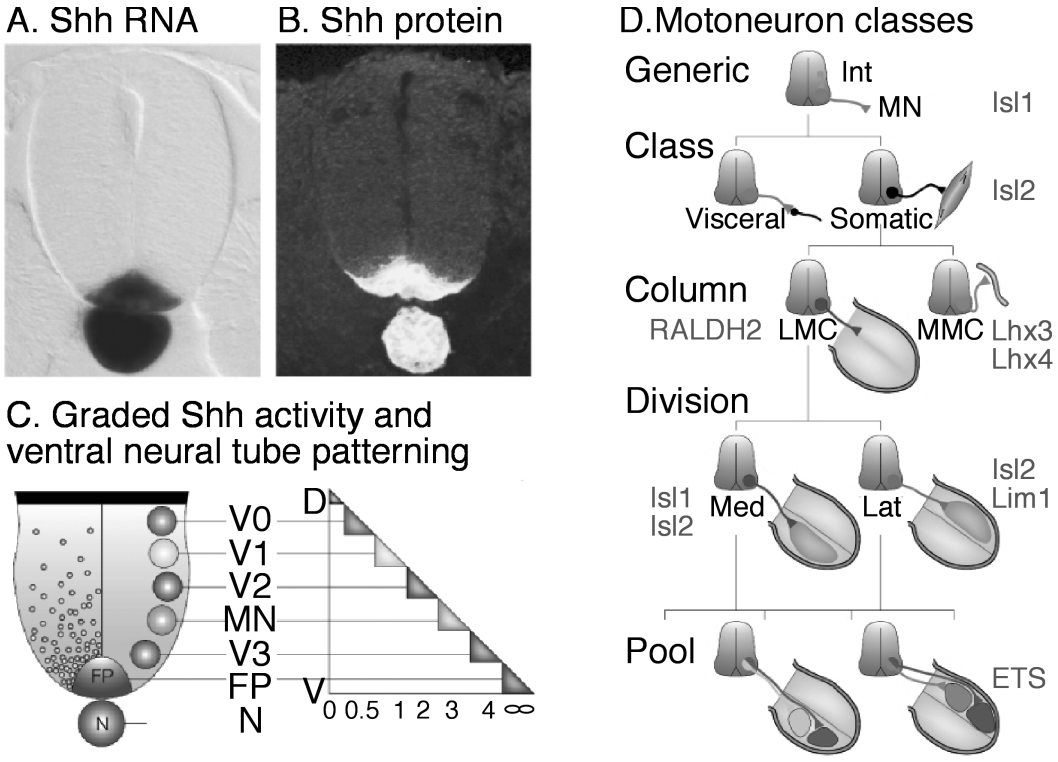

Fig. 3.1. Early development of spinal interneurons and motoneurons. Cross-section through the chick spinal cord (stage 18) showing the expression of *Shh* RNA **(A)** and Shh protein **(B)**. **(C)** A model for the affects of Shh on the specification of ventral neuronal types. The left side of C shows a gradient of Shh from the notochord (N) and floor plate (FP). The right side shows the proposed relationship between Shh concentration and the neuron type that the progenitor cell becomes. **(D)** Summary of progressive specification of motoneuron subtype. Generic: features common to all motoneurons; Class: somatic vs visceral subtype; Column: lateral vs medial column; Division: motor axon projection to particular limb compartment; Pool: motoneuron pool. The particular transcription factors that are important in specifying motoneuron subclass are shown in lighter type. Modified from Jessell (2000).

secreted by cells of the notochord and floor plate (Briscoe et al., 1999, 2000), is important in establishing the dorsoventral patterning of neurons in the spinal cord. Developing neurons throughout the ventral horn and intermediate zone are exposed to Shh. Those located closest to the floor plate and notochord are exposed to Shh in the highest concentration, while those located more dorsally, to lower levels. Jessell and coworkers (Jessell, 2000) identified five classes of neuron whose fates are determined by the graded concentration of Shh. The most ventral class is an interneuron (possibly Renshaw cell), followed dorsally by motoneurons, and then, farther dorsally, by three interneuron classes.

Much more is known about the fate of somatic motoneurons than of other spinal neuron classes (Fig. 3.1(D)). Recall that the mediolateral location in the ventral gray matter determines if the motoneuron will innervate axial (medial location; medial motor nuclei) or limb (lateral location; lateral motor nuclei) muscle. Within the lateral motor nuclei, the dorsoventral position of the developing motoneuron determines the location of the limb muscle that it innervates. Finally, within one division, the location of the motoneuron within the pool determines precisely which muscle is innervated. Particular transcription factors have been identified that play a role in determining a motoneuron's mediolateral location, its position within the lateral motor nuclear column, and in which motoneuron pool it is located (Fig. 3.1(D)). Importantly, the process by which a motoneuron's identity is established can be replicated in vitro: embryonic precursor cells can be raised to become motoneurons with a particular phenotype by exposing them to the appropriate proteins and transcription factors in the proper sequence and at the proper concentrations (Wichterle et al., 2002). This has important implications, both for studying the biology of motoneurons, but also for replacement strategies in motoneuron disease.

Motoneuron dendrite morphology and the pattern of input a motoneuron receives is refined by the actions of descending pathways and primary afferent inputs and by experience. In a particularly striking example, Kalb and colleagues (Inglis et al., 2000) have shown in rats that the dendritic tree of developing medial (i.e. axial) motoneurons is significantly reduced under conditions of weightlessness during space flight. The vestibulospinal system projects preferentially to medial motoneurons (Kuypers, 1981) for axial control and balance. Weightlessness changes the pattern or reduces the level of descending drive by vestibulospinal axons. This can, in turn, affect activity-dependent processes in the motoneurons during a critical period. Earlier it had been shown that the levels of particular molecules in developing motoneurons are regulated by activity during a discrete period (Kalb and Hockfield, 1992, 1994). Similarly, transection of the spinal cord during early postnatal development, to eliminate the actions of all descending pathways, impairs the proper formation of spinal reflex circuits (Petersson et al., 2004). Motor cortex lesion or blockade of motor cortical activity changes the levels of expression of activity-dependent genes in spinal interneurons and motoneurons (Gibson and Clowry, 2003; Clowry et al., 2004). These findings show that while the fate of a precursor cell is strictly determined by genetic and molecular factors and thereby establish the cell's identity within spinal motor circuits, activity-dependent processes can affect how the cell functions within the circuit.

3.3. Development of CS projections to the brainstem and spinal cord

Development of the CS projection to the spinal cord consists of many discrete steps, each of which is likely to reflect multiple component parts (Fig. 3.2). The outcome of this process is to establish stable circuits with spinal cord neurons. While our understanding of the mechanisms underlying these processes is incomplete, insights have been obtained in identifying some of the molecular factors and other determinants shaping development of the descending CS projection. Moreover, dysfunction at several stages leads to CS developmental and motor impairments in animals and humans. Both basic science studies and observations in patients point to an important interplay between developmentally regulated molecules on the one hand and neural activity and an individual's motor experiences, on the other.

3.3.1. Pathfinding and corticospinal axon growth to the spinal cord

Layer 5 pyramidal neurons in a variety of cortical motor and somatic sensory areas project to the spinal cord within specific white matter regions. Significant progress has been made recently in determining the factors important for early specification of these neurons.

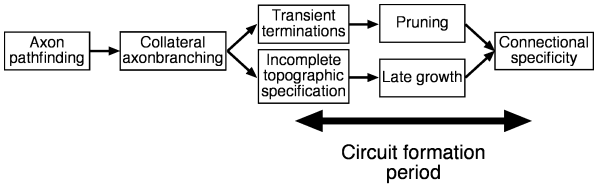

Fig. 3.2. CS tract development. Five key steps in specifying connections of developing CS axons. The key period for circuit formation is shown by the horizontal arrow.

Macklis and colleagues (Arotta et al., 2005) have identified a particular set of transcription factors in the mouse that are expressed in cortical neurons projecting to the brainstem and spinal cord. Mutant mice for one molecule in particular, Fezl (forebrain embryonic zinc finger-like), do not have any corticospinal projection (Molyneaux et al., 2005). Early in development, the distribution of corticospinal neurons is extensive – reaching from the frontal to the occipital poles – but later in development the distribution becomes restricted mostly to the posterior frontal and anterior parietal cortex (Stanfield et al., 1982; Galea and Darian-Smith, 1995). The reduction in number of CS neurons is principally due to elimination of spinal branches, not wide-spread cell death (Oudega et al., 1994; Galea and Darian-Smith, 1995). A small contingent of 'pioneer' axons leads the way into the cord, followed later by waves of axons that further populate the corticospinal tract (Joosten et al., 1987). Pathfinding is organized by complex tissue molecular cues that are detected by the primary growth cone of the axon (Tessier-Lavigne and Goodman, 1996; Mueller, 1999). The cues for guiding developing CS axons to the spinal cord are likely to be extraordinarily complex. Several studies have shown the importance of a member of the immunoglobulin superfamily of cell adhesion molecules, the L1 cell adhesion molecule (L1-CAM; Cohen et al., 1998; Kamiguchi et al., 1998). L1CAM is expressed along many developing pathways, including the corticospinal tract, and has been implicated in a variety of important developmental processes, especially in neurite growth and axon fasciculation (Kamiguchi et al., 1998). L1 knockout mice display profound errors in CS axon guidance. There are two striking features. First, significant numbers of CS axons fail to cross the midline in the caudal medulla. Figure 3.3(A) shows the aberrant pattern of incomplete axonal decussation in a L1 knockout mouse; the normal pattern of complete decussation is shown in B for a wild-type mouse (Itoh et al., 2004). In the knockout mouse, many of the axons fail to cross. Rather, they follow a mirror symmetrical course through the ipsilateral medulla (see inset in Fig. 3(A)) into the ipsilateral dorsal column. At more rostral brainstem levels, corticofugal axons have a normal location suggesting that the impairment is in decussation and subsequent caudal guidance, not collateral branching and outgrowth. Interestingly, the proteoglycan CD24, which is a ligand for L1, is localized at the pyramidal decussation (Cohen et al., 1998) and may be involved in guiding axons across the midline. Second, in the spinal cord, there are significantly fewer CS axons in the L1 knockout mice; note that the amount of label in the tract is much less in the knockout (Fig. 3.3(C)) than in the wild-type (Fig. 3.3(D)). Interestingly, there are no ipsilateral CS axons in the spinal cord in the knockout mouse despite incomplete decussation (Fig. 3(C)). (In rodents most corticospinal axons are located in the dorsal column, in contrast to the carnivores, monkeys and humans, where most CS axons are in the lateral column.) Thus, L1CAM is important for CS axons to project to the appropriate spinal targets on the contralateral side.

Consistent with an important role in CS system development, human L1 mutations produce hypoplasia of the

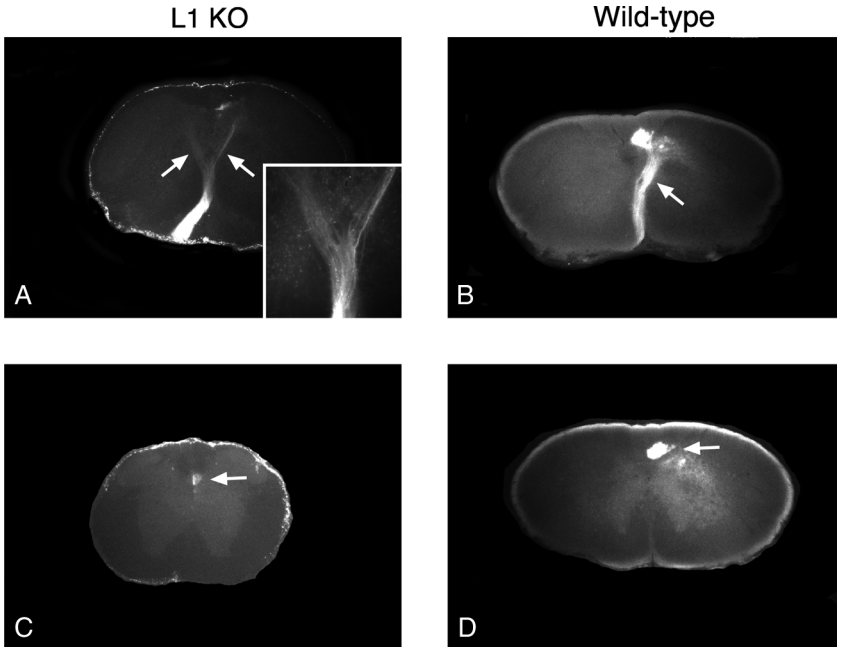

Fig. 3.3. L1CAM and CS development. Each panel shows a transverse section through the mouse CNS. Left column shows sections from the L1CAM knockout mouse; right column shows the wild type. CS axons are labeled white. **(A)** Section through the caudal medulla at the level of the pyramidal decussation. Arrows point to bilateral projections of L1CAM knockout mouse. **(B)** Arrow points to normal decussation. **(C)** Arrow points to reduced descending CS projection on the contralateral side; note there is no ipsilateral label. **(D)** Arrow points to normal descending contralateral projection. Micrographs courtesy of Dr Vance Lemmon; Itoh et al. (2004).

CS tract and spasticity as well as a variety of other brain structural and functional impairments. There are several classes of L1 mutations in humans that, to varying degrees, have reduced survival and diverse structural and cognitive impairments. Spastic paraparesis and hypoplasia of the CS tracts is almost always present (Kamiguchi et al., 1998). While detailed neuroanatomy of the CS projection in humans with L1 gene mutations is lacking, recently Eyre and colleagues (Dobson et al., 2001) used transcranial magnetic stimulation (TMS) to examine the functional organization of the defective CS system in humans. They found clear evidence for impaired CS physiology in patients with L1 mutations. The thresholds for evoking limb motor responses using TMS were elevated and response latencies were longer; more so for the lower than the upper extremity. The greater defects in evoking lower extremity responses could be due to impaired fasciculation of developing axons and myelination, which might lead to a greater conduction distance-dependent impairment. They also identified reduced inhibition of motor responses, which is evidence for a reduced projection to inhibitory interneurons in the cord. Eyre and colleagues found impairments in performance of several tests of hand/digit dexterity in individuals with L1 mutations. Since the behaviors they examined normally depend on the CS system for their execution, this study greatly extends earlier genetic findings by showing a strong correlation between skilled motor impairment and the L1 mutation.

Other molecules have been implicated in guidance and decussation of developing CS axons. The roundabout (robo) family of transmembrane proteins prevent axons from crossing the midline inappropriately. First identified in *Drosophila*, axons in robo mutants cross and recross the midline (Tear et al., 1993). Two Robo proteins, Robo 1 and 2, are receptors for Slit, which is a protein that has axon growth repulsive properties. Robo is strongly expressed on CS axons. CS axons may be prevented from aberrant recrossing the midline through interactions between these Robo proteins on developing axons and Slit at the floor plate of the hindbrain and spinal cord. A third member of this family, Robo 3, has different properties. While Robo 1 and 2 mediate axonal repulsive responses to Slit and thereby prevent recrossing the midline, Robo 3 is required for decussation (Sabatier et al., 2004). In patients with a rare genetic disorder, horizontal gaze palsy with progressive scoliosis (HGPPS), mutation in the Robo 3 gene prevent decussation of the corticospinal tract. TMS in these patients produces ipsilateral motor evoked responses. Interestingly, the ascending dorsal column-medial lemniscal pathway, which also decussates in the medulla, fails to cross in these patients. Somatosensory evoked potentials are ipsilateral to stimulation, rather than the normal contralateral (Jen et al., 2004). In addition to CS and somatic sensory path abnormalities, these patients also have profound hindbrain structural defects. Several other syndromes present with defective decussation of CS axons in the medulla, including Kallmann's syndrome and Klippel-Feil syndrome. As we elucidate the mechanisms of these syndromes we will better understand the molecules necessary for CS axon decussation. I discuss below the role of Ephrin and eph receptors in preventing re-crossing of CS axons in the spinal gray matter.

3.3.2. CS axonal branching within the gray matter

Long distance growth of the primary descending CS axon is followed by formation of side or collateral, branches that extend into the surrounding gray matter after a variable delay period (Bastermeyer and O'Leary, 1996). Gray matter innervation is mediated by target-specific chemotropic factors that induce branching. In tissue explant experiments, for example, neurites from a portion of the prospective forelimb area of sensory-motor cortex grow toward a cervical spinal explant but not to a lumbar explant (Kuang and Kalil, 1994). This indicates the importance of target-derived factors that diffuse into the local environment to attract and guide growing corticospinal axons. One factor that may play a role in CS axon outgrowth is neurotrophic factor-3 (NT-3). In mature animals, exogenous NT-3 in the spinal gray matter or in collagen implants causes increased branching of regenerating CS axons (Houweling et al., 1998; von Meyenburg et al., 1998). NT-3 is present in spinal gray matter during the period of CS axon outgrowth (Friel and Martin, preliminary findings). Motor cortex neurons in culture express mRNA for the receptor tyrosine kinase (TRK) C (Giehl, 2001), which is the receptor for NT-3. Interestingly, CS neurons anterogradely transport NT-3 into the descending axon. Thus, the role for NT-3 in CS development is complex, with both target-derived functions and possible autocrine affects (Giehl, 2001).

3.3.3. Development of connectional specificity in the spinal gray matter

When CS axons initially grow into the spinal gray matter their termination pattern bares little resemblance to the mature pattern (Li and Martin, 2000, 2001). A dominant pattern – which is present in developing cats, rats, opossums and likely to be present in humans (see below) – is that CS axons have a widespread spinal termination pattern early in development that is subsequently refined to a more restricted distribution (Fig. 3.4) (Cabana and Martin, 1985; Theriault and Tatton, 1989; Alisky et al., 1992; Curfs et al., 1994, 1995; Li and Martin, 2000, 2001).

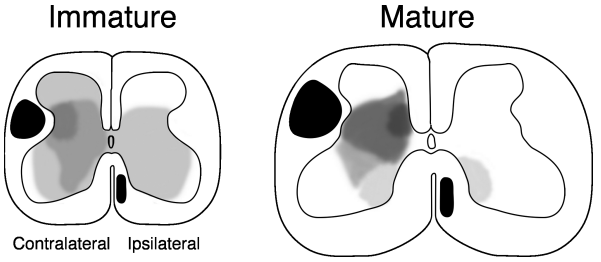

Fig. 3.4. Change in the pattern of CS terminations in the developing spinal cord. Semi-schematic transverse sections of the spinal cord. The density of gray shading shows the density of CS terminations. The lateral and ventral CS tracts are shown in black.

In the cat, individual CS segmental axon terminals have a broad dorsoventral (i.e., laminar) distribution at 1 month (Fig. 3.5; top), but by 7–8 weeks is refined to a mature pattern that is restricted to the deeper dorsal horn layers and the ventral horn. This restricted pattern is maintained into maturity (Fig. 3.5; top, adult). Axons that are present in a particular area early in development but subsequently eliminated are often termed transient terminations. Note that in the cat there are abundant terminations in the motor nuclei during development but few in maturity (Fig. 3.4) (Li and Martin, 2000; Salimi and Martin, 2004). Transient terminations extend into the ipsilateral gray matter (Cabana and Martin, 1985; Theriault and Tatton, 1989; Alisky et al., 1992) and, in the rodent, into the white matter (Curfs et al., 1994) where they may be synapsing on distal dendrites of motoneurons and interneurons. As transient branches are eliminated, there is a parallel increase in local branching and presynaptic site development in the terminals that survive (Li and Martin, 2002). In the cat, there is age-dependent growth of local branches that depends on CS neural activity (see below). Development of local branching and presynaptic sites is expected to lead to stronger connections. Maturation of the physiology of CS connection matches the anatomy. In immature animals, stimulation of the CS system evokes postsynaptic responses throughout the extent of the gray matter, although the amplitude of the postsynaptic responses is largest in the middle layers (Fig. 3.5; bottom left). By contrast, in mature animals (Fig. 3.5; bottom right) responses are largely limited to the middle layers only. Moreover, there is a remarkable increase in the amplitude of responses during development.

There is a close correspondence between our findings in the cat and the findings of Sakurai and colleagues using a rat corticospinal co-culture (Takuma et al., 2002; Ohno et al., 2004). At 7 days in vitro, CS axons innervate neurons widely throughout the spinal gray matter but by 14 days most of the ventral terminations are largely eliminated. The loss of ventral connections is activity dependent (Ohno and Sakurai, 2005) and was NMDA receptor-dependent (Ohno et al., 2004).

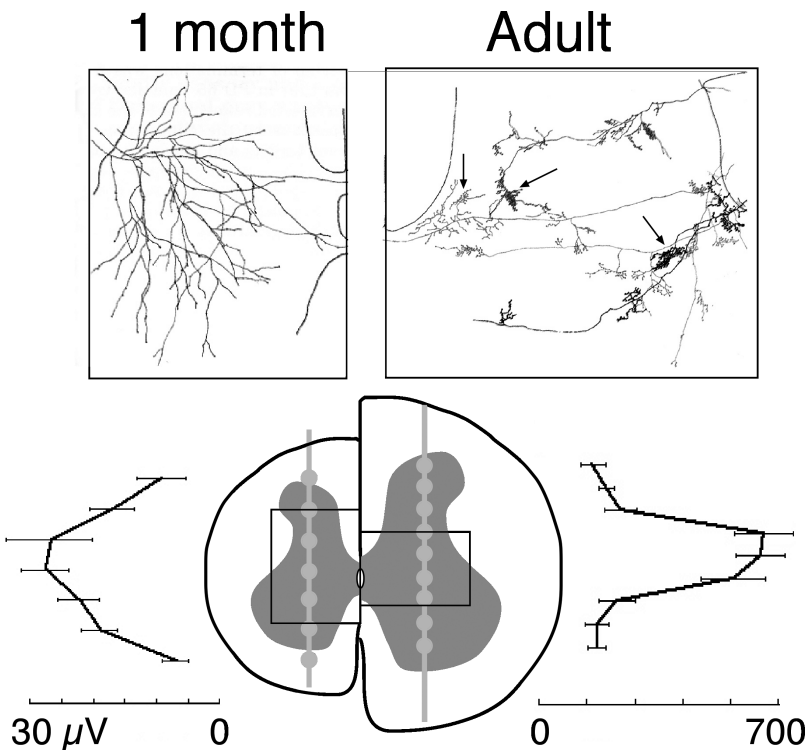

Fig. 3.5. For full color figure, see plate section. Development of the specificity of CS connections. Anatomical specification is shown in the top two panels. Individual CS axon terminals are shown in different colors for immature cats (left) and in mature cats (right). Presynaptic boutons correspond to the small dots. Arrows on the right point to terminations with a high density of presynaptic sites and branches. The lower panel shows refinement of the postsynaptic responses evoked by pyramidal tract stimulation. The graphs plot the amplitude of the postsynaptic field potential as a function of distance from the dorsal pial surface (note vertical electrode tract and recording sites at the dots). Top panel modified from Li and Martin (2002). Bottom panel modified from Meng et al. (2004).

The presence of postsynaptic responses in the young animal suggests that the CS system is functional at very young ages. However, this is misleading. Although electrical stimulation of the CS system in the young animal can evoke spinal responses (Meng and Martin, 2003), the responses are small and insufficient to evoke muscle responses unless the electrical stimulation currents are very high (Meng et al., 2004). What can account for the development of strong functional connections between CS terminals and spinal neurons? Not only do CS axon terminals develop more branches and presynaptic sites during early postnatal life, but there is also an enhanced capacity to summate descending control signals. Using pairs of electrical stimuli to CS axons in the pyramid or trains of stimuli of varying duration, we recorded the evoked spinal and muscle responses (Meng et al., 2004). We found that the spinal postsynaptic response to the second of a pair of stimuli, a characteristic termed facilitation, was larger at all ages. Importantly, facilitation increases with age. With stronger facilitation as the corticospinal system matures, the motor cortex can activate spinal motor circuits with lower levels of neural activity. As an animal matures, CS axon terminals grow denser branches and develop more presynaptic sites. This leads to stronger connections with spinal motor circuits and, together with more facilitation, a greater capacity of the CS system to evoke movements.

While in the human there are no comparable anatomical data charting CS system maturation, several electrophysiological studies point to an early period of transient ipsilateral and ventral terminations, similar to the anatomy of the developing cat CS system. Eyre et al. (2001) have shown that TMS in the late term premature infant and up to about 1 year evokes bilateral motor responses (Fig. 3.6(A,B)). There is a reduction in the amplitude of the ipsilateral response relative to the contralateral response during the first year or two of life (Fig. 3.6(B)). As discussed below, our studies in the cat suggest that the reduction in the ipsilateral response reflects competitive interactions between the developing contralateral and ipsilateral CS terminations and that the contralateral terminals win. Several studies report that TMS thresholds in humans decrease between 1 and 10 or more years (Eyre et al., 1991; Nezu et al., 1997); there is also a progressive decrease in TMS threshold in monkeys during the first year (Olivier et al., 1997). This could reflect increased CS terminal branching and an increased capacity for synaptic facilitation, as we have seen in the cat (Li and Martin, 2001; Meng et al., 2004). It is interesting that there is also a disproportionate reduction in the central motor conduction delay for contralateral not ipsilateral responses (Fig. 3.6(C)), which is likely due to enhanced myelination.

Despite subtle differences in the results of electrophysiological studies of the developing CS systems in

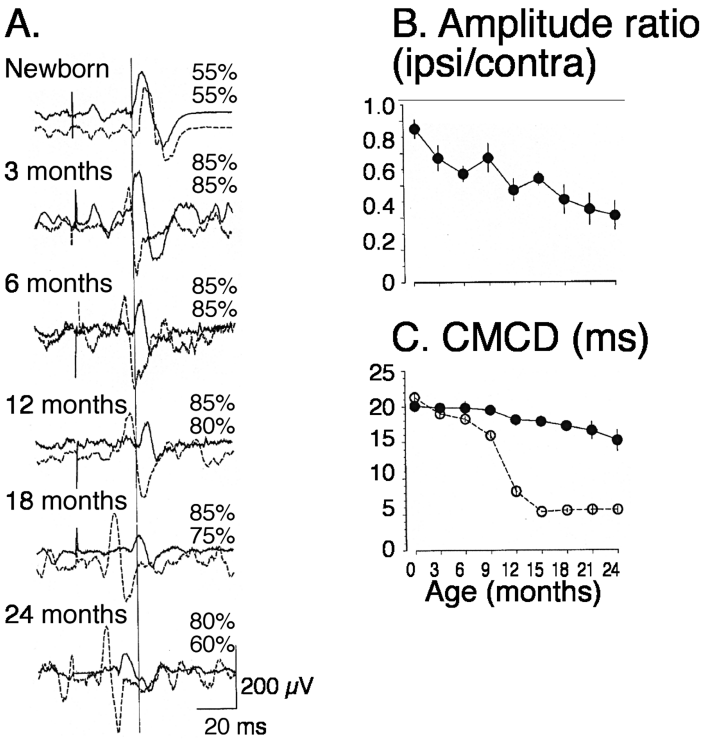

Fig. 3.6. Normal human CS system development. (**A**) Ipsilateral (solid) and contralateral (dashed) motor potentials evoked by TMS. Threshold is expressed as a percentage of maximum stimulus intensity. (**B**) The value of the ipsilateral response divided by the contralateral response. (**C**) Change in the central motor conduction delay. From Eyre et al. (2001).

cats and humans, there is a remarkable concordance: CS synapses at very early ages are capable of activating their spinal target neurons. While the connections may not be strong enough (in the cat, to activate muscle) or have the proper pattern to engage circuits for movement control, it is plausible that active CS terminals are helping to shape the organization of intrinsic spinal circuits to which they connect. Experiments in rats point directly to the possibility that developing CS terminals help to eliminate transient muscle afferent terminals. In mature rats, more muscle afferent boutons are present in the spinal gray matter after an early postnatal lesion of the motor cortex (7 days) than after a lesion made in adults (Gibson et al., 2000). This suggests that developing CS terminals can shape the distribution of intrinsic spinal connections.

Surprisingly, there is no evidence for transient CS terminations in the rhesus monkey, neither the extent of contralateral terminations nor the presence of ipsilateral terminations. Newborn monkeys appear to have CS terminals that are limited to the contralateral intermediate zone and deep dorsal horn (Kuypers, 1962; Armand et al., 1997), a distribution that falls short of the mature pattern. Over the next 3–8 months, there is outgrowth ventrally into the motor nuclei. The time course of this late CS development correlates both with the time course of emergence of strong TMS-evoked motor effects (see below) and development of individuated finger use (Olivier et al., 1997). The absence of ipsilateral transient CS terminations in the rhesus monkey may be due to a molecular barrier, similar to what has been shown in mice. Transient ipsilateral terminations are not present in mice and this is probably due to the presence of a midline growth barrier established by Ephrin B, the membrane bound ligand for the ephA4 receptor. Ephrin B or ephA4 knockout mice have a bilateral corticospinal termination pattern in maturity (Coonan et al., 2001; Kullander et al., 2001; Yokoyama et al., 2001). In vitro, the Ephrins are potent repellent molecules for growing axons (O'Leary and Wilkinson, 1999). This, however, does not explain the absence of transient dorsal or ventral contralateral terminations. Alternatively, transient terminations – both contralateral and ipsilateral – could be present earlier in development and eliminated prenatally. The prenatal distribution of CS terminations has yet to be examined.

3.3.4. Conclusion

Connectional specificity determines with which spinal circuits a corticospinal neuron can engage, and therefore the neuron's functions. Cell intrinsic factors determine whether a neuron becomes a CS neuron. Target-derived diffusible substances determine the initial pattern of growth into the gray matter. While this process is highly complex and undoubtedly well-regulated, only coarse patterns of connectivity are achieved. Whether corticospinal neurons ultimately form functional connections with one or another spinal circuit, which is the basis for its motor control functions, depends on a more refined pattern of connectivity. This is achieved later in development, by 6–7 weeks in the cat, 8 months in the monkey and much later in the human, probably after several years. Importantly, the response of developing neurons depends on more than intrinsic factors. This is because the actions of intrinsic factors depend also on the neuron's biochemical and functional state. Studies of neurons in culture show that guidance cues that are attractants can become repellents by manipulating cyclic nucleotide levels within cells (Song et al., 1998). Thus, the final termination pattern of a developing neuron depends both on the target and the internal state of the neuron.

3.4. Does specification of CS connections during postnatal development depend on motor cortical neural activity and limb use?

While growth of CS axon terminals is initially extensive in many species and in vitro, the density of terminal branches and presynaptic sites is sparse. Immature CS synapses are minimally effective in activating spinal neurons and in driving muscle contraction. We propose that refinement of the pattern of terminations depends, in part, on competitive synaptic interactions among CS terminations and between corticospinal and other spinal terminals. We envisage these activity-dependent interactions to stem from basal levels of neural activity, phasic activity that reflects motor control signaling, and the patterns of limb use. To examine this hypothesis further, we have conducted experiments in which the level of CS activity was manipulated. We have also examined the role of motor experience in refining terminations.

3.4.1. The pattern of contralateral and ipsilateral CS terminations depends on the balance of neural activity in motor cortex on the two sides

Synaptic competition among developing CS terminals in vivo is best studied by manipulating the activity of the CS system on one side and examining the terminals of the manipulated and non-manipulated (control) systems independently. Activity of the CS system can be reduced by intracortical infusion of the GABA agonist muscimol or augmented by electrical stimulation. Figure 3.7 summarizes the salient results of the effects of silencing CS neurons during development. During normal development of corticospinal axons in the cat, each motor cortex

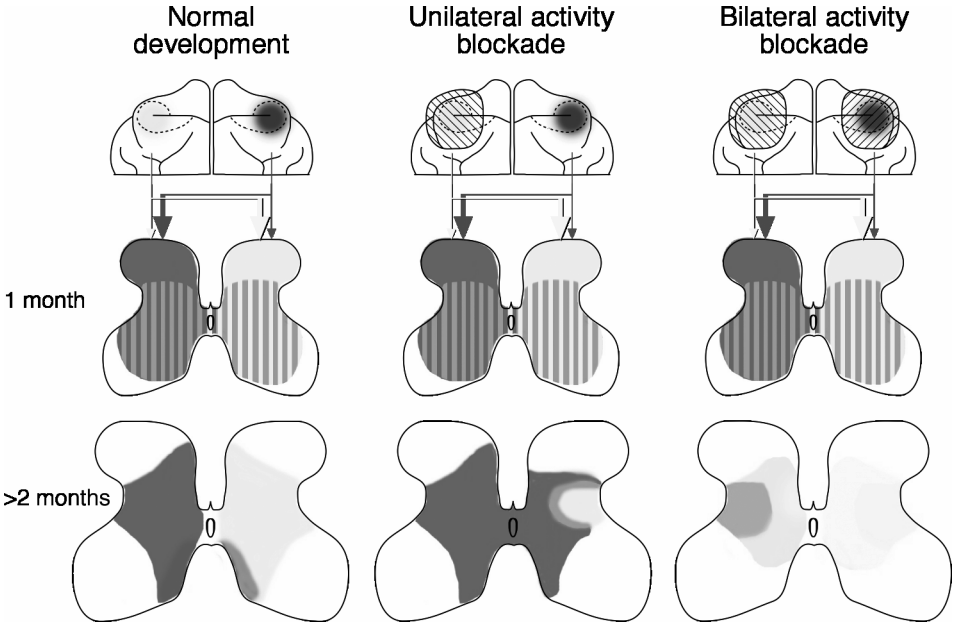

Fig. 3.7. For full color figure, see plate section. Activity-dependent development of the cat CS system. Each panel shows a schematic view of the frontal lobes of the cat cortex (top). The CS system from each side is shown in a different color. Regions of overlap are striped. **(A)** Normal development. **(B)** Unilateral activity blockade. The left (yellow) side is inactivated (cross-hatched). **(B)** Bilateral activity blockade.

projects bilaterally to the spinal gray matter by 1 month, but most ipsilateral (i.e. re-crossed) terminations are eliminated by about 2 months (Fig. 3.7; left) (Theriault and Tatton, 1989; Alisky et al., 1992; Li and Martin, 2000). If the activity of neurons in one motor cortex is blocked by intracortical Muscimol infusion (Martin et al., 1999), the silenced corticospinal system fails to populate most regions of the spinal gray matter (Fig. 3.7; middle, shaded cortex is inactivated; yellow terminations). This impairment reflects a failure to maintain silenced axons within territories normally occupied by CS axons. The axons of silenced CS neurons have very sparse terminal branches, with few presynaptic boutons (Friel and Martin, 2005). This pattern is similar to the one we observed after limb disuse (see below).

In contrast to the contracted distribution of the silenced corticospinal system, the contralateral active system not only develops the normal contralateral projection but also maintains significant ipsilateral terminations in the intermediate zone and dorsal horn. Thus, the reduction in termination space of the silenced side is balanced on that side by maintenance of more ipsilateral terminations of the active system, which are the terminations that are normally eliminated (Martin et al., 1999). These topographic changes persist into maturity. The presence of ipsilateral terminations of the active side implies that these terminals can compete effectively with terminals of other spinal afferent systems on that side. When the motor cortices on the two sides are both silenced, a relatively normal pattern is present with contralateral terminations, however the overall density of terminations is less than expected (Fig. 3.7; right) (Martin and Lee, 1999). This suggests that the changes in the distribution of CS terminals occurring after activity blockade are due to activity-dependent competition between developing corticospinal terminals and other spinal neural systems.

Our findings show that the active side maintains bilateral terminations at the expense of the silenced side. Another way to examine the role of activity on the two sides in shaping the pattern of terminations is to augment activity unilaterally. Electrical stimulation of CS axons in the medullary pyramid for several weeks during early postnatal life results in maintenance of transient ipsilateral (and contralateral) terminations at 8 weeks (Fig. 3.8). Normally there are predominantly contralateral terminations at this age. We also found that projections from the non-stimulated side were displaced dorsally and laterally as a consequence of this stimulation. Both the maintenance of transient terminations and the topographic displacement of non-stimulated axons are consistent with the activity-dependent competition model: the stimulated axons are more competitive at securing spinal synaptic space at the expense of the non-stimulated axons. I discuss below how electrical

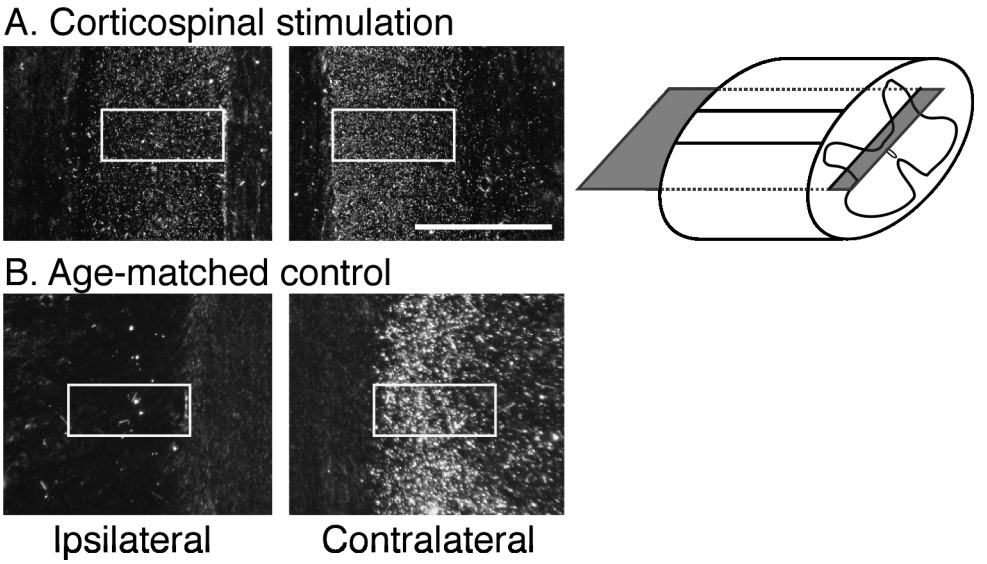

Fig. 3.8. For full color figure, see plate section. Effect of electrical stimulation on development of CS terminations. CS axon were traced (wheat germ agglutinin conjugated to horseradish peroxidase or WGA-HRP; golden-brown color) and their distribution is shown on horizontal sections through the cervical cord (inset). **(A)** The pyramidal tract was stimulated from 5–7 weeks and the tissue was harvested from the animal at 7 weeks. **(B)** Age-matched control. Note that labeling is bilateral in A (compare the amount of label within the yellow boxes) but contralateral in B. Modified from Salimi and Martin (2004).

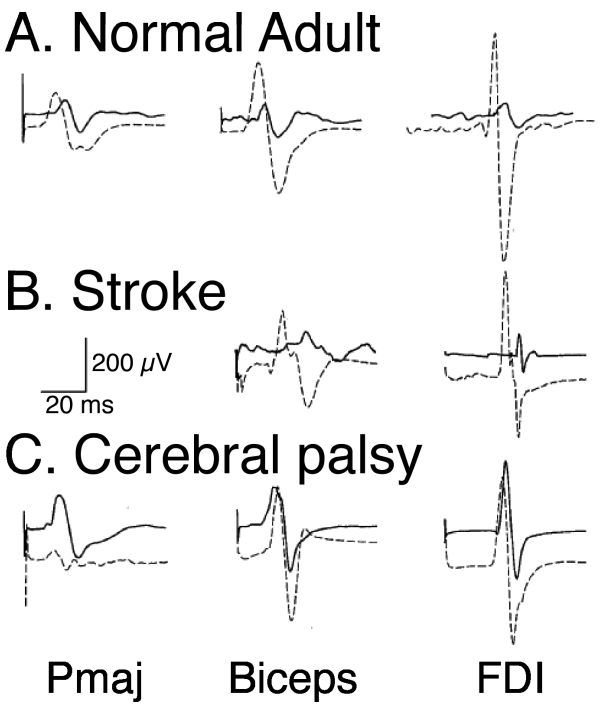

Fig. 3.9. Effects of neonatal and adult stroke on TMS-evoked motor potentials in humans. **(A)** Normal adult. **(B)** A subject with a stroke. **(C)** Hemiplegic cerebral palsy. From Eyre et al. (2001).

stimulation of the CS system might be used therapeutically to promote connections and function.

The pattern of bilateral CS projections from the active side and sparse contralateral projections from the silenced side after unilateral inactivation (Fig. 3.7(B)) is remarkably similar to the laterality of evoked motor responses following TMS of the motor cortex in patients with spastic hemiplegia after perinatal brain trauma (Farmer et al., 1991; Carr et al., 1993; Eyre et al., 2001). As described above, TMS of the motor cortex on one side in the neonate evokes bilateral motor responses (Fig. 3.6). By contrast, TMS in the adult evokes predominantly contralateral responses (Fig. 3.9(A)). Several studies have reported that, in hemiplegic cerebral palsy (Farmer et al., 1991; Carr et al., 1993; Eyre et al., 2001), TMS of the less impaired side evokes bilateral responses (Fig. 3.9(C)). TMS of the severely impaired side fails to evoke significant responses (Carr et al., 1993; not shown in figure). By contrast, strokes in adults that produce hemiparesis do not augment the ipsilateral response (Fig. 3.9(B)), showing that the effect in cerebral palsy is linked to damage during early development, possibly at a time when the CS system has bilateral connections with the cord. These findings are consistent with the hypothesis that the impaired side is rendered much less competitive in securing and maintaining spinal synaptic space than the normal or less impaired side.

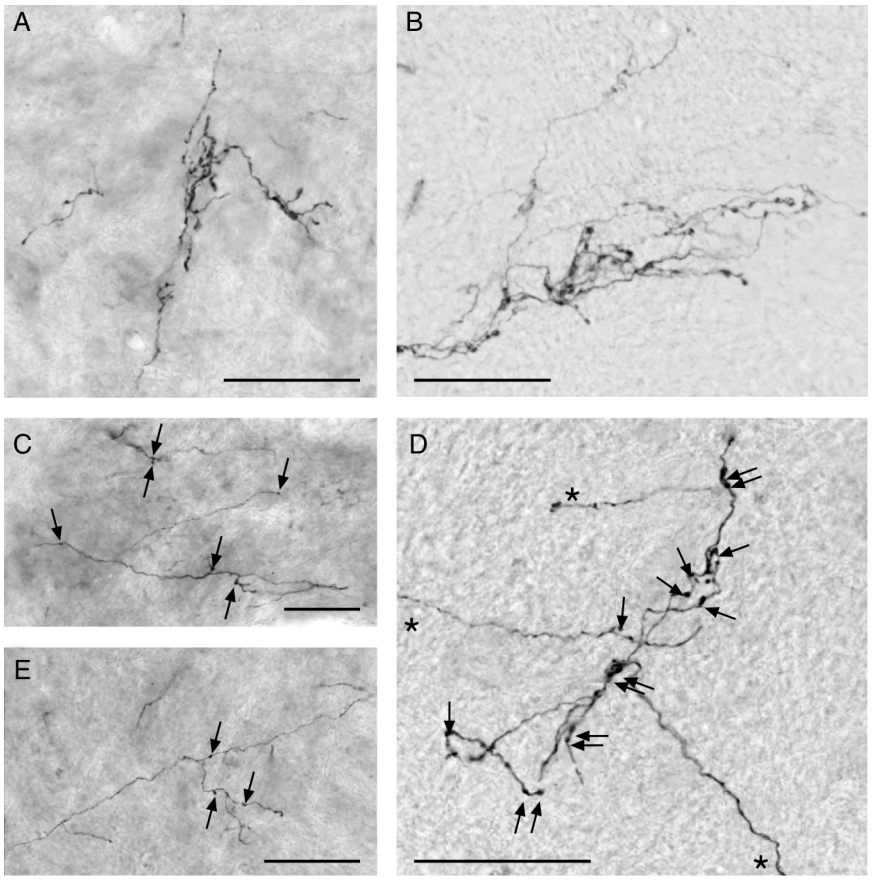

Fig. 3.10. Effect of limb disuse on development of CS axon terminal morphology. Individual CS axon terminals, anterogradely labeled after BDA injections in motor cortex. **(A)** Normal cat, 8 weeks. **(B)** Normal adult cat. **(C, D)** CS terminals from an 8 week old cat that was subjected to BOTOX forelimb muscle injections between weeks 3 and 8 (see text for details). **(E)** CS terminals from a mature cat that was subjected to BOTOX forelimb muscle injections between weeks 3 and 8. Calibration: 50 μm. From Martin et al. (2004).

3.4.2. Normal topography and morphology of corticospinal axon terminals depends on motor experience

Preventing limb use during early development has a profound effect on CS axon terminal development, similar to the effects of motor cortex activity blockade. To prevent limb use in kittens, we injected botulinum toxin A into several forelimb muscles (Martin et al., 2004). The weakening effect of the toxin resulted in the animal not using the limb despite the presence of several intact muscle groups. Limb muscle weakening was maintained by repeated BOTOX injections between weeks 3 and 8. Like motor cortex activity blockade, preventing limb use prevents the late developmental growth of CS axon terminals and presynaptic sites. Compare the control terminal of an 8 week old animal in Figure 3.10(A) with terminations in parts C and D, which are from another 8 week old animal in which limb movements were prevented. And also like activity blockade, when limb use is regained CS axon terminals do not recoup lost connections. Compare the control terminal of a mature animal in Figure 3.10(B) with that of a mature animal the did not use its limb between weeks 3 to 8 (Fig. 3.10(E)). While there is some branch growth during the intervening period of limb use, it is much less than in controls (Martin et al., 2004). This effect is persistent; the reduction in axon branching and presynaptic site density improves little later in development.

3.4.3. Normal skilled motor behavior depends on motor cortex activity and experience

Skilled movement control in cats is profoundly impaired after early postnatal motor cortex activity blockade and after preventing limb use. We examined performance of animals in a variety of tasks in which vision was needed for controlling the affected limb after cessation of activity blockade. Considering the importance of the CS system in distal limb control, it is not surprising that motor cortex activity blockade and forelimb disuse disrupted the ability of the animal to grasp objects using the affected side (Martin et al., 2004). In addition, activity blockade resulted in aiming errors during reaching (Martin et al., 2000) and foot placement errors during visually-guided locomotion (Friel et al., 2004). In both tasks, the end-point of the animals' movements was hypermetric (i.e. over-reach and over-step). The common phenotype suggests a common underlying mechanism, such as an impairment in using visual information to compute the end-point of the

movement or an impairment in expressing the planned motor output.

3.4.4. Conclusions

Spinal cord neurons receive convergent inputs from multiple peripheral, spinal and supraspinal targets. And many of the inputs are located on distal dendrites. Activation of spinal neurons is likely to require cooperation between multiple sources of facilitatory inputs to bring a neuron to firing level. The presence of effective collaboration of particular corticospinal terminals with other inputs in the gray matter, such as other descending pathways or primary afferent fibers, would be expected to lead to strengthening and stabilizing of CS synapses (Clowry et al., 2004). These findings have important clinical significance. The effects of perinatal activity and limb use manipulations in animals are permanent. We propose that this means that when activity or limb use returns, the developing CS axons have lost ground in competing for and securing stable synaptic space. A clinical implication of the basic finding of activity-dependent CS axon terminal development is to harness activity for therapeutic applications (see below).

3.5. Role of activity and limb use in development of the cortical motor map

Much of the CS tract originates in the primary motor cortex (Porter and Lemon, 1993). A characteristic feature of the motor cortex is the representation of body muscles, or the motor map. The cortical motor representation integrates subcortical and premotor control signals to access output circuits for controlling particular joints. While TMS has been used to study development of the CS projection to the cord in humans and monkeys, development of the motor representation has only been studied in the cat. The motor map is assessed using microstimulation, whereby a microelectrode is used to excite a small population of cortical neurons. The representation is first detected at 2 months in the cat (Bruce and Tatton, 1980; Chakrabarty and Martin, 2000). Prior to about 2 months, motor cortex stimulation does not evoke motor responses (Bruce and Tatton, 1980). During the following month, four changes in the motor representation occurred (Chakrabarty and Martin, 2000; Fig. 3.11). First, there is an increase in the percentage of sites from which stimulation evokes a motor response (Fig. 3.11). Second, there is a concomitant decrease in

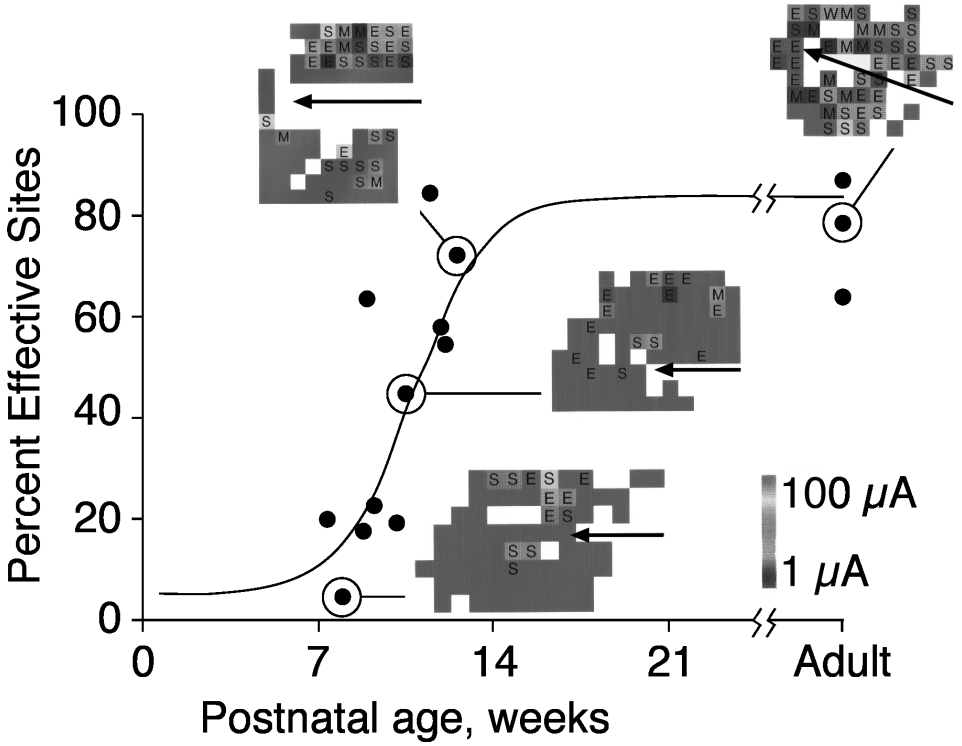

Fig. 3.11. For full color figure, see plate section. Development of the M1 motor map in the cat. The graph plots the percentage of sites in motor cortex from which electrical stimulation evoked a motor response. The color insets are maps from the arm area of motor cortex in representative animals at the ages indicated. The letters indicate the particular motor effect produced (see text for explanation). Arrows mark the location of a sulcus (cruciate) that separates the rostral representation of the motor map from the caudal representation. Modified from Chakrabarty and Martin (2000).

the current threshold. This reduction can be seen on the maps of stimulation effects as a change from a preponderance of high-threshold (red) to low-threshold sites (blue). The maps are from the forelimb area of the cat motor cortex. The threshold reduction suggests more efficient transduction of electrical stimuli into muscle control signals. Third, there is an elaboration of the motor map, from initially representing only proximal muscles (Fig. 3.11; shoulder, "S" elbow; "E") to one that represents all forelimb joints (shoulder, elbow, wrist and multijoint 'M' effects, including the digits). Fourth, as animals get older there is a higher percentage of sites from which effects at multiple joints are produced. These multijoint sites could play a role in encoding interjoint synergies. The proximal to distal progression in motor map development is reminiscent to the proximal to distal control strategy of human infants during arm movement development (Berthier et al., 1999).

Many features of motor map development can be modified by early motor experiences. By promoting early experiences with prehension training, the electrical threshold for evoking responses decreased and the percentages of effective sites and multi-joint sites both increased (Martin et al., 2005). In contrast, preventing limb experience during development of the motor map results in an increase in the threshold for evoking responses and a decrease in the other measures. However, all of these features revert to control values several months after normal experience returns (Martin et al., 2005). This return back to control levels reflects cortical representational plasticity that persists throughout the animal's life (Kleim et al., 2003).

3.6. The integrity of the descending cortical projection after perinatal trauma correlates with distal motor skill

It is well known that, in humans, perinatal damage to the developing CS system has profound, long-term effects on skilled motor control (Porter and Lemon, 1993). Recently, Olivier and colleagues (Duque et al., 2003) studied the relation between performance and structure of corticofugal systems. They examined patients with congenital hemiplegia when they were between 8 and 19 years. Performance on a battery of motor and somatic sensory tests, as well as coordination between fingertip forces in the tests, were correlated with dysgenesis of corticofugal pathways in the midbrain, by measuring the symmetry of the cross-sectional area of the cerebral peduncles on magnetic resonance images. They found that all of the measures of manual and digital dexterity correlated significantly with peduncular cross-sectional area, including a measure of fingertip coordination (Fig. 3.12). Their findings show a remarkably precise relationship between an anatomic measure of CS organization and fine distal motor skill. Their findings suggest that the more corticofugal axons available the better off the patient is. It is attractive to think that the number of CS axons is key. This is plausible since the direct corticomotoneuronal connection is thought to be the basis for individuated finger movements in adult humans (Porter and Lemon, 1993).

3.7. Implications for therapeutic intervention by harnessing CS activity

Activity- and use-dependent development of the CS system assures that neural events and experience, at the time connections are being made, play an important role in shaping the circuits for controlling movement that the animal makes throughout life. However, activity- and use-dependence also creates a vulnerability to deviations from an optimal functional state of the motor systems. The clinical significance of activity and use (or experience) dependence is well-established for the visual system, where periods of monocular visual deprivation produced by cataracts or strabismus can lead to impairments in sight (Mitchell, 1991; Mitchell et al., 2003). Our work and that of in vitro studies (Takuma et al., 2002; Ohno et al., 2004) and even humans (Eyre et al., 2001) lead to a similar conclusion for the CS system and skilled movement control. There are many parallels between hemiplegic cerebral palsy in humans after perinatal trauma and CS developmental impairments in cats after activity or use blockade. It is plausible that reduced activity-dependent competition in surviving or damaged CS neurons contributes to some of the anomalous organization of the motor systems and motor dysfunction in cerebral palsy. It is also plausible that variance in postnatal motor experiences contributes to variance in normal motor skills in maturity. Certainly, when pushed to the limit in cultures that severely restrict motor experiences in babies, development of motor skills are significantly altered (Solomons and Solomons, 1975).

The importance of CS activity and early limb motor experience during development should be taken into account in attempting to ameliorate developmental motor impairments. We need to devise ways to ensure that the CS system on the damaged side remains competitive after brain injury. Constraint induced therapy may be just such a way (Echols et al., 2001; Charles and Gordon, 2005). Similar to what has been tried after adult stroke, children with spastic hemiplegia have their unimpaired (or less impaired) arm physically restrained, thereby forcing them to use their affected arm during daily activities. This approach holds promise as an important rehabilitation strategy because studies have shown

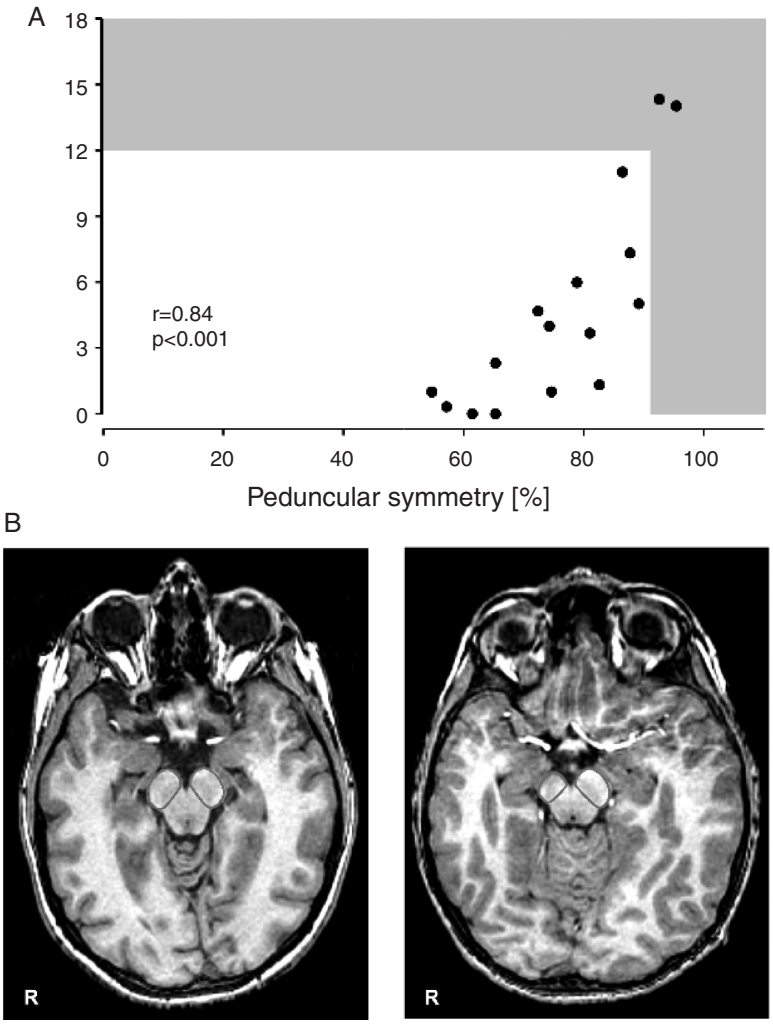

Fig. 3.12. For full color figure, see plate section. Effects of perinatal damage to the CS system on manual dexterity in maturity. **(A)** Graph showing the relationship between the symmetry of the cerebral peduncle measure and digit dexterity. The gray band is within normal limits. **(B)** Magnetic resonance images through the midbrain of a normal subject (left) and a subject with perinatal CS system damage (right). The red lines are drawn around the cerebral peduncles. Note that the two peduncles are the same size in the normal (**B**, left). By contrast, the right peduncle in the patient (**B**, right) is much smaller than the left peduncle. Images kindly provided by Dr E. Olivier. Modified from Duque et al. (2003).

that children engaged in constraint induced therapy show improvement in several performance assessments (Echols et al., 2001; Wolf et al., 2002; Gordon et al., 2005). It is plausible that this improvement occurs by making connections from the affected side stronger and more competitive than other inputs in maintaining synapses on spinal cord neurons. However, one possible disadvantage of this approach is that strengthening the damaged side occurs at the expense of the less impaired side. This concern derives from our animal studies showing permanent CS impairments and motor deficits after preventing postnatal limb use or motor cortex activity (Martin et al., 2004, 2005). This is also similar to the loss of visual capabilities in the unaffected eye after patching the other eye (Mitchell et al., 1984; Mitchell, 1991).

Whether or not there is a contraindication, constraint induced therapy is predicated on playing one side against the other and therefore requires that one side be more functional than the other. Many forms of perinatal damage to the motor systems produce bilateral impairments, which cannot benefit from constraint induced therapy. A biological-based therapy is indicated; one that promotes function of the damaged CS system independent of the functional state of the other side. One such approach is to augment the competitive advantage of damaged CS terminations using electrical stimulation, similar to rescuing transient terminations during development (Salimi and Martin, 2004). A biological, not training, based approach is especially needed for very early human development when babies cannot comply with the exercise regimens set by a therapist. TMS and deep brain stimulation, which are routinely used in the diagnosis and treatment of neurological disorders (Wassermann and Lisanby, 2001), could be applied to promoting CS system function perinatally.

Is CS system activity- and use-dependence unique to development or does the mature system share this dependency for maintaining spinal terminations and function? We have recently begun to investigate the importance of activity of the CS system in CS axon terminal morphology as well as in maintenance of the cortical motor map. Decreasing CS system activity in mature animals profoundly disrupts the organization of the cortical motor map (Chakrabarty and Martin, unpublished observations) and decreases the density of presynaptic sites in the spinal gray matter (Salimi et al., 2004; Friel and Martin, 2005). In contrast, augmenting activity by electrical stimulation results in more presynaptic sites in the spinal cord (Salimi et al., 2004). A reduction in presynaptic sites predicts a reduced capacity to activate spinal motor circuits and some degree of motor impairment if a sufficiently sensitive motor assessment is used. These effects can be accounted for by a reduction in the efficacy of cortical activation of spinal motor circuits. It is also likely that the activity reduction has significant effects on the strength of intracortical synapses (Kleim et al., 2003).

What about ameliorating the devastating motor signs of perinatal CS damage in adults, well after the topography of connections is set? Our finding that neural activity is necessary for maintaining CS axon terminal morphology and the cortical motor map in maturity does not imply that activity manipulations in maturity, after early postnatal insults, can reverse CS development defects. Indeed, animal studies indicate that without a requisite level of activity during development, or pattern of limb use, permanent changes occur in CS axon terminal morphology and topography (Martin, 2005). While stimulation might augment presynaptic sites in maturity, the effects are probably limited to the local regions of the spinal cord where the remaining CS axon branches are present. Failure to rebalance the pattern of CS projections is because of maturational changes both in the spinal gray matter as well as in CS neurons themselves that limit or even preclude long axon growth (Schwab et al., 1993). However, there is long-term optimism. Several pharmacological approaches are being explored to promote CS axon regeneration after spinal and brain injury in animals (David and Lacroix, 2003; Filbin, 2003) that could be applied to the CS system damaged during development, such as in cerebral palsy. For example, important regulation of axon growth depends on the cyclic nucleotide cAMP (Gao et al., 2003). While also important for a myriad of intracellular processes, the downstream targets of cAMP-mediated axon regeneration are being investigated (Gao et al., 2004) and could provide the means for promoting axon regeneration. Regeneration is not enough, however, because there is no assurance that the correct circuits will re-form. By combining localized delivery of a drug that is *permissive* for axon growth together with augmenting CS activity or motor training – which is *instructive* for forming the proper connections – intrinsic activity- and use-dependent mechanisms could be recruited to reestablish corticospinal motor circuits and restore motor function.

Acknowledgments

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Chapter 4

Molecular mechanisms of motor neuron degeneration in amyotrophic lateral sclerosis

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4.1. Introduction

Motor neuron disease (MND), referred to in many countries as amyotrophic lateral sclerosis (ALS), is amongst the most common of adult-onset neurodegenerative diseases. There are several high incidence foci of disease, e.g. on the Western Pacific island of Guam and the Kii peninsula of Japan, but in most parts of the world the incidence of 1–2/100,000 population is fairly uniform. The life-time risk of developing ALS is of the order of 1 in 2000. Some epidemiological evidence indicates that the incidence of the disease is increasing, though undoubtedly the altered age structure of many human populations and better developed neurological clinical services are likely to contribute to this observation.

The clinical and pathological features of ALS/MND are described in detail in Chapters 13 and 5 respectively of this volume. The individual afflicted by ALS typically develops relentlessly progressive neuromuscular failure resulting from combined degeneration of both upper and lower motor neuron groups in the motor cortex, brainstem and spinal cord. Clinical variants of the disease may apparently affect only spinal lower motor neurons, upper motor neurons or motor neurons innervating the bulbar muscles at the onset of disease. However, with disease progression, the majority of patients will develop clinical signs of upper and lower motor dysfunction affecting both limb and bulbar musculature and will be classified as having the commonest ALS subtype of disease.

There is a tendency in ALS for the disease to start focally, with propagation of the disease process to contiguous groups of motor neurons. The rate of disease progression varies between individuals, but the average survival is only 2–3 years from symptom onset. The predominant cause of death is respiratory failure.

The mechanisms of motor neuron injury and cell death in ALS are complex and multifactorial. Several genetic mutations can set the scene for motor neuron degeneration in familial ALS (fALS), but much remains to be learned about the genetic and environmental factors underlying the commoner sporadic form of the disease. Most insights into the molecular mechanisms of motor neuron injury have arisen from study of the subtype of disease caused by SOD1 (Cu/Zn superoxide dismutase) mutations, but even in this circumscribed disease subtype there appears to be a complex interplay between multiple pathogenetic processes as described below. New evidence is emerging for the role of non-neuronal cells in the vicinity of motor neurons as contributors to cellular injury. Evidence has also accumulated that cell death of motor neurons is likely to occur by a caspase-dependent programmed cell death pathway resembling apoptosis.

This chapter reviews the current state of knowledge of the interacting molecular mechanisms considered important in contributing to motor neuron injury and cell death (summarized in Fig. 4.1), as well as insights into cell-specific factors which may underlie the selective vulnerability of motor neurons to the neurodegenerative process in ALS.

4.2. Genetics

The majority of ALS cases are sporadic, where the cause of disease is unknown, but 5–10% of cases are genetic. Familial ALS follows Mendelian genetics and can be either dominant or recessive, with several loci

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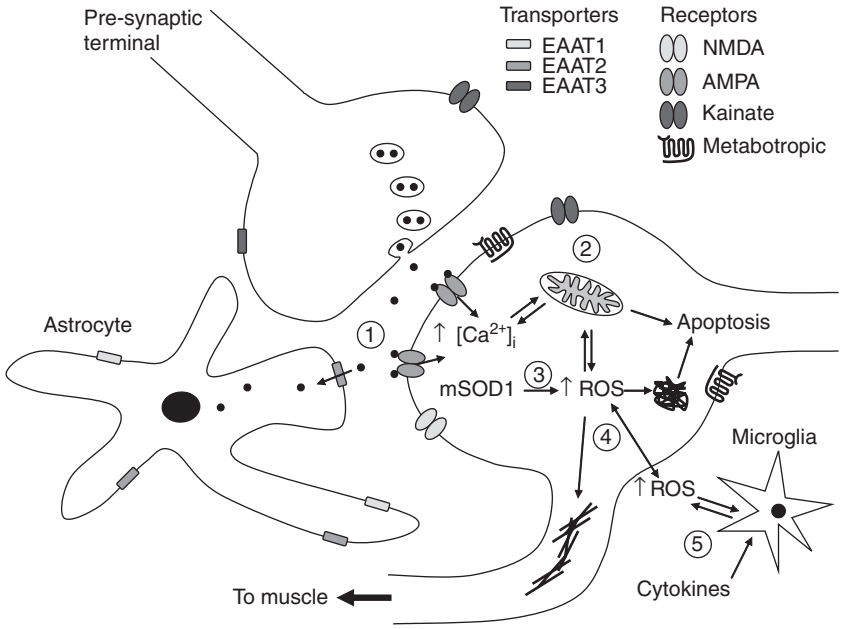

Fig. 4.1. Summary of mechanisms of neurodegeneration in ALS. (1) Glutamate release from pre-synaptic terminal activates AMPA receptors leading to increased intracellular calcium signaling. Altered AMPA receptor subunit expression can cause increased calcium ion permeability. Reduced glutamate re-uptake due to decreased transporter expression/activity exacerbates excitotoxicity. Low levels of CaBP and reduced mitochondrial sequestration of Ca^{2+} results in increased Ca^{2+} signaling. (2) Mitochondrial dysfunction triggering increased ROS generation, decreased calcium buffering and apoptosis. (3) Increased ROS production from mutant SOD1 chemistry and/or mitochondrial dysfunction, causing oxidation of protein, lipid and DNA. (4) Protein aggregation and neurofilament changes. (5) Microglial activation by cytokines and ROS further increase ROS production.

having been identified for both modes of inheritance. Dominant fALS is usually clinically and pathologically indistinguishable from sporadic ALS (sALS), although large studies have led to identification of variation in the timing of disease onset and progression (Cudkowicz et al., 1997; Camu et al., 1999). In contrast, recessive fALS cases, which are rarer than dominant fALS, often have juvenile onset and slow progression. Genetic linkage analysis in fALS cases is not easy since the disease does not usually present before the 4th–5th decade and disease progression is often rapid. Despite this, familial cases have been linked to several distinct loci in the human genome (Table 4.1). The genes responsible for disease have been identified for some of these loci, giving insights into disease pathogenesis. Other genes that by themselves do not cause disease, but are risk factors, have also been identified. The diversity of genes involved in fALS illustrates the complexity of the disease and it is hoped will provide vital clues for new therapeutic strategies.

4.2.1. ALS1: Copper, zinc superoxide dismutase 1 (SOD1)

A major breakthrough in ALS research came with the identification that mutations in copper, zinc superoxide dismutase 1 (SOD1) account for ~20% of familial cases (Rosen et al., 1993). This finding was exciting since the major function of SOD1 is in antioxidant defense and oxidative stress is believed to play a role in ALS pathogenesis (see oxidative stress section), but also surprising because SOD1 is ubiquitously expressed, whereas ALS targets motor neurons relatively selectively. Why motor neurons are particularly vulnerable to injury in the presence of SOD1 mutations is still not known. SOD1 removes toxic superoxide radicals in a two-step redox reaction involving a copper ion at the active site and produces oxygen and hydrogen peroxide (see oxidative stress section). More than 100 different SOD1 mutations leading to ALS have now been identified (http://alsod1.iop.kcl.ac.uk/) and these are spread throughout all five exons, as well as a small number in untranslated regions (Orrell, 2000). The majority of mutations are mis-sense mutations, where one amino acid is substituted for another. There are also a small number of insertions or deletions, which shift the reading frame, thereby changing the downstream amino acid sequence and often giving rise to a premature stop codon. An early attempt to screen SOD1 mutations found that the A4V mutation in exon 1, which is involved in β barrel and dimer formation, is the most common mutation (Deng et al., 1993). The other 11 mutations identified in the study were also

Table 4.1

Genes linked to motor neuron disorders. Continually updated information on MND-associated mutations is available at *http://alsod1.iop.kcl.ac.uk/*

Disease subtype	OMIM number		Locus	Gene	Disease features	References
	Disease	Gene				
ALS1	105400	147450	21q22.1	*SOD1*	Dominant (except D90A), adult onset	Siddique et al. (1991); Rosen et al. (1993)
ALS2	205100	606352	2q33	*ALS2/alsin*	Recessive, juvenile onset; Slow progression; Some patients have a pure upper motor neuron phenotype; RFALS type 3	Hadano et al. (2001); Yang et al. (2001)
ALS3	606640		18q21	Unknown	Dominant, adult onset	Hand et al. (2002)
ALS4	602433	608465	9q34	*senataxin*	Dominant, juvenile onset; Slow progression, normal life span	Chance et al. (1998); Blair et al. (2000)
ALS5	602099		15q15.1-q21.1	Unknown	Recessive, juvenile onset; Most common recessive fALS; RFALS type 1	Hentati et al. (1998)
ALS6	608030		16q12.1-2	Unknown	Dominant, adult onset	Abalkhail et al. (2003); Ruddy et al. (2003); Sapp et al. (2003)
ALS7	608031		20ptel	Unknown	Dominant, adult onset	Sapp et al. (2003)
ALS8	608627	605704	20q13.33	*VAPB*	Dominant, adult onset; Varied clinical presentation, often slow progression	Nishimura et al. (2004b)
ALS-FTD	105550		9q21-q22	Unknown	Dominant, adult onset	Hosler et al. (2000)
ALS with parkinsonism and dementia	600274	157140	17q21	*tau*	Dominant, adult onset	Majoor-Krakauer et al. (1994); Clark et al. (1998); Hutton et al. (1998)
X-linked ALS			Xp11-q12	Unknown	Dominant, adult onset	Siddique et al. (1998a)
Progressive LMN disease	601143	607641	2p13	Dynactin p150	Dominant, adult onset; Slow progression; Affects lower MN	Puls et al. (2003)

predicted to affect β strand formation rather than the active site, leading the authors to suggest that mutant SOD1 would be structurally defective and have less activity rather than being completely inactive or more active. Exon 3, which encodes the active site, was initially thought to be protected from mutations, but a few exon 3 mutations have since been reported. A particularly intriguing mutation is the D90A mutation, found on the periphery of the SOD1 molecule, which was originally reported as a recessive mutation (Andersen et al., 1995, 1996), but has subsequently been shown to be dominantly inherited in other pedigrees (Robberecht et al., 1996; Andersen et al., 1997). This apparent discrepancy led to a world-wide study of D90A pedigrees, which showed all 20 recessive D90A pedigrees studied shared a common ancestor, whereas the eight dominant D90A families arose from several unrelated ancestors (Al-Chalabi et al., 1998). This led the authors to suggest that D90A is a dominant mutation but in the recessive pedigrees there is a tightly linked protective factor that reduces the toxicity of the mutation. Mutations in SOD1 are also responsible for a small proportion of apparently sporadic ALS cases (Jones et al., 1993; Jackson et al., 1997), although sometimes the mutation may have low penetrance and may appear to skip several generations, making the diagnosis of familial ALS difficult (Orrell et al., 1996).

Although SOD1 mutations are only responsible for ~2% of all ALS cases, much attention has been focused on SOD1 because it provides an important tool that allowed development of animal and cell models. Transgenic mice carrying a variety of human SOD1 mutations develop motor neuron degeneration that is clinically and pathologically similar to human ALS (Gurney et al., 1994; Ripps et al., 1995; Wong et al., 1995; Bruijn et al., 1997b). Curiously, transgenic mice overexpressing mutant SOD1 in only neurons (Pramatarova et al., 2001; Lino et al., 2002) or astrocytes (Gong et al., 2000) do not develop ALS, suggesting a complex interplay between the two cell types in disease pathogenesis. In mouse chimeras produced by injecting wild-type embryonic stem cells into SOD1 mutant blastocysts, non-neuronal cells expressing only endogenous SOD1 delayed degeneration of mutant SOD1 expressing motor neurons, and motor neurons expressing endogenous SOD1 develop ALS pathology when adjacent to mutant SOD1 expressing non-neuronal cells, indicating that non-neuronal cells are likely to play a role in ALS pathogenesis (Clement et al., 2003). Development of cell models expressing human SOD1 mutations (Pasinelli et al., 1998; Roy et al., 1998; Cookson et al., 2002) has enabled molecular and cellular biological investigation of how SOD1 mutations cause disease. Together, they have helped to show that SOD1-mediated ALS is due at least in part to gain of a toxic function of SOD1. How this causes disease is still not fully understood, but is thought to involve oxidative stress, mitochondrial dysfunction, protein aggregation, excitotoxicity and inflammation. These mechanisms of motor neuron injury are discussed in later sections.

4.2.2. ALS2: Alsin

Recently, other genes associated with fALS have been identified. A rare, recessively inherited juvenile onset form of ALS with slow disease progression has been shown to be caused by mutations in the *ALS2/alsin* gene, located on chromosome 2 (Hadano et al., 2001; Yang et al., 2001). In addition, mutations in *ALS2* also cause juvenile onset forms of primary lateral sclerosis and hereditary spastic paraplegia (Yang et al., 2001). Most of the known mutations are small deletions that disrupt the reading frame, resulting in truncated proteins. Recently, a nonsense mutation has also been identified, although this introduces a premature stop codon, so it still results in production of a truncated protein (Devon et al., 2003). These truncated proteins are rapidly degraded by the proteasome (Yamanaka et al., 2003), suggesting that disease is caused by loss of alsin function. The *ALS2/alsin* gene encodes two mRNA transcripts that are widely expressed in human tissues, including the brain and spinal cord, with the long form being more abundant in most tissues (Hadano et al., 2001). Like mutant alsin, the short form of alsin is also rapidly degraded by the proteasome (Yamanaka et al., 2003), so it is probable that only the long form is functional.

The precise function of alsin is not known, although evidence is growing for a role in endosomal trafficking. The long form of alsin contains three putative guanine-nucleotide exchange factor (GEF) domains, for Ran, Rho and Rab5 GTPases respectively, as well as multiple membrane occupation and recognition nexus (MORN) repeats, which are often associated with phosphatidyl inositol signaling proteins. GEFs are responsible for activation of small GTPases by causing the GTPase to release GDP in exchange for GTP. Rab5 GTPases are involved in regulating vesicle trafficking in the endocytic pathway, and the Rab5 GEF domain of alsin is known to be functional (Otomo et al., 2003; Topp et al., 2004). Since all known disease-causing mutations lead to truncation and loss of the C terminal Rab5 domain, this activity is probably important in alsin function. The Rho GEF domain is also enzymatically active and selectively triggers GDP release from Rac1 (Topp et al., 2004). To date, the Ran GEF has not been shown to be functional but may instead be important for protein–protein interactions. One study suggested that Ran GEF negatively regulates association with the endosome

(Otomo et al., 2003), whilst another reported that it is necessary for recruitment to the endosome membrane (Yamanaka et al., 2003). How loss of alsin leads to development of a variety of motor neuron disorders is not understood, but several hypotheses have been proposed. Most of these focus on disruption of actin dynamics and endosomal trafficking. For example, if alsin is involved in glutamate receptor endocytosis, a loss of alsin activity could cause glutamate excitotoxicity (Topp et al., 2004). Another recent study showed an unexpected interaction between alsin and mutant SOD1, but not wild-type SOD1, where the Rho GEF domain of alsin was capable of rescuing mutant SOD1 phenotypes in a motor neuron cell line (Kanekura et al., 2004). Further analysis showed that this protection is mediated via interaction between the Rho-GEF domain of alsin and Rac1, which leads to activation of the PI-3-Kinase/Akt pathway (Kanekura et al., 2004).

4.2.3. ALS4: Senataxin

A juvenile onset autosomal dominant form of ALS which affects upper and lower motor neurons but has a slow rate of progression and does not affect lifespan has been designated as ALS4. Genetic linkage analysis of a single large pedigree found that ALS4 mapped to 9q34 (Chance et al., 1998) and was subsequently found to be caused by mutations in the *Senataxin* gene, *SETX* (Chen et al., 2004). The *SETX* gene encodes a 302.8 kDa protein of unknown function that shows a wide tissue distribution including brain and spinal cord. The C terminal of the SETX protein contains a superfamily I DNA/RNA helicase domain that is well conserved in rat and mouse orthologues, but the remainder of the protein contains no other recognizable motifs. DNA/RNA helicases are involved in replication, recombination and repair of DNA, transcription, RNA processing and initiation of translation. However, only one of the three disease-causing mutations so far identified is within the helicase domain. Since little is known about the function of SETX, it is difficult to predict how mutations cause disease. Curiously, loss-of-function mutations in *SETX* have been shown to lead to an unrelated disorder, ataxia-oculomotor apraxia type 2 (AOA2), with a recessive inheritance (Moreira et al., 2004). It has been proposed that loss of function mutations may lead to recessive AOA2, whereas either a partial loss of function or gain of toxic function mutation may cause dominant ALS4 (Chen et al., 2004).

4.2.4. ALS5 (RFALS Type 1)

The most prevalent form of recessive fALS, initially named recessive familial ALS type 1 (RFALS Type 1), was mapped to 15q15.1-q21.1 in four families by Hentati et al. (1998) and the locus has been named ALS5. Three further RFALS families showed no linkage to chromosome 15. Three of the four 15q linked families showed juvenile onset and slow progression of symptoms, typical of RFALS, whereas the fourth family showed unusually rapid disease progression. Genetic heterogeneity at the ALS5 locus suggests that there may be more than one mutation, so the differences in disease progression may be due to different mutations. The gene(s) affected in ALS5 have not yet been identified, although candidate genes within the genetic locus include microtubule associated protein 1A (MAP1A) and inositol 1,4,5-triphosphate 3-kinase A (Hentati et al., 1998).

4.2.5. ALS8: Vesicle-associated membrane protein/ synaptobrevin-associated membrane protein B

Recently, a large Brazilian Caucasian family with an atypical form of ALS showing onset earlier than classical ALS and slow progression that had no linkage to known fALS loci was reported by Nishimura et al. (2004a). They mapped the locus of this new ALS subtype, ALS8, to 20q13.3. Subsequent analysis of a further six families, which are believed to share a common ancestor, showed the same mutation but presented with differing clinical features from typical rapidly progressing ALS, atypical ALS and late onset spinal muscular atrophy (SMA) (Nishimura et al., 2004b). This heterogeneity suggests that other factor(s), either environmental or genetic, combine to affect the clinical progression of disease. However, since the mutation was not present in unaffected family members or unrelated normal controls, it is sufficient to cause disease. The mutation was identified as substitution of proline for serine at codon 56 (P56S) of vesicle-associated membrane protein/synaptobrevin-associated membrane protein B (VAPB). This mutation is predicted to remove a kink between two regions of β-strand, disrupt the hydrogen bonding pattern between the β-sheets and make the protein more flexible and capable of forming a new conformation. Wild-type VAPB is predominantly localized to the endoplasmic reticulum, whereas P56S-VAPB does not co-localize with endoplasmic reticulum or Golgi markers, but is found in intracellular aggregates (Nishimura et al., 2004b).

4.2.6. Other autosomal dominant fALS loci

After linkage of a subset of fALS cases to chromosome 21 (Siddique et al., 1991), there has been much effort in screening adult onset autosomal dominant fALS not linked to chromosome 21 or SOD1 to identify other

genes that cause ALS. These studies have revealed disease associated loci within chromosomes 16, 18 and 20. A genome wide analysis of a large European pedigree showed that a familial form of disease, which presented as classical ALS with no atypical features, was linked to 18q21 (ALS3) (Hand et al., 2002). Three separate studies have linked three adult onset autosomal dominant fALS pedigrees to 16q12 (ALS6) (Abalkhail et al., 2003; Ruddy et al., 2003; Sapp et al., 2003). Another adult onset autosomal dominant fALS pedigree has been linked to 20ptel-p13 (ALS7) (Sapp et al., 2003). A deletion in the intermediate filament protein peripherin, leading to production of a truncated protein that disrupts neurofilament assembly, has recently been identified in one sALS patient (Gros-Louis et al., 2004).

4.2.7. ALS with dementia and/or Parkinsonism

In a small proportion of cases, ALS occurs in conjunction with other neurodegenerative features, such as dementia or Parkinsonism. As with pure ALS, these disorders, which span the traditional disease classifications, can be genetic. ALS with fronto-temporal dementia (ALS-FTD), characterized by behavioral abnormalities and personality changes which precede dementia, in addition to the motor symptoms, has been linked to 9q21-q22 (Hosler et al., 2000). ALS with dementia and Parkinsonism (ALS-PD) was found with high incidence on the Western Pacific island of Guam in the 1960s (OMIM 105500) and, after several attempts to determine a genetic component, it was concluded that the cause was environmental rather than genetic (Blake et al., 1983; Garruto et al., 1983). This view has increased with the observed decrease in the incidence of disease in recent years, coinciding with increased travel between Guam and the rest of the world (Garruto et al., 1985; Plato et al., 2003). However, in a study of non-Guamanian families with ALS-PD, it was found that ALS, dementia and PD occur together within families more frequently than expected by chance, suggesting a genetic component (Majoor-Krakauer et al., 1994). Hereditary disorders causing FTD and Parkinsonism have been linked to mutations in Tau on chromosome 17q21-22 (Clark et al., 1998; Hutton et al., 1998). However, some patients with fALS, dementia and Parkinsonism do not carry mutations in Tau (Wilhelmsen et al., 2004), indicating that other loci may also be involved.

4.2.8. X-linked ALS

Males appear to be more susceptible to ALS than females, with an approximate ratio of 2:1, suggesting that there may be a sex-linked factor involved. Garofalo et al. (1993) investigated whether variation in the number of CAG trinucleotide repeats in the androgen receptor gene, which maps to Xq11-12 and is involved in the progressive motor neuron degeneration seen in Kennedy's disease, can also lead to ALS. Although this study found only borderline significance for involvement of the androgen receptor, another study has also found evidence of X-linked inheritance (Siddique et al., 1998a). However, the significance of these findings is difficult to determine since a full report of this pedigree has not been published and Dejager et al. (2002) have highlighted the difficulty in distinguishing Kennedy's disease from ALS.

4.2.9. Dynactin

An early adult-onset, slow progressing form of motor neuron disease that affects lower motor neurons only has been linked to a mutation in the p150 subunit of the transporter protein dynactin in one family (Puls et al., 2003). Dynactin is involved in dynein-mediated retrograde transport of vesicles and organelles along microtubules. The mutation is a single base change causing an amino acid substitution from glycine to serine at position 59 (G59S) of the p150 subunit. This mutation occurs in a highly conserved region of the protein, involved in binding to microtubules, although Gly59 is not thought to be directly involved in binding. It is proposed that the larger serine side-chain causes steric hindrance and disrupts the folding of the microtubule binding domain and the mutant p150 dynactin subunit shows reduced binding to microtubules compared to the wild-type protein. A subsequent study which screened the p150 subunit of dynactin revealed three missense mutations in ALS patients, which are absent in non-neurologic controls (Munch et al., 2004). However, unlike the original patients with the G59S mutation, these patients all showed upper motor neuron involvement and differing disease progression. Inactivation of the dynactin complex by overexpression of the dynamitin (p50) subunit has previously been shown to inhibit retrograde axonal transport and cause late onset motor neuron degeneration in transgenic mice (LaMonte et al., 2002).

4.2.10. Genetic risk factors for ALS

The variability seen in clinical presentation, age of onset and survival time seen in ALS cases, even amongst familial cases with a defined genetic mutation, suggests there is a complex involvement of environmental and/or genetic risk factors that affect both susceptibility and progression of disease. Some of the genetic risk factors suggested to be involved are discussed below and are summarized in Table 4.2.

Table 4.2

Genetic risk factors associated with ALS

Gene	Mutation	Phenotype	Reference
Neurofilament heavy subunit, NEFH	Insertion/deletion of KSP repeats	~1% of sALS cases	Figlewicz et al. (1994); Tomkins et al. (1998); Al-Chalabi et al. (1999)
GluR2	Altered RNA editing		Kawahara et al. (2004)
Vascular endothelial growth factor, VEGF	Polymorphisms in 5′ untranslated region	Increased risk of ALS	Oosthuyse et al. (2001); Lambrechts et al. (2003)
Ciliary neurotrophic factor, CNTF	Homozygous null mutation	Early onset of SOD1-ALS	Giess et al. (2002)
Survival motor neuron-1 and 2, SMN1 and SMN2	Reduced copy number	Lower copy number correlated with shorter survival times	Veldink et al. (2001); Corcia et al. (2002)
Apolipoprotein E ε4	Presence of allele	Shorter survival/bulbar onset	Al-Chalabi et al. (1996); Drory et al. (2001a)

4.2.10.1. Neurofilaments

Accumulation of aggregated neurofilaments is a common feature seen in ALS pathology and overexpression of either the neurofilament light- or heavy-subunit in transgenic mice leads to motor neuron degeneration (see cytoskeletal dysfunction and axonal transport section) suggesting a role for neurofilaments in ALS. Neurofilaments are ~10 nm intermediate filaments composed of light, medium and heavy subunits present in neuronal cells where they are important in determining axonal calibre and providing a framework for intracellular transport (Lee and Cleveland, 1996). The subunits have a central rod domain with globular head and tail regions. Several studies looking for neurofilament mutations in ALS have highlighted the importance of a repeated KSP sequence in the tail of the heavy neurofilament subunit (NF-H). An early study showed codon deletions in five sporadic ALS cases (Figlewicz et al., 1994) and further reports showed either insertion (Tomkins et al., 1998) or deletion (Figlewicz et al., 1994; Al-Chalabi et al., 1999) of complete KSP repeat motifs in a small number of cases, representing approximately 1% of ALS. Most NF-H mutations are seen in sALS cases, suggesting that neurofilament alterations are risk factors for disease.

4.2.10.2. Glutamate handling

A body of circumstantial evidence suggest a role for glutamate toxicity in ALS pathogenesis, with multiple reports describing reduced glutamate transport in motor cortex and spinal cord from ALS patients (Rothstein et al., 1992; Shaw et al., 1994) and decreased expression of the glial-specific glutamate transporter EAAT2 (GLT-1) (Rothstein et al., 1995). Disease-specific aberrant splicing of the EAAT2 mRNA transcript leading to loss of expression was initially suggested (Lin et al., 1998), but further studies showed no significant difference in splice variants between ALS and control patients (Meyer et al., 1999; Flowers et al., 2001). Recent work by Kawahara et al. (2004) has also investigated RNA processing mechanisms. The mRNA transcript of the GluR2 subunit of the glutamate AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptor usually undergoes RNA editing to change a glutamine to an arginine, with virtually 100% efficiency. This editing decreases calcium ion permeability through the receptor. A sub-population of motor neurons in ALS patients has recently been shown to have reduced GluR2 editing efficiency compared to Purkinje cells or motor neurons in controls, a change that would be predicted to result in AMPA receptors with increased calcium permeability (Kawahara et al., 2004).

4.2.10.3. Growth factors

Vascular endothelial growth factor (VEGF) was first described and named for its role in angiogenesis, but has subsequently been shown to act as a neurotrophic factor (Brockington et al., 2004; Van Den Bosch et al., 2004). Expression of VEGF increases during hypoxia, due to binding of hypoxia-inducible factors to a hypoxia-response element (HRE) in the VEGF promoter. Deletion of the VEGF allele in mice is embryonic lethal, but deletion of the HRE in the promoter leads to adult onset motor neuron degeneration reminiscent of ALS

(Oosthuyse et al., 2001). Screening of the HRE revealed no sequence alterations in ALS cases from four European populations, but single nucleotide polymorphisms in the 5′ untranslated region previously reported to correlate with reduced VEGF expression were found to be over-represented in ALS cases and conferred an increased risk of ALS of 1.8-fold (Lambrechts et al., 2003). Both disease and control individuals carrying the 'at risk' polymorphisms were found to have lower VEGF levels in serum compared to those without the polymorphisms. However, one of the four populations studied and another independent report (Brockington et al., 2005) failed to show this association between the 'at risk' polymorphisms and ALS, suggesting greater complexity of the effect of VEGF as a risk factor. Injection of a lentiviral vector expressing VEGF into various muscles with retrograde transport to spinal motor neurons has recently been reported to significantly delay onset and slow progression of disease in G93A-SOD1 mice, even when the injection is given after onset of symptoms (Azzouz et al., 2004). Intracerebroventricular delivery of recombinant VEGF protein into G93A-SOD1 rats has also recently been shown to significantly extend survival when given either before or at the time of onset of symptoms (Storkebaum et al., 2005).

A homozygous mutation in a splice site of ciliary neurotrophic factor (CNTF), present among ~2% of European and Japanese populations, results in production of a biologically inactive protein that causes a 15–20% reduction in motor neuron number, but does not itself cause disease. One individual in a family carrying the V148G-SOD1 mutation developed disease at an unusually early age and subsequent analysis showed this patient to be the only family member to carry the V148G-SOD1 mutation and have the homozygous null mutation in CNTF (Giess et al., 2002). Analysis of *G93A-SOD1/CNTF*$^{-/-}$ mice also showed significantly earlier onset of disease compared to *G93A-SOD1 CNTF*$^{+/+}$ mice, suggesting that the homozygous null CNTF mutation may be a risk factor for early onset of disease.

4.2.10.4. Survival motor neuron genes

Survival motor neuron genes 1 and 2 (*SMN1* and *SMN2*) are highly conserved paralogous genes present on chromosome 5, both of which are often present with more than one copy. Typically, there are two copies of SMN1 and one or two copies of SMN2 (Corcia et al., 2002). Deletion of *SMN1* copies causes recessive spinal muscular atrophy, but the disease phenotype is determined by the copy number of *SMN2*, with a milder phenotype correlating to more copies (Gavrilov et al., 1998). Various studies have investigated whether differences in *SMN* copy number are also associated with ALS. One study showed that *SMN2* gene deletions were approximately four times more common in sALS patients than in controls and that survival times were shorter in patients with homozygous *SMN2* deletions compared to patients without any deletions (Veldink et al., 2001). Conversely, a subsequent study found no differences in *SMN2* copy number between ALS and control groups, whereas the proportion of ALS patients with either one or three copies of *SMN1* was significantly increased compared to controls (Corcia et al., 2002). Further work is needed to clarify the roles of SMN1 and SMN2 as genetic modifying factors in ALS.

4.2.10.5. Apolipoprotein E

Apolipoprotein E ε4 allele is a risk factor in various neurodegenerative diseases, including Alzheimer's disease, where it is associated with earlier onset. Investigations into the role of ε4 as a risk factor in ALS have produced conflicting results. Two studies showed there was no significant difference in the frequency of the ε4 allele in control and ALS populations, although they found different effects of the allele (Al-Chalabi et al., 1996; Drory et al., 2001a). Drory et al. (2001a) found that the ε4 allele correlated with a shorter survival time, whereas Al Chalabi et al. (1996) reported that in the ALS population there was significant correlation between carrying the ε4 allele and bulbar onset disease. These differing results make it difficult to interpret the influence of Apo E ε4 on ALS, especially when combined with other studies that failed to show any association (Siddique et al., 1998b).

4.3. Oxidative stress

Reactive oxygen species (ROS), such as superoxide and hydroxyl radicals, hydrogen peroxide and peroxynitrite, are generated as by-products of aerobic metabolism. If allowed to persist, these molecules will disrupt the normal reducing conditions within the cell, causing oxidative damage to proteins, lipids and DNA. This damage is collectively called oxidative stress. Because these ROS produced during normal aerobic metabolism are potentially capable of causing oxidative stress, there are various 'housekeeping' or homeostatic mechanisms to defend against them. The cell can attempt to avoid production of free radicals, attempt to scavenge them and convert them into less toxic species or remove the oxidized products. The CNS uses a large amount of oxygen, has a high lipid content and fewer antioxidant enzymes compared to other tissues, making it particularly vulnerable to oxidative stress (Coyle and Puttfarcken, 1993).

There are now numerous reports showing oxidative stress in ALS pathogenesis. Together, they show elevated

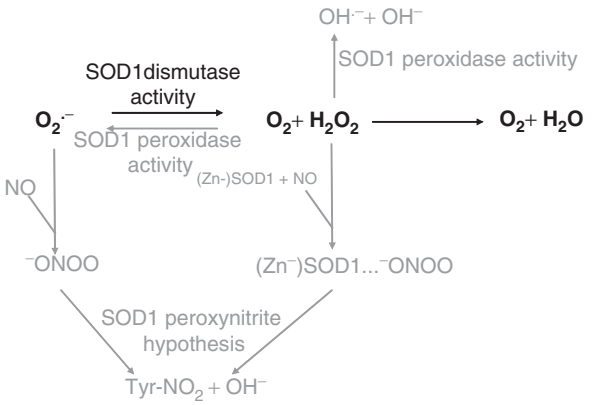

Fig. 4.2. Summary of SOD1 activity. Beneficial dismutase activity is shown in black. Other reactions that increase oxidative stress are shown in gray.

levels of free radicals and oxidative damage to protein, lipid and DNA in human ALS spinal cord (Shaw et al., 1995b; Fitzmaurice et al., 1996; Ferrante et al., 1997; Shibata et al., 2001). The initial discovery of genetic linkage to the SOD1 locus was therefore exciting, since the primary role of SOD1 is in antioxidant defense. SOD1 catalyses dismutation of superoxide radicals ($O_2^{\cdot -}$) to hydrogen peroxide and oxygen in a two-step redox reaction involving reduction and re-oxidization of the copper ion in its active site (Fig. 4.2 and Table 4.3) (Fridovich, 1975). Each monomer in the 32 kDa SOD1 homodimer has a cave-like active site containing one copper ion and one zinc ion. The channel leading to the active site is lined with highly conserved positively charged residues, which helps guide the superoxide anions to the active site (Cudd and Fridovich, 1982). The channel narrows down and the active site is small, enabling the small, negatively charged $O_2^{\cdot -}$ to enter and lie very close to the copper atom, but excluding molecules of larger size. The subsequent dismutation can occur rapidly (Klug et al., 1972). H_2O_2 is not attracted to these cations and is therefore free to leave the active channel and is subsequently broken down to oxygen and water by glutathione peroxidase or catalase.

How do mutations in SOD1 lead to selective motor neuron death in fALS? Transgenic mouse models of ALS carrying human ALS-linked SOD1 mutations support the human studies showing increased oxidative damage to protein, lipid and DNA (Ferrante et al., 1997; Andrus et al., 1998; Liu et al., 1998, 1999). Mutation of SOD1 causing unstable or mis-folded enzyme with reduced dismutase activity initially seemed an attractive hypothesis for the increased oxidative stress seen in ALS (Beckman et al., 1993; Deng et al., 1993), since SOD1 activity in fALS patient red blood cell lysates was shown to be less than 50% of control levels in two separate studies (Deng et al., 1993; Robberecht et al., 1994) and SOD1 activity is reduced in fALS cortical tissue (Bowling et al., 1993). However, different fALS-linked human SOD1 mutations have different levels of enzyme activity (with some, such as the G37R mutant, retaining full dismutase activity (Borchelt et al., 1994)) and no deletions of SOD1 have been found in fALS cases. Several transgenic mouse models expressing ALS-linked SOD1 mutations show that disease is not caused by a loss of dismutase activity (Gurney et al., 1994; Wong et al., 1995; Bruijn et al., 1997b). Similarly, one study has shown that increasing or decreasing levels of wild-type SOD1 have no effect on mutant SOD1-related ALS (Bruijn et al., 1998) and a SOD1 knock-out mouse does not develop ALS (Reaume et al., 1996).

Table 4.3

SOD1 chemistry

Type of reaction	Mechanism			Reference
Dismutation	$SOD\text{-}Cu^{2+} + O_2^-$	$\rightarrow$	$SOD\text{-}Cu^{+} + O_2$	Fridovich (1975)
	$2H^+ + O_2^- + SOD\text{-}Cu^{+}$	$\rightarrow$	$H_2O_2 + SOD\text{-}Cu^{2+}$	
	Net reaction:	$\xrightarrow{SOD1}$		
	$2H^+ + 2O_2^-$	$\rightarrow$	$H_2O_2 + O_2$	
Peroxidase	$SOD\text{-}Cu^{+} + H_2O_2$	$\rightarrow$	$SOD\text{-}Cu^{2+}\text{-}{}^{\bullet}OH + OH^-$	Wiedau-Pazos et al. (1996)
	$SOD\text{-}Cu^{2+}\text{-}{}^{\bullet}OH + HCO_2^-$	$\rightarrow$	$SOD\text{-}Cu^{2+} + CO_2^- + H_2O$	
Peroxynitrite	$O_2^- + NO^{\bullet}$	$\rightarrow$	^-ONOO	Beckman et al. (1993)
	$^-ONOO + SOD\text{-}Cu^{2+}$	$\rightarrow$	$SOD\text{-}CuO\ldots NO_2^+$	
	$^-OH + NO_2\text{-tyr-protein}$	$\leftarrow$	H-tyr-protein	
Peroxynitrite, Zn deficient SOD1	$(Zn^-)SOD\text{-}Cu^{+} + O_2$	$\rightarrow$	$(Zn^-)SOD\text{-}Cu^{2+}\ldots O_2^-$	Estevez et al. (1999)
	$(Zn^-)SOD\text{-}Cu^{2+}\ldots O_2{}^{\cdot -} + {}^{\bullet}NO$	$\rightarrow$	$SOD\text{-}Cu^{2+}\ldots OONO^- \leftrightarrow SOD\text{-}Cu^{2+}\ {}^-ONOO$	

These data suggest that in SOD1-linked fALS it is not a loss of normal SOD1 activity that causes disease, but rather that mutant SOD1 gains a toxic function.

A proposed mechanism for this toxic gain of function was that mutations in SOD1 may lead to a more open conformation, allowing other aberrant substrates to enter the active site and react with the copper ion. Two such hypotheses have been proposed: (i) peroxidase reaction and (ii) peroxynitrite involvement. SOD1 reactions are shown in Table 4.3.

4.3.1. Peroxidase reaction

In addition to its dismutase activity, SOD1 also has a peroxidase activity, where it either catalyses the reverse reaction of the dismutase reaction or uses the hydrogen peroxide produced in the dismutation as a substrate to produce hydroxyl radicals through the Fenton reaction (Yim et al., 1990, 1993). When performed in isolation, this reaction quickly slows as the SOD1 becomes inactivated. However, other small anions, such as formate or glutamate, are also able to bind to the positively charged active channel, and some of these scavenge the highly reactive hydroxyl radicals to produce more stable radicals that are able to leave the active channel (Yim et al., 1993). In this way, the peroxidase activity of SOD1 can continue (Yim et al., 1993). This free-radical generating activity has been implicated in ALS; although the dismutase activity of the G93A mutant of SOD1 is equivalent to wild-type SOD1 (Gurney et al., 1994), the free radical-generating activity of G93A-SOD1 is greater (Yim et al., 1996). Significantly lower levels of superoxide radicals and higher levels of hydrogen peroxide and hydroxyl radicals have been reported in G93A-SOD1 mice compared to normal mice and mice expressing wild-type SOD1 (Liu et al., 1999). Similarly, membrane lipid peroxidation and oxidation of protein and DNA were higher in the G93A mice (Liu et al., 1999). A4V-SOD1 produces detectably higher free radical levels than G93A and the use of Cu^{2+} chelators in neural cells expressing mutant SOD1 helps to rescue cells from serum withdrawal-induced apoptosis (Wiedau-Pazos et al., 1996). However, these findings have been questioned since a separate study failed to see a difference in hydroxyl radical production between normal and A4V/G93A mutant SOD1 (Singh et al., 1998).

4.3.2. Peroxynitrite hypothesis

There are two components to the peroxynitrite hypothesis. First, superoxide can react spontaneously with nitric oxide to produce peroxynitrite, which can be used by SOD1 to cause nitration of tyrosine residues within proteins (Beckman et al., 1993). Second, several ALS-linked SOD1 mutations show reduced zinc binding and altered geometry, which allows reducing agents other than superoxide (for example ascorbate and glutathione) to react rapidly with the oxidized Cu^{2+} at the active site (Lyons et al., 1996; Crow et al., 1997). The reduced SOD1-Cu^{+} is then able to catalyse the reverse of its usual dismutase reaction and actually produce superoxide. The superoxide can then react with nitric oxide that diffuses into the active site to produce peroxynitrite (Estevez et al., 1999). This mechanism is therefore restricted to SOD1 mutations which retain copper binding, but are zinc depleted.

The relevance of peroxynitrite involvement in SOD1 toxicity is controversial. The requirement for nitric oxide (NO) suggests that inhibiting neuronal NO synthase should reduce mutant SOD1 toxicity, but this was found not to be the case (Facchinetti et al., 1999; Upton-Rice et al., 1999). Higher levels of free 3-nitrotyrosine have been reported in G37R- and G93A-SOD1 transgenic mice compared to mice carrying human wild-type SOD1 (Bruijn et al., 1997a; Ferrante et al., 1997), but no increases in nitrated neurofilaments have been found in G37R- or G85R-SOD1 mice (Bruijn et al., 1997a; Williamson et al., 2000). Similarly, 3-nitrotyrosine was seen to be elevated in both sALS and fALS in one study (Beal et al., 1997), but in another study no increases in protein-bound nitrotyrosine could be seen in sALS or A4V fALS cases compared to age-matched non-ALS controls (Bruijn et al., 1997a).

The relevance of copper-mediated oxidative stress to ALS pathology becomes further confused in light of work investigating the activity of copper-deficient SOD1. Subramaniam et al. (2002) showed that ablation of the copper chaperone for SOD1 (CCS), which is required for at least 90% of copper loading into SOD1, had no effect on disease onset, progression or pathology of G93A, G37R or G85R mutant mice. Increased motor neuron death within facial nuclei was observed after axotomy, reminiscent of *SOD1*$^{-/-}$ mice, suggesting that the CCS is required for normal SOD1 function, but CCS deficiency does not affect the course of disease. Additionally, a transgenic mouse carrying a SOD1 mutation in which copper binding is abolished by mutation of the four histidine residues that hold the copper ion in the active site develops a motor neuron disease that is clinically and pathologically similar to other mouse models of ALS (Wang et al., 2003). These findings cast doubt on the hypothesis that oxidative stress caused by aberrant copper chemistry is a common mechanism in ALS pathology.

With the understanding that we currently have of the role of oxidative stress in ALS pathology, it is perhaps not surprising that efforts to treat ALS in animal and cell models by reducing oxidative stress have had

mixed success. Various antioxidant drugs have been tested in cell and animal models, including N-acetyl cysteine (Andreassen et al., 2000a), iron porphyrin (Wu et al., 2003) and a cocktail of antioxidants, in the form of lyophilized red wine (Esposito et al., 2002) and, whilst many show a mild protective effect, none have so far translated successfully into a treatment for human disease (Dib, 2003). For example, the antioxidant vitamin E (α-tocopherol) delayed the onset of clinical symptoms and slowed disease progression in G93A-SOD1 transgenic mice, but did not prolong their survival (Gurney et al., 1996). In clinical trials, vitamin E caused an increase in antioxidant activity and patients were less likely to progress to a severe state of the disease, but no increases in survival were seen (Desnuelle et al., 2001). A subsequent trial using 10× more vitamin E in conjunction with riluzole also failed to show a significant increase in survival times (Graf et al., 2004). It is possible that the effect of mutant SOD1 on oxidative stress extends beyond its own catalytic activity, since a recent study showed that expression of mutant SOD1 caused a decrease in the cellular anti-oxidant response (Kirby et al., 2005).

4.4. Excitotoxicity

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system, acting through a wide variety of receptors that can be either ionotropic (ion channels) or metabotropic (G protein-coupled). Excitotoxicity occurs in motor neurons when excessive signaling through ionotropic AMPA receptors, triggering increased calcium signaling and generation of reactive oxygen species, leads to neuronal injury and death (reviewed by Heath and Shaw 2002). In motor neurons, excitotoxicity is usually avoided by removal of glutamate from the synapse by the glial glutamate transporters, predominantly EAAT2 (Rothstein et al., 1996). However, in a subset of ALS patients there appear to be defects in glutamate handling, resulting in elevated levels of glutamate in cerebrospinal fluid (Rothstein et al., 1990, 1991; Shaw et al., 1995a; Spreux-Varoquaux et al., 2002) although other reports found no significant differences (Perry et al., 1990; Camu et al., 1993).

How glutamate signaling goes awry in ALS is not fully understood. Expression of the glutamate transporter EAAT2 is reduced (Rothstein et al., 1995; Fray et al., 1998) and there is decreased glutamate uptake in motor cortex and spinal cord from ALS patients (Rothstein et al., 1992; Shaw et al., 1994). EAAT2 expression is also reduced in mutant SOD1 rodent models (Bruijn et al., 1997b; Bendotti et al., 2001; Howland et al., 2002). In *Xenopus* oocytes co-expressing A4V or I113T-SOD1 with EAAT2, addition of hydrogen peroxide caused selective inactivation of the glutamate transporter through a mechanism thought to involve mutant SOD1 peroxidase activity and oxidation of residue(s) at the C terminal of the glutamate transporter (Trotti et al., 1999). The changes in glutamate handling in these models suggest that in at least some ALS cases, glutamate toxicity is a secondary event rather than a primary cause of disease. However, despite this the anti-glutamatergic drug riluzole prolongs survival in both human ALS patients (Lacomblez et al., 1996) and mutant SOD1 mouse models (Gurney et al., 1996).

Motor neurons expressing mutant SOD1 are more susceptible to glutamate toxicity than wild-type cells (Roy et al., 1998; Kruman et al., 1999) and show alterations in glutamate receptor subunit expression (Spalloni et al., 2004), suggesting mutant SOD1 affects glutamate transmitter signaling pathways as well as glutamate levels. Glutamate toxicity in motor neurons is primarily mediated through AMPA receptors (Rothstein et al., 1993; Carriedo et al., 1996; Van Den Bosch et al., 2000) and administration of AMPA receptor antagonists to *mnd* mice (Mennini et al., 1999) or G93A-SOD1 mice (Canton et al., 2001; Van Damme et al., 2003) significantly reduced symptoms and increased survival times. AMPA receptors are functionally diverse, in that they are composed of various combinations of four subunits, designated GluR1–4. The permeability of a particular AMPA receptor is determined by its subunit composition; the presence of the GluR2 subunit in a receptor renders that receptor impermeable to calcium (Hollmann et al., 1991; Burnashev et al., 1992). The GluR2 subunit is widely expressed, so most functional AMPA receptors have low permeability to calcium (Hollmann and Heinemann, 1994). Investigations into changes in AMPA receptor subunit expression in ALS have identified reduced expression of the GluR2 glutamate receptor subunit in motor neurons compared to other neuronal populations (Williams et al., 1997), but failed to find clear differences in subunit expression in motor neurons from ALS and control patients (Kawahara et al., 2003; Petri et al., 2004). These differences in glutamate receptor profile between motor neurons and other neuronal populations may partly explain the selective vulnerability of motor neurons, but do not suggest changes in subunit expression lead to ALS. A recent study investigating AMPA receptor properties showed no changes in calcium ion permeability in AMPA receptors in G93A-SOD1-expressing motor neurons, but increases in sodium and potassium permeability, which could then trigger calcium influx through voltage gated calcium channels (Pieri et al., 2003).

Other neuronal populations, such as GABAergic cortical neurons, also express large numbers of calcium permeable AMPA receptors and are susceptible to

excitotoxicity mediated through this glutamate receptor subtype (Yin et al., 1994; Carriedo et al., 1998), suggesting some other characteristic of motor neurons is also responsible for their selective vulnerability to excitotoxicity in ALS. Calcium signaling is usually attenuated by rapid buffering of free calcium ions, either by binding to cytosolic calcium binding proteins (CaBPs) or by uptake into organelles. Whereas GABAergic neurons have strong expression of several CaBPs (Hendry and Jones, 1991; Rogers, 1992), motor neurons express only low levels of calcium binding proteins and there appears to be correlation between low CaBP expression and susceptibility to ALS (Ince et al., 1993; Alexianu et al., 1994; Reiner et al., 1995; Appel et al., 2001). In support of this, the effects of glutamate toxicity in mutant SOD1-expressing motor neurons could be reduced by increasing expression of the CaBP calbindin (Roy et al., 1998). It may be due to the low CaBP level in motor neurons, that AMPA receptor activation in motor neurons causes greater mitochondrial calcium uptake than in GABAergic neurons and results in mitochondrial ROS generation and cell death (Carriedo et al., 2000).

4.5. Mitochondrial dysfunction

Mitochondria are not only responsible for generation of ATP, but are also the major site for the generation of reactive oxygen species and have roles in calcium buffering and initiation of apoptotic cell death, all of which are considered important in ALS pathogenesis (reviewed by Menzies et al. 2002c). Morphologically abnormal mitochondria have been described in motor neurons from ALS patients (Nakano et al., 1987; Siklos et al., 1996; Sasaki and Iwata, 1999), mutant SOD1 transgenic mice (Dal Canto and Gurney, 1995; Wong et al., 1995) and a motor neuron cell line expressing mutant SOD1 (Menzies et al., 2002a). In G93A-SOD1 mice, mitochondrial abnormalities, including mitochondrial swelling, broken outer membranes and dilation of cristae, were apparent before onset of muscle weakness (Kong and Xu, 1998). Massive mitochondrial vacuolation occurred with the onset of symptoms, suggesting that mitochondrial dysfunction may be an important early event in disease pathogenesis and constitute part of the toxic gain of function seen in mutant SOD1.

Biochemical studies of mitochondria from ALS patients and cell models have shown reduced electron transport chain activity (Jung et al., 2002; Mattiazzi et al., 2002; Menzies et al., 2002a; Wiedemann et al., 2002), decreased mitochondrial membrane potential (Carri et al., 1997), disrupted calcium homeostasis (Carri et al., 1997; Swerdlow et al., 1998) and changes to the mitochondrial proteome (Fukada et al., 2004). Activity of other free radical scavenging enzymes was increased in cybrid cell lines (neuroblastoma cells containing mitochondria from ALS patients), indicating elevated levels of reactive oxygen species (Swerdlow et al., 1998). It is therefore not surprising that partial deficiency of mitochondrial SOD (MnSOD/SOD2) in G93A-SOD1 mice increased disease severity and decreased survival compared to G93A-SOD1 mice with normal mitochondrial SOD function (Andreassen et al., 2000b). The elevated levels of reactive oxygen species have been proposed for causing the increased frequency of mitochondrial DNA mutations in the motor cortex and spinal cord of ALS patients (Dhaliwal and Grewal, 2000; Wiedemann et al., 2002). One atypical MND case was found to carry a mitochondrial DNA mutation resulting in production of a truncated cytochrome c oxidase subunit (Comi et al., 1998). Several studies have shown increased translocation of cytochrome c from mitochondria into the cytoplasm, a trigger for apoptosis, during disease progression in G93A-SOD1 mice, that is not observed in age-matched non-transgenic littermates (Guegan et al., 2001; Zhu et al., 2002).

SOD1 is generally considered to be a cytosolic protein (Fridovich, 1975), but it has recently been shown that there is a pool of enzymatically active SOD1 in the mitochondrial intermembrane space (Okado-Matsumoto and Fridovich, 2001; Sturtz et al., 2001; Higgins et al., 2002; Mattiazzi et al., 2002). Since mitochondria are the major site of ROS generation, through incomplete reduction of oxygen during respiration (Lenaz, 1998), it is perhaps not surprising that SOD1 enters the mitochondrion, although the mechanism of mitochondrial import of SOD1 is not known. Curiously, the proportion of SOD1 that entered mitochondria was greater in brain than in liver (Mattiazzi et al., 2002), suggesting a neuronal-specific mechanism for mitochondrial import of SOD1.

Like endogenous SOD1, mutant SOD1 can also enter mitochondria and analysis of four ALS-linked SOD1 mutants showed selective mitochondrial import of mutant SOD1 over wild-type SOD1 in spinal cord mitochondria (Liu et al., 2004). As with wild-type SOD1, such accumulation was greater in spinal cord than in non-neural tissue. An age-dependent increase in mitochondrial mutant SOD1 accumulation has been reported by several groups and has been shown to precede onset of symptoms (Liu et al., 2004) and to coincide with increased oxidative damage and decreased respiratory activity of mitochondria (Mattiazzi et al., 2002) and the appearance of mitochondrial swelling and vacuolization (Jaarsma et al., 2001). The role that mitochondrial mutant SOD1 plays in ALS pathogenesis is currently unknown, although localization of mutant SOD1 to mitochondria has been shown to be sufficient to cause cell death in neuroblastoma cells

(Takeuchi et al., 2002a). A recent study by Pasinelli et al. (2004) showed that mitochondrial SOD1 directly binds to the anti-apoptotic protein Bcl-2 and, in the case of mutant SOD1, both dimeric and high molecular weight aggregates of SOD1 bind Bcl-2. Either mitochondrial SOD1 aggregates, sequestration of Bcl-2, or a combination of both may be toxic to mitochondria and trigger programmed cell death (Green and Reed, 1998).

Drugs targeted to correct mitochondrial dysfunction are now being investigated in SOD1 transgenic models of ALS. Creatine was proposed as a potential therapy since it maintains ATP levels, enabling maintenance of the mitochondrial membrane potential and calcium buffering, and inhibits opening of the mitochondrial permeability transition pore and apoptosis. Treatment of G93A-SOD1 mice with creatine significantly improved motor performance, delayed loss of motor neurons and extended survival (Klivenyi et al., 1999, 2004). However, creatine failed to have any beneficial effect in two clinical trials in human ALS patients (Groeneveld et al., 2003; Shefner et al., 2004). The tetracycline derivative minocycline, which inhibits cytochrome c release from the mitochondria, has been shown to delay disease onset and extended survival in G93A-SOD1 mice (Zhu et al., 2002). Human trials of minocycline in ALS are in progress.

4.6. Protein aggregation

Protein aggregates formed from mis-folded mutant proteins are a common feature in many neurodegenerative diseases, but whether they are a primary cause of disease pathogenesis, a harmless by-product or even a cellular defense mechanism to sequester potentially toxic proteins is not known (reviewed by Caughey and Lansbury, 2003; Ross and Poirier, 2004). Protein aggregates that are immunoreactive to antibodies against ubiquitin (a protein tag that targets proteins for proteolytic degradation) are present in virtually all ALS cases (Ince et al., 1998, 2003; Piao et al., 2003). SOD1 immunohistochemistry has shown positive staining of protein aggregates in human fALS cases with SOD1 mutations and also in a proportion of sporadic cases (Chou et al., 1996; Shibata et al., 1996a,b; Bruijn et al., 1998).

Analogous cytoplasmic protein aggregates have also been observed in mutant SOD1 transgenic mouse models (Gurney et al., 1994; Dal Canto and Gurney, 1995, 1997; Bruijn et al., 1997b, 1998) and cell culture models (Durham et al., 1997; Johnston et al., 2000; Lee et al., 2002). The number of inclusions increased with age in G93A and G85R mice, but inclusions were rarely seen in G37R mice (Watanabe et al., 2001). Most inclusions immunostained for SOD1 in G85R mice, whereas there was more variability in G93A mice with some being strongly positive and others negative. In early stages before obvious disease pathology appeared, many inclusions were also immunoreactive for glial fibrillary acidic protein (GFAP), suggesting they were in astroglial cells. At later stages, more inclusions were immunoreactive for neuronal markers. No inclusions were seen in control mice. In cultured spinal motor neurons, expression of mutant SOD1 resulted in abnormal cytoplasmic aggregation of SOD1 and increased apoptosis, that was not seen in motor neurons expressing wild-type SOD1 (Durham et al., 1997). Not all neurons expressing mutant SOD1 developed aggregates, but only neurons containing aggregates became apoptotic. Such aggregates and apoptosis were not observed in other neuronal populations that are not affected by ALS. Conversely, although differentiated PC12 cells expressing mutant SOD1 contained significantly more SOD1 aggregates and had reduced viability compared to cells expressing wild-type SOD1, the cells that contained aggregates were not necessarily the ones that became apoptotic (Lee et al., 2002).

Whether protein aggregates contribute to ALS pathogenesis has long been debated. Several hypotheses for aggregate-mediated toxicity have been proposed, including: (1) Sequestration of proteins in aggregates thereby inhibiting their normal function. Other proteins identified in SOD1-containing aggregates include ubiquitin, heat shock proteins involved in chaperone and proteasome function, the anti-apoptotic protein Bcl-2, the copper chaperone for SOD1 CCS, GFAP, glutamate transporters and p38MAPK (Kato et al., 2001; Watanabe et al., 2001; Niwa et al., 2002; Bendotti et al., 2004; Pasinelli et al., 2004). (2) Inhibition of organelle function, such as mitochondria, through formation of aggregates within the organelle (Pasinelli et al., 2004). (3) Inhibition of the proteasome allowing subsequent accumulation of damaged or mis-assembled proteins. Protein aggregation has been shown to inhibit the ubiquitin-proteasome system (Bence et al., 2001). Since free ubiquitin levels were not depleted, the authors suggested that inhibition could be caused by the proteasome becoming conjugated to ubiquitinated aggregates that it is unable to unfold and degrade or by sequestration of proteins required for proteasome function in the aggregates. In support of this hypothesis, reduced chaperone activity (Bruening et al., 1999; Tummala et al., 2005) and proteasome activity (Allen et al., 2003) have been reported in mutant SOD1 transgenic mouse and cell culture models, respectively. Resistance to mutant SOD1 toxicity in NIH 3T3 fibroblasts was associated with elevated chaperone activity and increased expression of several molecular chaperones (Bruening et al., 1999). Over-expression of the molecular chaperone HSP-70 (Bruening et al., 1999; Takeuchi et al., 2002b) and the

E3 ubiquitin ligase dorfin (Niwa et al., 2002) partly protected against mutant SOD1 toxicity in cell culture models. Similarly, pharmacological induction of heat shock proteins using arimoclomol increased survival in G93A-SOD1 mice by 22% (Kieran et al., 2004).

4.7. Cytoskeletal dysfunction and axonal transport problems

Neurofilaments form a major component of the intermediate filament network present in neuronal cells, composed of light (NF-L), medium (NF-M) and heavy (NF-H) subunits, and have important roles in maintaining cell shape and determining axonal diameter (reviewed by Lee and Cleveland, 1996). They are particularly important in motor neurons, with their characteristic large size and long axons. Evidence supporting a role for neurofilaments in ALS pathogenesis is clear. Aggregation of neurofilaments is a pathological feature of many neurodegenerative disorders including ALS (Hirano et al., 1984a,b) and have also been seen in SOD1 transgenic mice (Gurney et al., 1994; Wong et al., 1995; Morrison et al., 1996; Tu et al., 1996; Dal Canto and Gurney, 1997; Zhang et al., 1997). Mutations in the KSP repeat region of the NF-H gene are present in approximately 1% of sporadic cases (Figlewicz et al., 1994; Tomkins et al., 1998; Al-Chalabi et al., 1999) and are thought to constitute a risk factor for disease (see Genetics section). Reduced expression of NF-L, causing changes in the stoichiometry of NF subunits, has been reported in sporadic and familial ALS cases, G93A-SOD1 transgenic mice and a cell culture model of ALS (Bergeron et al., 1994; Zhang et al., 1997; Wong et al., 2000; Menzies et al., 2002b). Transport along neurofilaments was also shown to be disrupted in mutant SOD1 transgenic mice (Zhang et al., 1997; Williamson and Cleveland, 1999). How mutant SOD1 causes neurofilament changes is not known, although a recent report described how mutant SOD1, but not wild-type SOD1, bound directly to the 3′ untranslated region of NF-L mRNA, thereby leading to destabilization of the mRNA and increased mRNA degradation (Ge et al., 2005). NF-L is required for NF-M and NF-H to assemble into filaments, so changes in expression may disrupt neurofilament assembly and trigger neurofilament aggregation.

The involvement of neurofilament dysfunction as a primary event in ALS pathogenesis was revealed in transgenic mouse models highly over-expressing mouse NF-L (Xu et al., 1993) and human NF-H (Cote et al., 1993). Both models developed pathology reminiscent of ALS and defects in axonal transport were also demonstrated in the NF-H over-expressing mice (Collard et al., 1995). However, no significant loss of motor neurons was observed in either model, raising suggestions that the pathology was caused by massive over-expression of NF subunits rather than through an ALS-associated mechanism. A subsequent transgenic mouse expressing modest levels of a mutant NF-L caused massive selective death of motor neurons and skeletal muscular atrophy, more reminiscent of ALS (Lee et al., 1994). Deletion of NF-L in G85R-SOD1 mice significantly delayed onset and progression of disease and reduced the selective toxicity to motor neurons (Williamson et al., 1998). Counter-intuitively, over-expression of human NF-H in G85R-SOD1 transgenic mice also resulted in increased survival, with lifespan being extended by up to 65% compared to G85R-SOD1 mice (Couillard-Despres et al., 1998). In both of these transgenic models an increase in perikaryal neurofilaments and a decrease in axonal neurofilaments were observed, suggesting that neurofilament distribution may be important in ALS toxicity. Protection may be due to changes in neurofilament distribution, with increased perikaryal neurofilament levels and/or reduced axonal neurofilaments. This hypothesis has been disputed by Kong and Xu (2000), who reported increased survival in G93A-SOD1 mice over-expressing either mouse NF-H or NF-L. The authors observed similar increases in survival with both neurofilament subunits, even though axonal neurofilaments were not reduced in the NF-L transgenic mice. Other hypotheses propose that increased neurofilament protein may act as a sink for oxygen radical species or increase calcium buffering and protect against rises in intracellular calcium caused by oxidative stress and mitochondrial dysfunction.

Another intermediate filament protein, peripherin, has also been implicated in ALS. Peripherin has been identified in intermediate filament inclusions in human ALS cases (Corbo and Hays, 1992; Migheli et al., 1993; Wong et al., 2000) and SOD1 transgenic mice (Tu et al., 1996; Beaulieu et al., 1999; Julien and Beaulieu, 2000). Over-expression of peripherin in mice caused a late-onset selective motor neuron disease with intermediate filament aggregates containing peripherin, which was exacerbated when NF-L was knocked out (Beaulieu et al., 1999). Peripherin over-expression induced death of cultured motor neurons (Robertson et al., 2001) and it was subsequently shown that only one of the three known peripherin splice variants, the 61 kDa isoform, was responsible for this toxicity (Robertson et al., 2003). Peripherin-61 was detected in motor neurons from G37R-SOD1 mice and human sporadic ALS. A frame-shift mutation in the *peripherin* gene giving rise to a truncated protein that disrupted the neurofilament network has recently been identified in an ALS patient (Gros-Louis et al., 2004). Therefore, although up-regulation or suppression of peripherin

expression had no effect on disease onset, severity or progression caused by G37R-SOD1 (Lariviere et al., 2003), peripherin may be involved in disease pathogenesis in a small proportion of ALS cases.

Intracellular transport of proteins and organelles is of particular importance in motor neurons, which in humans may have axons up to 1 meter in length. Since the protein synthesis machinery is located in the cell body and dendrites, transport mechanisms are required to move proteins and organelles along the axon using the microtubule network (reviewed by Vallee and Bloom, 1991). Both anterograde and retrograde axonal transport have been shown to be disrupted in mutant SOD1 transgenic mice (Zhang et al., 1997; Williamson and Cleveland, 1999; Murakami et al., 2001). The transport defects occurred before the onset of pathological changes in G85R-SOD1 mice (Williamson and Cleveland, 1999). Different molecular motors are believed to be involved in controlling anterograde and retrograde transport. Whilst no mutations affecting proteins controlling anterograde transport have yet been identified in ALS, a mutation in dynactin, a protein involved in dynein-mediated retrograde transport, caused a progressive lower motor neuron disorder (Puls et al., 2003) (see Genetics section). Two point mutations in a dynein subunit caused progressive motor neuron degeneration in heterozygous mice (Hafezparast et al., 2003). Similarly, disruption of the dynactin complex, thereby reducing activation of cytoplasmic dynein, inhibited retrograde transport and triggered late-onset motor neuron degeneration in genetically engineered mice (LaMonte et al., 2002).

4.8. Inflammatory cascades and the role of neighboring cells

There has recently been increased interest in the role of non-neuronal neighboring cells in the pathogenesis of ALS (reviewed by Sargsyan et al., 2005). Genes involved in an inflammatory response were up-regulated in pre-symptomatic mutant SOD1 transgenic mouse spinal cord (Yoshihara et al., 2002) and activated microglia and astrocytes have been observed in spinal cord from both ALS patients and mutant SOD1 transgenic mice (Kawamata et al., 1992; Hall et al., 1998; Alexianu et al., 2001). Although mutant SOD1 damaged motor neurons in culture (Durham et al., 1997), neuron-specific expression of mutant SOD1 was not sufficient to cause motor neuron degeneration in transgenic mice (Pramatarova et al., 2001; Lino et al., 2002), suggesting that non-neuronal cells play an essential role in disease pathogenesis. Astrocyte-specific expression of mutant SOD1 was also not sufficient to cause disease (Gong et al., 2000), indicating that ALS pathogenesis may require involvement from both neuronal and non-neuronal cells. In chimeric mice, composed of mixtures of normal cells and mutant SOD1-expressing cells, normal motor neurons neighboring mutant SOD1 non-neuronal cells developed ubiquitinated proteinaceous inclusions, whereas normal non-neuronal cells extended survival of mutant SOD1-expressing motor neurons (Clement et al., 2003). Results from a cell culture model, co-culturing human neuroblastoma and glioblastoma cell lines expressing mutant SOD1, showed a vicious cycle where the glial cells became activated and the inflammatory response caused the neurons to become apoptotic and release more pro-inflammatory signals (Ferri et al., 2004). This cycle was not seen in co-cultures of mutant neuroblastoma and wild-type glioblastoma or wild-type neuroblastoma and mutant glioblastoma.

Several inflammatory cytokines and enzymes, including various interleukins, tumor necrosis factor-α (TNFα), cyclooxygenase 2 (COX2) and prostaglandin E2 (PGE2) have been shown to be up-regulated in the spinal cord of ALS patients (Almer et al., 2002; Henkel et al., 2004) and mutant SOD1 mice (Elliott, 2001; Hensley et al., 2002, 2003; Yoshihara et al., 2002). Microglia, the resident macrophage population in the central nervous system become activated by these inflammatory mediators and release further pro-inflammatory cytokines and reactive oxygen species (Gonzalez-Scarano and Baltuch, 1999). Although microglial activation can be neuroprotective (Watanabe et al., 2000; Polazzi et al., 2001), it can often be detrimental (Chao et al., 1992; Kim et al., 2000) and there is growing evidence to suggest that inhibition of microglial activation is protective in ALS. Microglia cultured from adult pre-symptomatic mutant SOD1 mice showed increased TNFα release upon stimulation, compared to wild-type microglia (Weydt et al., 2004) and TNFα antagonists significantly increased survival in mutant SOD1 mice (West et al., 2004). Similarly, reduction of PGE2 levels, by inhibition of COX2, protected against motor neuron loss and increased survival in mutant SOD1 mice (Pompl et al., 2003; Klivenyi et al., 2004). Cerebrospinal fluid from ALS patients was toxic to motor neurons in mixed spinal cord cultures and this toxicity could be reduced by inhibition of microglial activation using minocycline (Tikka et al., 2002).

4.9. Apoptosis

Apoptosis is a process of programmed cell death where a cell actively pursues a pathway leading to its death. There are three major pathways of apoptosis, initiated through different routes, but converging on activation of a family of proteases called caspases that destroy

proteins (reviewed by Sathasivam et al., 2001; Guegan and Przedborski, 2003). The death receptor pathway starts with activation of cell surface death receptors (members of the tumor necrosis factor receptor family), triggering activation of caspase-8. In the mitochondrial pathway, pro-apoptotic members of the Bcl-2 family translocate to the mitochondria, inducing release of cytochrome c from mitochondria into the cytosol where it activates caspase-9. In the endoplasmic reticulum pathway, stress to the endoplasmic reticulum activates caspase-12. Activation of these initiator/upstream caspases triggers the caspase cascade, leading to activation of executioner/downstream caspases, including caspases 3 and 7. These pathways are regulated by other proteins, including the Bcl-2 family, which contain both pro- and anti-apoptotic members, and the apoptosis inhibitor proteins which inhibit caspase activity and therefore block apoptosis.

There have been conflicting reports on whether apoptotic cell death occurs in ALS, with some studies showing evidence of apoptosis and others failing to uncover evidence for this mode of neuronal death (reviewed by Sathasivam et al., 2001; Guegan and Przedborski, 2003). The rapid time-course of apoptosis makes identification of apoptotic cells in post-mortem tissue, fixed at a particular time-point, difficult. The general opinion is now that apoptosis does occur, but whether it is the major mechanism of cell death is unknown. A detailed study by Martin (1999) showed that motor neuron degeneration in ALS structurally resembled apoptosis and also reported changes in the sub-cellular distribution of pro- and anti-apoptotic proteins of the Bcl-2 family in a direction favoring of apoptosis, increased caspase-3 activity and DNA fragmentation. Models of SOD1-mediated ALS also show apoptotic death. Expression of mutant SOD1 increased apoptotic cell death in motor neurons (Durham et al., 1997) and in serum-starved immortalized neural cells (Rabizadeh et al., 1995; Cookson et al., 2002). Under basal conditions, neural cell lines expressing mutant SOD1 showed increased activation of caspase-1 (Pasinelli et al., 1998) and caspase-9 (Sathasivam et al., 2005) as well as increased expression of phosphatidyl serine residues at the cell surface (Sathasivam et al., 2005) without increased cell death compared to control cells, suggesting these cells are 'primed' for death and are therefore more susceptible to further insults. In G93A-SOD1 mice, age-dependent changes in protein expression (Vukosavic et al., 1999) and distribution (Guegan et al., 2001) indicative of mitochondrial apoptotic pathways that are not seen in age-matched non-transgenic littermates have been observed. Overexpression of Bcl-2 has been shown to attenuate neurodegeneration, delay activation of caspases-1 and -3 and prolong survival in G93A-SOD1 mice (Vukosavic et al., 2000). Similarly, inhibition of caspases by a pan-caspase inhibitor reduced oxidative stress-induced death in mutant SOD1-expressing neuroblastoma cells (Pasinelli et al., 1998) and increased motor function and prolonged survival in G93A-SOD1 mice (Li et al., 2000).

4.10. Environmental risk factors

In approximately 90% of ALS cases there is no current evidence for a genetic pre-disposition to disease development, suggesting that other external factors may be implicated. The best evidence to date for an environmental trigger to disease development is the unusually high incidence of ALS-PD among the Chamorro people on the Pacific Island of Guam. Failure to identify a genetic component to Guam ALS-PD (Blake et al., 1983; Garruto et al., 1983) and the decreased incidence of disease that correlates with increased travel between Guam and the rest of the world (Garruto et al., 1985; Plato et al., 2003) suggested an environmental toxin might be responsible. The neurotoxic non-protein amino acid β-methylamino-L-alanine (BMAA) produced by cyanobacteria living symbiotically in the corraloid roots of cycad trees was shown to cause clinical and pathological changes similar to Guam ALS-PD when fed to macaques (Spencer et al., 1987). Recently, evidence has emerged that human exposure to BMAA may occur through consumption of flying foxes, popular amongst the Chamorro people, and BMAA has been detected in brain tissue from Chamorro people who died of ALS-PD (Banack and Cox, 2003; Cox et al., 2003).

Other putative environmental risk factors have also been proposed, but as yet the significance of these has not been reported consistently. Some epidemiological studies have suggested that there may be a causal link between physical exercise and risk of developing ALS (Granieri et al., 1988; Strickland et al., 1996; Scarmeas et al., 2002; Al-Chalabi and Leigh, 2005), although other studies have found no such association (Longstreth et al., 1998). Results from mutant SOD1 transgenic mice studies are similarly conflicting. Some studies reported that exercise had no effect on disease onset or survival (Gurney et al., 1996; Liebetanz et al., 2004), whereas other studies showed that exercise either decreased survival (Mahoney et al., 2004) or delayed disease onset and prolonged survival (Kirkinezos et al., 2003; Veldink et al., 2003). Variations in exercise regimes between these studies suggest that the type and duration of exercise undertaken may determine the effect on disease progression. Each study reporting an exercise-dependent change in survival also reported male/female differences, adding yet further complexity to the role of exercise in ALS pathogenesis.

A small scale study found that moderate physical exercise by ALS patients in early stages of disease had short-term positive effects on the perception of fatigue and the quality of life, but did not affect the decline in muscle strength (Drory et al., 2001b).

The hypothesis that viruses may cause ALS has received continuing interest over the years, but clear evidence for viral involvement remains elusive (reviewed by Jubelt, 1992; Karpati and Dalakas, 2000). Although several studies using PCR techniques have identified enterovirus nucleic acids in spinal cord from ALS patients (Woodall et al., 1994; Berger et al., 2000; Giraud et al., 2001), other PCR based studies have failed to detect enterovirus in ALS spinal cord (Swanson et al., 1995; Nix et al., 2004). Antibodies against retroviral proteins have been found in serum from ALS patients (Westarp et al., 1995) and reverse transcriptase activity, indicative of retroviral infection, has been found in serum from a greater proportion of ALS patients than unrelated controls (Steele et al., 2005). An ALS-like disorder seen in a small proportion of HIV patients has been successfully treated using antiretroviral treatment (MacGowan et al., 2001; Moulignier et al., 2001; Nishio et al., 2001).

Other putative risk factors include an imbalance in essential trace elements, particularly heavy metals (Kapaki et al., 1997; Yase et al., 2001; Kamel et al., 2002; Gellein et al., 2003), cigarette smoking (Kamel et al., 1999; Nelson et al., 2000; Weisskopf et al., 2004), electric shock (Sirdofsky et al., 1991), exposure to electromagnetic fields (Davanipour et al., 1997; Johansen and Olsen, 1998; Savitz et al., 1998; Hakansson et al., 2003) and service during the Gulf war of 1990–1991 (Haley, 2003; Horner et al., 2003; Rose, 2003; Armon, 2004). Increased rates of ALS have been reported in airline pilots (Nicholas et al., 1998, 2001) and the hypothesis has been suggested that prolonged periods of hypobaric hypoxia in the cabin may combine with adverse polymorphisms in the regulatory regions of the VEGF gene to trigger disease (Pamphlett, 2002).

4.11. Vulnerability of motor neurons to neurodegeneration in ALS/MND

Although ALS is now regarded as a multi-system disease, affecting some non-motor populations of the central nervous system (Ince, 2000), the most severe changes are seen in motor neurons. Why motor neurons are more vulnerable to degeneration in ALS remains an enigma. Selective vulnerability is even seen amongst motor neurons, with certain populations, including those controlling eye movement and the motor neurons of Onuf's nucleus controlling pelvic floor muscles, being characteristically spared (Ince, 2000). Adaptations of the motor neuron that allow it to perform its unique function may leave it vulnerable to the degeneration seen in ALS (Fig. 4.3, reviewed by Shaw and Eggett, 2000). A major adaptation of the motor neuron is its large size, with a cell body of approximately 50–60 μm and an axon of up to 1 m long in humans. A cell of this size and shape needs a large, strong cytoskeleton to support it and will rely heavily upon its intracellular transport system to deliver proteins and membrane constituents, most of which are made in the cell body, around the cell. Maintaining a cell of this size will require a huge metabolic input, necessary for production of membrane components and proteins, transportation of these around the cell and maintaining a membrane potential along the length of the axon. Mitochondria are able to provide the energy, but they are also the major

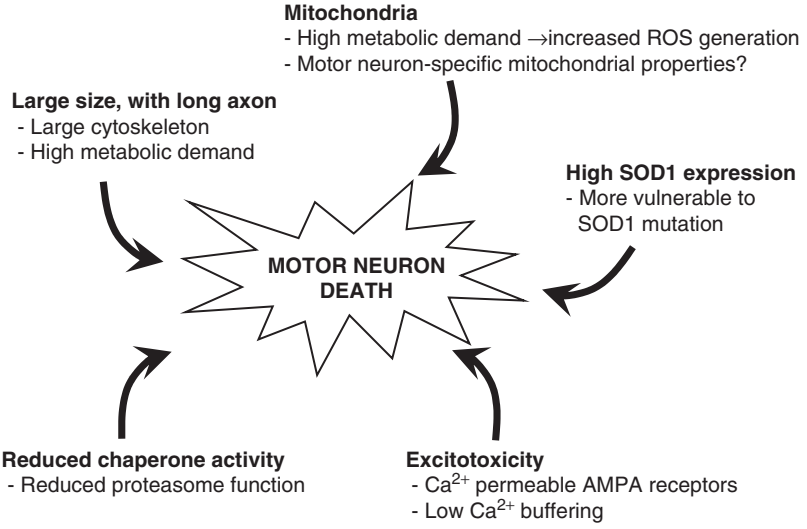

Fig. 4.3. Features of motor neurons that may render them selectively vulnerable in ALS.

site of reactive oxygen species production in the cell. The energy demands of the cell may therefore cause the ROS levels to be higher in motor neurons than other cells, making them more vulnerable to oxidative stress. The extensive cytoskeleton and large amount of plasma membrane provide targets for oxidative modification. Mitochondrial DNA is also a recognized target for free radical attack and, combined with the high energy demand, may make motor neuron mitochondria more prone to dysfunction. There is increasing evidence to suggest that properties specific to mitochondria from the spinal cord render them more vulnerable to toxic insult (Liu et al., 2004; Sullivan et al., 2004). SOD1 expression in motor neurons is high (Pardo et al., 1995), suggesting that a strong antioxidant response is important in motor neurons. Such a reliance of motor neurons on normal SOD1 function may help explain why motor neurons are selectively vulnerable in the presence of mutant SOD1 protein.

Motor neurons have also been shown to have a poor heat shock response to toxic insult. Liver extract from mutant SOD1-expressing mice showed increased chaperone expression and activity compared to non-transgenic controls, whereas spinal cord extract from mutant SOD1 mice had reduced chaperone activity (Bruening et al., 1999). The authors suggested that most cells can up-regulate expression of chaperone proteins in response to mutant SOD1, whereas motor neurons are less able to up-regulate chaperone expression, resulting in sequestration of chaperones in SOD1 aggregates and inhibition of the proteasome.

Motor neurons appear to be more vulnerable to glutamate and calcium toxicity than other cell types (Carriedo et al., 1996). Expression of the glial glutamate transporter EAAT2 has been shown to be higher around motor neuron groups vulnerable to degeneration in ALS (Milton et al., 1997), suggesting that these populations are particularly susceptible to glutamate toxicity and would be more greatly affected by defects in EAAT2 function. Expression of the GluR2 AMPA receptor subunit, which renders AMPA receptors impermeable to calcium, was found to be unusually low in motor neurons (Williams et al., 1997). Therefore, release of glutamate into the synapse would trigger increased calcium entry into the post-synaptic motor neuron. The calcium buffering capacity of motor neurons was reported to be lower than in other cells (Ince et al., 1993; Alexianu et al., 1994), so combined with the increased calcium entry, there would be increased activation of calcium signal pathways, with potential toxic effects (Appel et al., 2001). Uptake of free calcium into mitochondria causes mitochondrial dysfunction and triggers increased ROS generation (Carriedo et al., 2000). The role of calcium signaling may be central to the selective vulnerability of motor neurons, since it connects excitotoxicity and oxidative stress, and there appears to be correlation between low calcium binding protein expression and susceptibility to ALS (Reiner et al., 1995).

It seems quite plausible that the selective vulnerability of motor neurons to degeneration in ALS is not a result of one of the factors discussed above, but is due to multiple susceptibility factors acting together. Mutant SOD1 is prone to form aggregates in motor neurons (Durham et al., 1997; Watanabe et al., 2001) and may contribute to mitochondrial dysfunction and oxidative stress, as well as glutamate toxicity and increased intracellular calcium levels in motor neurons (Kruman et al., 1999). Aberrant calcium signaling in motor neurons can lead to mitochondrial dysfunction and increases in reactive oxygen species generation (Carriedo et al., 2000; Appel et al., 2001) and misfolding of mutant SOD1 (Tateno et al., 2004), whereas ROS can inactivate the glial glutamate transporter EAAT2, thereby triggering excitotoxicity (Trotti et al., 1999). In this way, oxidative stress and glutamate toxicity could form a positive feedback loop, in which either could initiate the signaling cascade leading to motor neuron death (Rao and Weiss, 2004).

4.12. Conclusions

The mechanisms of motor neuron degeneration in amyotrophic lateral sclerosis are steadily being revealed. It is becoming increasingly evident that what was once believed to be a single disease is actually a collection of disorders that converge to produce a common disease phenotype. The diversity of toxic insults, from mutations in a gene encoding a ubiquitously expressed housekeeping enzyme to defects in the cytoskeleton and intracellular trafficking and as yet unidentified environmental factors, which all lead selectively to degeneration of motor neurons, emphasizes the multifactorial nature of ALS. This suggests that future efforts to understand and combat ALS should also be multifaceted. With the deciphering of the human genome and powerful modern genetic techniques, it will be possible to further understand the genetics of the disease and to identify otherwise elusive underlying genetic risk factors. Large scale epidemiological studies may reveal the role of environmental risk factors, particularly when combined with genetic data. The cellular and animal models that have been developed and extensively characterized provide tools to investigate the underlying biochemistry of motor neuron injury and to develop potential treatments. Given the diversity of neurodegenerative mechanisms implicated in ALS pathogenesis, it seems likely that an effective neuroprotective therapy

will employ a combination of treatments targeting multiple molecular pathways.

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Chapter 5

Cytopathology of the motor neuron

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5.1. Introduction

This chapter describes the pathology of disorders that demonstrate relative selectivity for involvement of the pyramidal motor system; the upper motor neurons, the corticospinal tracts and the lower motor neurons. Most of these diseases fall into the broad category of neurodegenerative disorders, but some other disorders resulting from infectious or toxic etiologies that also demonstrate preferential targeting of the motor system are included.

Several themes have emerged in the pathology of neurodegenerative diseases in recent years and are reflected in the disorders discussed in this chapter. These diseases include familial disorders that facilitate the study of molecular pathology in the context of defined gene defects. In diseases such as hereditary spastic paraparesis, a wide range of mutations in different genes result in similar clinicopathological phenotypes. Investigation of the detailed pathology in genetically defined cohorts of HSP cases is still in its infancy. However an investigation of the molecular pathology of familial motor system disorders has the potential to elucidate the manner in which perturbations of different protein functions may, via different pathogenetic mechanisms, converge upon final common pathologies. Amyotrophic lateral sclerosis (ALS) includes both familial and sporadic forms and, although there are some pathological differences between them, a detailed understanding of the pathology in familial forms with a known gene defect can illuminate the pathogenesis of sporadic disease.

Conceptual advances in the cellular pathology of neurodegeneration have particularly focused on the role of cellular inclusions. These vary in terms of the anatomy, cellular location, morphology and protein substrates among different neurodegenerative diseases and have a valuable role in autopsy diagnosis. When present in familial neurodegenerative diseases, inclusions often contain the abnormal protein derived from the mutated gene. The proteins within inclusions are generally poly-ubiquitylated, so they may be identified by immunohistochemistry to ubiquitin and p62 (Kuusisto et al., 2003). Polyubiquitylation is a process by which a protein or polypeptide is targeted for proteasomal degradation. The presence of aggregated, ubiquitylated proteins is therefore taken to indicate the accumulation of an abnormal, denatured or mis-folded protein. In sporadic ALS, the specific protein content of the characteristic ubiquitylated inclusions remains to be defined, but their presence has become central to autopsy diagnosis and in clarifying nosologic relationships. Indeed the better case definition that has resulted from the introduction of ubiquitin immunohistochemistry in the late 1980s means that studies on ALS in the older neuropathological literature need to be interpreted with caution. Immunohistochemical studies using antibodies to other proteins continue to contribute to conceptual advances in this field, as is illustrated by the use of antibodies that identify expanded polyglutamine tracts in X-linked spinobulbar muscular atrophy.

5.2. Spinal muscular atrophy

Spinal muscular atrophies (SMA) are pure lower motor neuron disorders that are slowly progressive and genetically determined. They usually produce symmetrical weakness that, despite the name, may involve bulbar as well as spinal musculature and that is not accompanied by clinical evidence of pyramidal or sensory involvement. This is a genetically heterogeneous group of disorders and this section is confined to the pathology of the most

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common autosomal recessive forms of SMA. These childhood, proximal, forms of SMA are classified according to age of onset and progression (Zerres and Davies, 1999). SMA I (Werdnig-Hoffman's disease) has an onset from the prenatal period to the first 6 months of life. There is a failure to reach early motor milestones, so that these children never sit unaided, and death usually occurs from respiratory failure at less than 2 years of age. Children with SMA II, the intermediate form, have an onset in infancy, before the age of 18 months; they achieve sitting but do not become ambulant. SMA III (Kugelberg-Welander disease) has an onset in childhood, usually after the age of 18 months. These children usually become ambulant and live into adulthood. SMA IV has been used as a designation for some late onset cases. These autosomal recessive forms are all linked to mutation of the SMN 1 gene on chromosome 5q13 (Schmalbruch and Haase, 2001). There is also a variety of other forms of SMA, with recessive, dominant and X-linked forms of inheritance. Many of these present with atypical SMA or 'SMA plus' syndromes and are not linked to 5q13 (Zerres and Rudnik-Schoneborn, 2003). They will not specifically be considered further here.

5.2.1. Neuropathology of autosomal recessive 5q13-linked SMA

5.2.1.1. SMA I

SMA types I, II and III all share involvement and loss of the lower motor neuron as the primary pathological lesion underlying the clinical phenotype. However, there are subtle differences in cytopathology between the three forms not just related to differences in severity and age of onset. Given its fatal outcome at a young age, the neuropathology of SMA I has been the best studied of the three forms. Autopsy studies have demonstrated extensive loss of motor neurons from spinal cord anterior horns and brainstem motor nuclei. Residual motor neurons show a ballooned or chromatolytic appearance, with swelling and pale staining in routinely stained sections because of loss of Nissl substance (Fig. 5.1). Atrophic, shrunken neurons may be seen and occasional neurons show neuronophagia, where the residual neuron is surrounded by microglia. Glial fibers may surround a space from which the neuron has been lost, so-called empty beds. The anterior horns generally show gliosis (Lippa and Smith, 1988; Fidzianska et al., 1990; Murayama et al., 1991; Simic et al., 2000). The ballooned neurons of SMA I show appearances superficially similar to chromatolysis, a morphological reaction of the neuronal cell body to axonal injury. However, immunohistochemical and ultrastructural studies have shown that the cytopathology of the ballooned neurons differs from that of chromatolysis, reflecting a different pathogenesis. The ballooned neurons of SMA I show an accumulation of intermediated filaments around the periphery of the cell with lysosomes and mitochondria in the center (Murayama et al., 1991; Chou and Wang, 1997). Phosphorylated neurofilaments are seen within the perikaryon of the neuron and are present in a band-like distribution around the periphery of the cell, corresponding to the ultrastructural accumulation of intermediate filaments. These alterations in the phosphorylation and distribution of neurofilament indicate that disturbance of cytoskeletal function plays a role in disease pathogenesis. Ubiquitin immunohistochemistry shows an abnormal, weak, diffuse pattern of staining (in contrast to the ubiquitylated inclusions of motor neuron disease) (Lippa and Smith, 1988; Kato and Hirano, 1990; Sobue et al., 1990; Murayama et al., 1991; Matsumoto et al., 1993). Synaptic boutons have been observed incarcerated within the periphery of these cells (Chou and Wang, 1997).

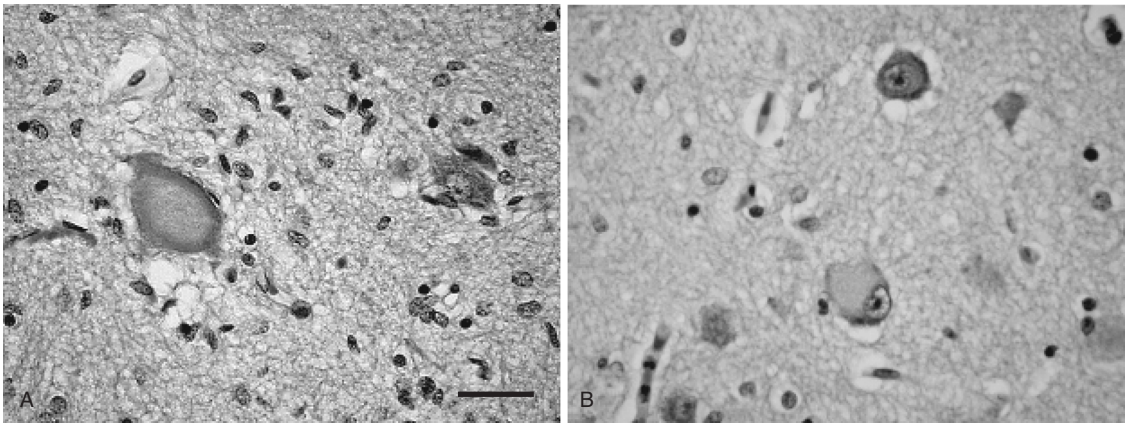

Fig. 5.1. A chromatolytic spinal motor neuron in the ventral horn in a case of SMA type 1 contrasts with a smaller and normal appearing motor neuron (**A**). Similar changes affect some non-motor neurons (**B**). [Hematoxylin & Eosin.]

Ballooned neurons show a loss of membrane reactivity for synaptophysin, but an increase in cytoplasmic staining for this synaptic marker (Ikemoto et al., 1996). Changes in the pattern of glycosylation have also been described (Chou and Wang, 1997).

Betz cells of the motor cortex and the pyramidal tract are preserved in SMA I. Ballooned neurons also affect the bulbar motor nuclei of the trigeminal, facial, hypoglossal nerves and the nucleus ambiguus. Extraocular motor nuclei may also show swollen neurons as may Onuf's nucleus, despite lack of clinical involvement. Swollen neurons are also seen in Clarke's nucleus of the spinal cord and in the ventrolateral nucleus of the thalamus (Iwata and Hirano, 1978; Sung and Mastri, 1980; Kato and Hirano, 1990). Autopsy in a long-term survivor, through assisted ventilation, has shown imaging abnormalities of the thalamus (Ito et al., 2003). EEG alterations that may be referable to disturbed thalamic functions were present and there was mild frontal cortical atrophy on imaging, suggested to be related to the thalamic involvement. Reports of more widespread pathology such as these and findings in longer survivors who have been ventilated provide evidence that, although the pathology of SMA is targeted to the motor neuron, in pathological terms it is a multisystem disorder with a hierarchy of involvement.

Skeletal muscles in SMA I show early denervation with some distinctive features that may reflect delayed maturation. Muscle biopsies show large groups of atrophic fibers. These tend to be rounded and are of both type 1 and 2 histochemical fiber types, distributed in a normal checkerboard pattern (as opposed to type grouping). Atrophic fibers may form large groups, sometimes involving whole fascicles. Amongst these groups are fibers with retained size or showing hypertrophy. These tend, uniformly, to show the staining pattern of type 1 fibers with myosin ATPase preparations. Muscle necrosis and inflammation are not present and muscle spindles tend to be unaffected (Anderson, 1985; Schmalbruch and Haase, 2001). Neural cell adhesion molecule (NCAM) is expressed on both large and small fibers in SMA I muscle (Walsh et al., 1987). NCAM is normally expressed in developing muscle but not in the adult, but it is re-expressed during denervation. NCAM expression on the small fibers in SMA I is consistent with their origin from denervation. Expression on the larger fibers has been used to argue that their innervation is unstable. It has also been suggested that surviving motor neurons and the muscles they supply are immature and therefore prone to on-going degeneration. The small muscle fibers have central nuclei and ultrastructural features reminiscent of the immature myotube (Fidzianska et al., 1990; Schmalbruch and Haase, 2001). The increased frequency of type 2C fibers may also reflect immaturity (Saito, 1985). Muscle fibers in SMA I show the expression of fetal isoforms of muscle proteins such as myosin heavy chain. Whereas the hypertrophic fibers express slow myosin, consistent with their type 1 histochemical pattern, atrophic fibers express prenatal myosin forms as well as fast or slow myosin. The type 1 predominance in the larger fibers suggest that slow twitch fibers may be relatively preserved or that transformation from type 2 to type 1 has occurred. The myosin isoform pattern and morphology of the small fibers may be due to prenatal onset of denervation, resulting in delayed fiber type maturation. Immunohistochemical studies have also shown abnormal, subsarcolemmal accumulation of desmin and titin in atrophic and hypertrophic fibers, so that even the fibers with preserved size are not completely normal (Soussi-Yanicostas et al., 1992).

5.2.1.2. SMA II

The neuropathology of SMA II is less well described as there are few reports of well characterized cases. As in SMA I, there is severe loss of spinal motor neurons with empty neuronal beds and anterior horn gliosis and there is neuronal loss from the nuclei of the hypoglossal and facial nerves. Phrenic motor neurons are relatively preserved, consistent with the preservation of diaphragmatic function that is characteristic of most 5q13-related SMA. Motor neurons may show atrophy, but ballooned or chromatolytic neurons appear not to be a feature, in contrast to SMA I. Also in contrast to SMA I, a decrease in Betz cells has been reported. Immunohistochemistry shows no abnormality of neurofilament protein or ubiquitin (Araki et al., 2003). There appear therefore to be cytopathological differences between SMA I and II, which may imply differences in pathogenesis. Abnormalities of neurofilament function may, for example, be more important in SMA I. However, given the paucity of SMA II cases reported, these findings need to be further defined in larger numbers of cases. The muscle pathology of SMA II appears to be similar to that of SMA I

5.2.1.3. SMA III

SMA III and adult onset SMA also show loss of anterior horn motor neurons and gliosis with no changes in upper motor neurons or the pyramidal tracts (Namba et al., 1970; Huang and Luo, 1983). The muscle pathology of SMA III differs somewhat from that of SMA I and II in that the appearances are more typical of chronic neurogenic atrophy (Kugelberg and Welander, 1956; Namba et al., 1970; Anderson, 1985). Small angulated fibers and small group atrophy reflect denervation. Fiber type grouping demonstrable in myosin ATPase preparations indicates re-innervation. Secondary myopathic changes may develop, which may sometimes cause difficulties in the differential diagnosis of biopsy appearances.

5.2.2. Apoptotic and cell stress mechanisms in SMA

The question of the mechanism by which neurons die in SMA is still unresolved and, as for many neurodegenerative disorders, the issue of whether death occurs by apoptosis is a difficult problem. Studies using the terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) method, which relies on the labeling of DNA strand breaks characteristic of apoptosis, have reported varying results for anterior horn cells in SMA I (Hayashi et al., 1998; Simic et al., 2000). TUNEL positive cells have also been detected in the ventrolateral thalamus, also a site of cytopathology in SMA I. A study using the related in-situ end labeling method (ISEL) found only occasional labeled atrophic neurons in SMA II (Araki et al., 2003). The low rate of labeling observed with this method might be expected, given that cell death occurs over a considerable time, but the process of apoptosis for an individual cell is rapid. These methods suffer from a lack of specificity for apoptosis and show a positive reaction in necrosis or other forms of DNA damage (Grasl-Kraup et al., 1995). However, ultrastructural studies in SMA I suggest that apoptosis may be occurring. In addition there is evidence for changes in the expression of apoptosis-regulatory proteins, including loss of staining for the anti-apoptotic Bcl2, and strong expression of p53, a protein that may induce apoptotic pathways (Simic et al., 2000). In the case of SMA I, it may be that the key stage for the induction of apoptotic pathways occurs in utero, when the pathology and the onset of denervation are known to begin. The gestational period around 10 to 20 weeks is a time when increased physiological motor neuron death is seen, at a time when functional neuromuscular synapses are developing. A high proportion of TUNEL positive neurons are present at this time, associated with increased rates of motor neuron loss (Soler-Botija et al., 2002). This process is associated with altered patterns of BCL-family protein expression; a decreased level of Bcl-2 has been observed in fetuses with SMA I, compared to controls, at 15 and 22 weeks gestation and the normal developmental increase in Bcl-X appears to be irregular and delayed (Soler-Botija et al., 2003). Thus, an excess of developmental programmed cell death may be important in SMA I. Apoptotic bodies may also be found in skeletal muscle in SMA, associated with expression of the pro-apoptotic molecule Bax (Fidzianska et al., 1990; Tews and Goebel, 1997). The SMN gene is expressed in skeletal muscle (Lefebvre et al., 1995; Williams et al., 1999) so that the effects of the mutation may contribute to pathogenesis through a direct effect on muscle; this is supported by experimental studies using muscle and neuronal co-cultures (Braun et al., 1995; Guettier-Sigrist et al., 2002). However, unstable innervation and fiber immaturity may also be provoking factors. In summary, it can be said that the potential function of genes in the region affected in 5q-related SMA, including SMN and NAIP (see below) suggests a central role for dysregulation of apoptotic pathways in SMA. Although evidence is not unequivocal, cell death shows some of the features of apoptosis and there is evidence that apoptosis-regulatory pathways are altered.

There is also histopathologic evidence that oxidative stress may play a role in the pathogenesis of SMA. Immunohistochemical studies have shown the expression of 4-hydroxy-2-nonenal-modified protein, a reactive lipid aldehyde produced by membrane oxidation, in both SMA I and II (Hayashi et al., 2002; Araki et al., 2003). In contrast to findings in amyotrophic lateral sclerosis, increased nitrotyrosine content of proteins was not observed. These studies also showed staining for 8-hydroxy-2′-deoxyguanosine, a marker for oxidative DNA damage. Loss of expression of the glutamate transporter GLAST (EAAT1) has also been seen in the ventrolateral nucleus of the thalamus in SMA I, suggesting that there may be disturbances in glutamate metabolism at this site (Hayashi et al., 2002).

5.2.3. Molecular pathology

The region to which SMA types I, II and III map contains four genes; the survival motor neuron gene (SMN), the neuronal apoptosis inhibitory protein gene (NAIP), the p44 gene (which encodes a subunit of the transcription factor TFIIH) and the H4F5 gene. Duplication of this region has resulted in telomeric and centromeric copies of each gene. Mutation in the telomeric copy of SMN, designated SMN 1, is the cause of SMA, although there may be associated mutations in the other genes (Lefebvre et al., 1995; Biros and Forrest, 1999). Elevated levels of SMN 2 protein are associated with milder phenotypes, and there is an increased copy number of the centromeric gene, SMN 2, in the milder forms. This suggests a model to account for variation in disease severity. SMA I is associated with homozygous deletion or in a few cases more subtle mutations in SMN 1, whereas SMA II and III appear to be associated with a conversion of SMN 1 to 2. SMN 2 encodes predominantly for a transcript that lacks exon 7 because of a C to T transition at codon 280 (although some full length transcript is produced), so that SMN2 protein appears to compensate only partially for loss of SMN 1 function. The role of additional mutations in other genes in this region, such as NAIP, as disease modifiers is yet to be defined.

SMN protein is widely expressed, so that its activity appears fundamental to cell function, and is found in both the nucleus and cytoplasm (Gubitz et al., 2004).

The selectivity of pathology in SMA is therefore not simply due to a restricted distribution of the protein. It is expressed from early in fetal life, which is consistent with the effects of its loss being first manifested in utero (Tizzano et al., 1998). Disease severity is in proportion to the reduction of SMN production (Lefebvre et al., 1997). Within the nucleus, SMN protein is highly concentrated in nuclear bodies called gems (gemini of coiled bodies). These are closely associated with Cajal or coiled bodies with which they have a dynamic relationship. Although usually co-localizing, they appear to be separate in fetal tissues (Zimber et al., 2004). SMN forms a stable complex with gemins, the protein components of gems, and appears essential for gem assembly. SMN, through this interaction, appears to be involved in RNA metabolism. It appears to be important for pre-mRNA splicing through a role in assembly of the spliceosome (which catalyses removal of introns from mRNA precursors) and perhaps in the formation of other ribonucleoproteins (Terns and Terns, 2001; Gubitz et al., 2004). Thus, pathology in SMA may be related to disturbed RNA metabolism. Other roles, however, may yet be defined. For example, in a Zebra fish model antisense reduction of SMN levels leads to impaired motor axon outgrowth independently of cell death, suggesting that a development defect in axon growth and pathfinding may be an early event (McWhorter et al., 2003). SMN has also been shown to abrogate apoptosis induced by Bax and Fas through interaction with Bcl-2, so that a disturbance in apoptosis regulation is a further candidate mechanism.

Determining the reason for the specificity of SMA pathology for the lower motor neuron remains a key goal in this field (Sendtner, 2001). A loss of SMN function below a threshold that is critical in motor neurons is one possibility or SMN may have a unique function in motor neurons. Uniquely, adult motor neurons have more gems and Cajal bodies than at the fetal stage (Young et al., 2001). There are also some differences in the morphology of these structures in the motor neuron compared to other cells; in motor neurons they form large aggregates around the periphery of the nucleolus. There are also differences in the nuclear distribution of SMA. It is thus possible that disturbances to these organelles may have unique effects in motor neurons.

Whilst SMA with atypical features is often linked to chromosomal regions other than 5q (Zerres and Rudnik-Schoneborn, 2003), defining the genetic abnormality in cases of SMA has allowed the identification of 5q-linked cases with greater phenotypic variation. SMN 1 deletion may be associated with arthrogryposis (Bingham et al., 1997). Congenital cytoplasmic body myopathy, characterized by the presence of cytoplasmic bodies but not neurogenic changes at the time of muscle biopsy, has been associated with a homozygous deletion of exons 7 and 8 of the SMN gene (Vajsar et al., 1998). In cases of 5q13-linked SMA diaphragmatic function tends to be spared. This contrasts with 'SMA with respiratory distress (SMARD)' which is usually due to a mutation of the immunoglobulin μ-binding protein-2 gene located on chromosome 11. However, a variant of SMA with respiratory insufficiency, multiple joint contractures and bone fractures has been described in association with SMN 1 mutation (Garcia-Cabezas et al., 2004). The neuropathology of this case was typical of that of SMA I, although phrenic motor neuron involvement was not specifically reported. It seems likely that study of more, genetically defined, cases will identify further phenotypic variations associated with mutation of the SMN 1 gene.

5.3. X-Linked spinobulbar muscular atrophy

X-linked spinobulbar muscular atrophy (X-SBMA), or Kennedy's disease, is a motor system degeneration of males in which the brunt of the pathology is borne by the lower motor neuron. The clinical onset is usually in the third to fifth decades and is characterized by muscle cramps, fasciculations, weakness and atrophy affecting limb and bulbar muscles. The weakness is of lower motor neuron type; upper motor neuron signs are absent. The presence of mild sensory abnormalities in some cases indicates that, although lower motor involvement is a dominant feature, nervous system pathology is not confined to the motor system. Manifestations of X-SBMA beyond the nervous system include androgen insensitivity with gynecomastia, reduced fertility and testicular atrophy. Patients may also demonstrate a raised creatine kinase, whilst impaired glucose tolerance, hepatic dysfunction and raised plasma lipids imply pathology in visceral organs (Kennedy et al., 1968; Harding et al., 1982; Arbizu et al., 1983; Sobue et al., 1989; Amato et al., 1993). Female carriers, heterozygous for the abnormal gene, are generally clinically unaffected, but subtle phenotypic abnormalities may be present (Sobue et al., 1993).

5.3.1. Neuropathology

Autopsy studies have demonstrated that X-SBMA is characterized pathologically by severe loss of lower motor neurons from all levels of the spinal cord, and also motor nuclei of cranial nerves V, VII, XI and XII; there is relative sparing of the extra-ocular muscle groups. This is accompanied by mild gliosis (Kennedy et al., 1968; Nagashima et al., 1988; Sobue et al., 1989). Small neurons from the intermediate zone of the spinal cord may also be lost (Terao et al., 1994). Remaining lower

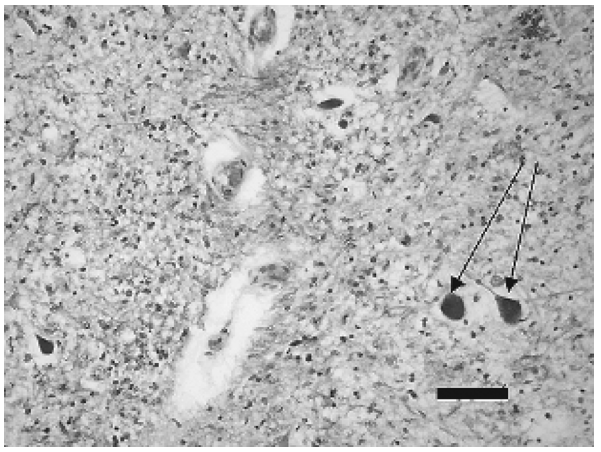

Fig. 5.2. Depletion of motor neurons in the spinal ventral horn associated with some attenuation of the spinal gray matter is a characteristic but non-specific finding in X-SBMA. The figure shows two dark and shrunken profiles of surviving lower motor neurons. [Hematoxylin & Eosin.]

motor neurons may show atrophy (Fig. 5.2), but chromatolysis is not a feature, suggesting that the motor neuron degeneration is not secondary to retrograde axonal degeneration (Sobue et al., 1989). Ubiquitylated cytoplasmic inclusions are not a feature of X-SBMA, in contrast to amyotrophic lateral sclerosis in which they are considered to be diagnostic and related to pathogenesis (see § 5.4.2.2.2 below). The pattern of expression and distribution of neurofilament is unaltered in X-SBMA (Sobue et al., 1990). The anterior roots of the spinal cord show loss of axons (Kennedy et al., 1968) and skeletal muscles demonstrate changes of severe denervation and reinnervation with fiber-type grouping (Harding et al., 1982; Arbizu et al., 1983; Sobue et al., 1989; Guidetti et al., 1996). Clusters of myonuclei (nuclear clumps) may be seen, reflecting severe fiber atrophy. As in other chronic denervating states, secondary myopathic changes may be seen with occasional necrotic fibers, the latter presumably being the origin of the raised serum creatine kinase which may be seen. A muscle biopsy of a female carrier of the mutation has shown type 2 fiber predominance and increased fiber size variation (Sobue et al., 1993).

As implied by the finding of sensory abnormalities, pathology may be demonstrated in the sensory system in X-SBMA (Wilde et al., 1987; Sobue et al., 1989; Amato et al., 1993; Li et al., 1995; Guidetti et al., 1996). Sensory neurons within dorsal root ganglia are preserved, but there is a central and peripheral sensory axonopathy, worse distally. There is myelinated fiber loss from fasciculus gracilis, more severe rostrally (i.e. distally from the cell body), without gliosis. Neurons within nucleus gracilis and cuneatus are preserved however. Sural nerve studies have revealed a reduction of myelinated fibers, particularly larger diameter fibers. Axonal atrophy and degeneration are seen, and increased segmental demyelination and remyelination are described. Given the preservation of sensory neuron cell bodies within dorsal root ganglia, it has been suggested that sensory pathology represents sensory neuron dysfunction without cell loss.

In addition to sensory pathology, evidence of involvement of cerebral structures in some cases further suggests that the selective involvement of the lower motor neuron in this disease is only relative. Imaging studies have shown cerebral atrophy in some cases and abnormalities of long-term memory and attention have been described (Guidetti et al., 1996). One report has described the development of pre-senile dementia in a patient with X-SBMA (Shaw et al., 1998). Neuropathological examination in this case revealed frontal subcortical gliosis, neuronal depletion from the hippocampus, particularly from the CA1 region and gliosis associated with microglial reaction in hippocampus, caudate, thalamus and subthalamus.

5.3.2. Molecular pathology and cytopathology

X-SBMA is one of the triplet-repeat group of disorders, resulting from an expansion of a CAG trinucleotide repeat in exon 1 of the androgen receptor gene, located at Xq11-q12 (La Spada et al., 1991). The mutation results in an expansion of the repeat from a normal range of approximately 11 to 33 repeats to an expansion of 40 to 62 in cases of the disorder (Brooks and Fischbeck, 1995). A greater size of the expansion appears to correlate with earlier disease onset (Doyu et al., 1992; Igarashi et al., 1992), although there is some variability in the findings of different studies (Amato et al., 1993). The expanded CAG repeat sequence is expressed, encoding an enlarged polyglutamine tract within the androgen receptor protein.

The normal androgen receptor is expressed in the nuclei and perikarya of neurons but not glia. Its wide expression in different neuronal groups, including those of cerebral cortex, hippocampus, basal ganglia, Purkinje cells, dorsal root ganglia, as well as motor neurons, indicates that restricted expression is not an explanation for the motor selectivity of this disorder (Clancy et al., 1992; MacLean et al., 1996; Li et al., 1998a). It is also expressed in non-neuronal tissues, including testes, muscle and scrotal skin, but not liver, spleen or kidney. The androgen receptor appears to have a role in mediating a trophic effect in motor neurons. However, expression of an androgen receptor with an expanded polyglutamine tract appears to cause disease through a gain of function mechanism, in contrast to point mutations in DNA and steroid binding regions of the gene that

lead to the androgen insensitivity syndrome through loss of function (Brooks and Fischbeck, 1995; Ross, 2002).

In common with the other CAG repeat diseases, which include Huntington's disease, dentatorubral-pallidoluysian atrophy, and several forms of spinocerebellar ataxia (SCA 1,2,3,6,7,17), pathological studies of X-SBMA have demonstrated the formation of neuronal intranuclear inclusions (NI) (Li et al., 1998; Ross, 2002). A tendency to aggregate is thus common to the disorders due to polyglutamine repeat region expansion because of the tendency of the elongated polyglutamine tracts to self-associate and potentially to associate with other poly-Q containing proteins essential to cellular function and survival (e.g. CREB-binding protein). The resultant nuclear inclusions contain the mutant protein and may therefore be identified immunohistochemically using antibodies, such as IC2, that recognize an expanded polyglutamine tract. The NI are reactive for the N-terminal portion of the androgen receptor but not the carboxy-terminal portion, implying either that the molecule has undergone proteolytic processing prior to incorporation in the inclusion or that epitopes are masked. Proteins within NI are ubiquitylated, so that, in common with many other types of inclusions in neurodegenerative disorders, they are recognized by ubiquitin immunohistochemistry in autopsy material. NI range from 1 to 5 μm in diameter and the ultrastructure is of non-membrane-bound granular and filamentous material (Yamada et al., 2000; Merry, 2001; Wood et al., 2003). NI are found in residual motor neurons in X-SBMA and also in non-CNS tissues, including testes, scrotal skin, kidney and heart but not liver, spleen or muscle (Li et al., 1998). Within the CNS the neurons in systems not clinically affected have been reported not to contain NI (Li et al., 1998), although a more recent study, using immunohistochemistry with the IC2 antibody, demonstrated NI in a widespread distribution within the CNS of X-SBMA patients (Adachi et al., 2005).

Although a cardinal feature and pathological marker of X-SBMA, the role of NI in pathogenesis is unclear. A number of possible pathogenic mechanisms have been suggested and have been reviewed (Gallo, 2001; Merry, 2001; Ross, 2002; Wood et al., 2003). NI contain a variety of other constituents including molecular chaperones (heat shock proteins) and transcription factors such as TATA-binding protein, CREB and CREB-binding protein (CBP; a co-activator in transcriptional responses to neurotrophic factors which contains a poly-Q sequence; McCampbell et al., 2000; Wood et al., 2003). In a recent transgenic study, down-regulation of CBP expression by mutant androgen receptor resulted in reduction of the expression of a CBP-target, vascular endothelial growth factor, which has been implicated in motor neuron survival (Sopher et al., 2004). Thus, sequestration factors regulating key transcriptional responses may play a role in the pathogenesis of X-SBMA. Nuclear localization of mutant androgen receptor appears to be important for toxicity. Animal models suggest that the nuclear localization and toxicity of the mutant androgen receptor are ligand dependent, and that both the clinical phenotype and histopathologic features of nuclear mutant androgen receptor accumulation can be ameliorated by androgen blockade (Katsuno et al., 2003). Ligand dependence of localization and toxicity would explain why a mutation that appears to cause disease through a gain-of-function does not manifest in heterozygous females.

The relative role of NI or smaller molecular intermediates of aggregation in pathogenesis is unclear (Ross, 2002). A recent immunohistochemical study with the IC2 antibody on autopsy cases has demonstrated that, in addition to NI, there is diffuse nuclear accumulation of mutant protein in X-SBMA. Diffuse accumulation appears to be more extensive than the distribution of NI and correlates better with the extent of CAG repeat expansion (Adachi et al., 2005). Use of the IC2 antibody in this study also demonstrated that cytoplasmic accumulation of mutant androgen receptor are present in X-SBMA, probably localized to the Golgi apparatus. In contrast to the nuclear accumulations, this protein was not ubiquitylated. This neuropathological finding raises the interesting possibility that cytoplasmic mechanisms may also contribute to pathogenesis of this disorder.

5.4. Amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) and related disorders

5.4.1. ALS, and the spectrum of ALS-related disorders

Classical ALS (Charcot's disease) presents as progressive weakness or spasticity in limb, bulbar or respiratory muscle groups ultimately leading to death either through respiratory failure or an associated infection (Brooks, 1994; Brooks et al., 2000). The pathological features are those of motor system degeneration that may affect all levels of the pyramidal pathways and the spinobulbar lower motor neuron populations. In contrast to Alzheimer's disease, Parkinson's disease and many other late-onset neurodegenerative disorders, the precise molecular basis for neuronal dysfunction and death are not established for sporadic ALS but an increasing number of genetic changes have now been described in patients with familial disease (i.e. Cu/Zn Superoxide dismutase [SOD1] and Alsin) (Deng et al., 1993; Rosen et al., 1993; Hadano et al., 2001; Yang et al., 2001). Pathological diagnosis still lacks a 'signature molecule' as the basis for a molecular pathological definition of the disorders (c.f. α-synuclein in Parkinson's disease,

Tau in Alzheimer's disease, Frontotemporal dementias and other tauopathies, β-amyloid in Alzheimer's disease). In the late 1980s a characteristic lesion was demonstrated in ALS using immunocytochemistry for ubiquitin (Leigh et al., 1988; Lowe et al., 1988). These lesions define the substrate for a spectrum of clinical disorders ranging from pure lower motor neuron degeneration (progressive muscular atrophy), spanning ALS and "ALS with dementia," through to pure upper motor neuron degeneration (primary lateral sclerosis) and a non-motor variant of frontotemporal dementia (Ince et al., 1998a; Ince, 2000). This concept of an ALS disease spectrum has also been proposed for other neurodegenerative disorders, such as the Parkinson's disease/a-synucleinopathy spectrum (Ince et al., 2000), and implies that a common molecular pathology defines a disease process which manifests clinically depending upon the relative severity of pathology throughout the nervous system. The genetic and environmental factors that underlie this pattern of susceptibility are not understood. An increased awareness of the extent of extra-motor "multisystem" pathology in ALS has weakened the concept of 'selective vulnerability' in ALS research. It seems that populations of neurons in the CNS and peripheral nervous system express a varying degree of susceptibility to the ALS disease process rather than absolute vulnerability or disease resistance. This has implications for research into the pathogenesis of ALS and suggests that specific biochemical or neurophysiologic properties of motor neurons that underlie vulnerability or resistance in various anatomic regions of the CNS are likely to be subtle and quantitative. The concept of selective vulnerability in ALS is valid but in a more complicated manner than has been assumed in the past. Patients may manifest the same disease process as different clinical phenotypes due to variations in the anatomic expression of the disease process (Ince et al., 1998b). This is illustrated in familial ALS; non-SOD1 linked families are described in whom the disease varies between affected members from those with classical ALS to those with a frontotemporal dementia syndrome. The genetic and environmental basis for such variability is likely to be highly informative in future research into ALS.

5.4.2. Classical ALS

5.4.2.1. Upper motor neuron (UMN) pathology in ALS

UMN pathology in ALS is variable both in pathological extent and in the resulting clinical phenotype. There is no consistent relationship between the extent of UMN involvement at autopsy and the clinical findings. UMN pathology is best demonstrated on the basis of three characteristic features:

5.4.2.1.1. Motor cortex pyramidal neurons The Betz cell population of the motor cortex (Brodmann area 4) may be reduced. This is traditionally interpreted as loss of these giant pyramidal neurons due to degeneration, but this has not been formally demonstrated (Brownell et al., 1970). The somata of Betz cells in the normal motor cortex are very prominent and many cases of ALS show a reduced or absent population of these profiles. There are no neurochemical or immunocytochemical markers that are specific for Betz cells as opposed to other populations of large pyramidal neurons in the cerebral neocortex. Therefore the possibility remains that atrophic changes in Betz cells reduce their size so that they become indistinguishable from adjacent pyramidal neurons. It is likely that Betz cells die in ALS, but there are cases with a clear history of UMN signs and with corticospinal tract pallor at brainstem or cervicothoracic levels in whom the motor cortex appears intact. Therefore the functional substrate of UMN features in ALS is likely to include a major component of distal denervation due to axonal loss which may precede somal degeneration and actual loss of Betz cells from the cortex. The classical molecular pathology of lower motor neurons in ALS, the ubiquitylated neuronal inclusion (UBI, see below), has not been convincingly described in Betz cells.

5.4.2.1.2. Glial pathology in the motor strip and adjacent neocortex The motor cortex in ALS may show variable astrocytic gliosis affecting both gray matter and the underlying subcortical white matter. Irregular immunoreactivity for GFAP has been reported within the motor strip (Kamo et al., 1987) but it is unclear whether this is distinguishable from the variable appearances that may be present in a spectrum of unaffected individuals. Astroglial hyperplasia and hypertrophy, that is most prominent in deeper cortical layers (i.e. layers 5 and 6) and in the cortex-white matter junction, is a feature in some cases of ALS (Ince, 2000) and may be very prominent in the lower two thirds of the cortical ribbon (Fig. 5.3(A, B)) and in the subcortical white matter. This astrocytic gliosis is associated with a significant increase in reactive microglia best observed using the lysosomal marker CD68 or an immunostain for HLA-DR. Microglial activation within the corticospinal tract in the brain and spinal cord is demonstrable clinically in PET studies using the ligand PK11195 to detect the peripheral benzodiazepine receptor (Cagnin et al., 2001; Sitte et al., 2001).

5.4.2.1.3. Pathological changes in the corticospinal tract Axonal loss within the descending pyramidal motor pathway, associated with secondary myelin pallor and gliosis of the corticospinal tracts (CST), is a cardinal feature of the pathology in ALS (Fig. 5.3(C)).

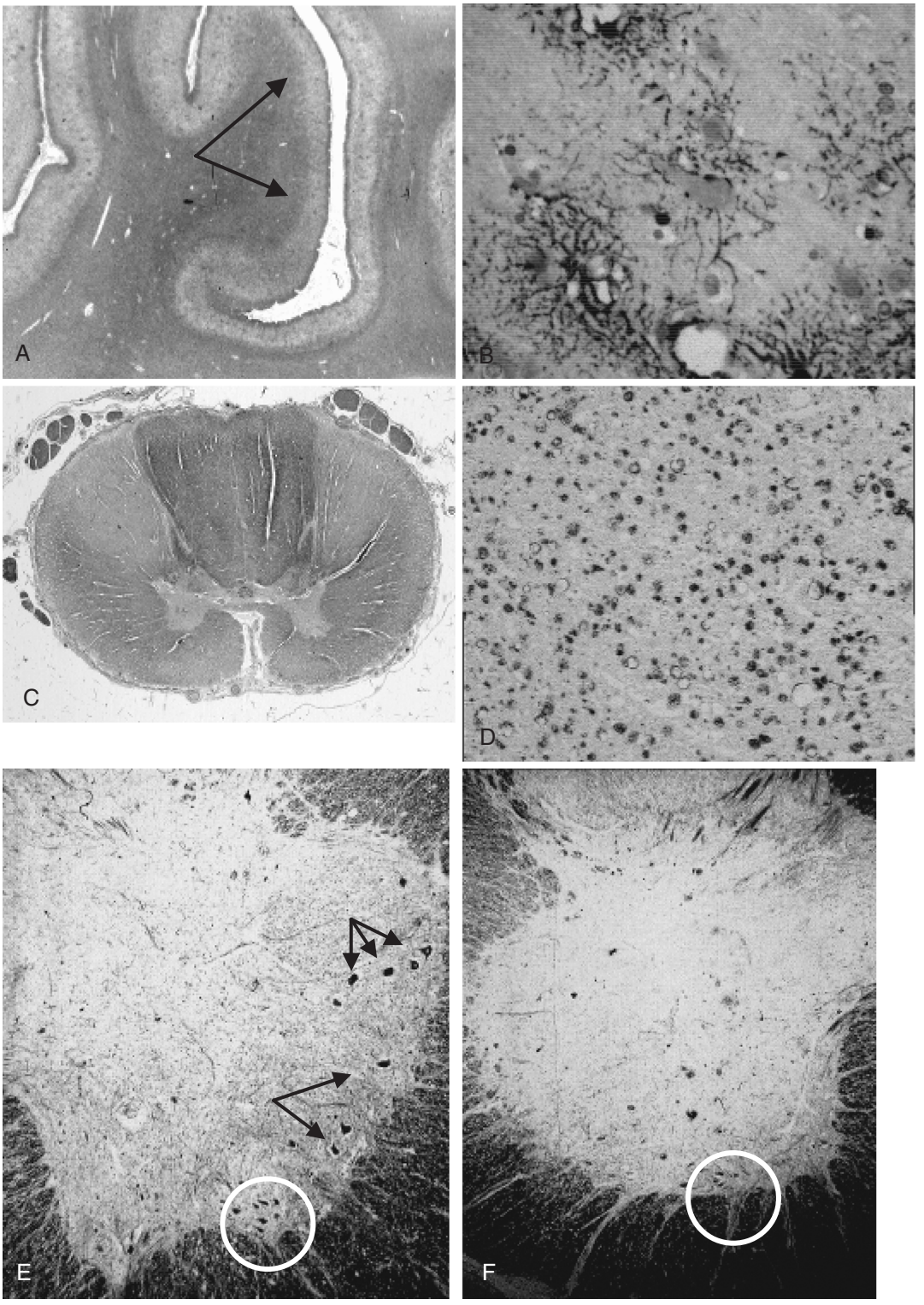

Fig. 5.3. The motor cortex in ALS may show a band of astrocytic gliosis in the lower cortical layers extending into the subcortical white matter (**A**). In other cases with preservation of cortical Betz cell bodies (arrow) the astrocytes show no reactive changes (**B**). The pallor of myelinated tracts in the spinal cord is classically symmetrical and involves the whole anteroventral funiculi (**C**). Loss of myelin in the dorsal column is also present in at least 50% of cases. The most sensitive marker of early myelin loss due to axonal degeneration is an increase in the number of cellular profiles immunoreactive for CD68, a marker of microglial activation and of active macrophages (**D**). In the sacral spinal ventral horn level S2 the normal cord (**E**) shows prominent lower motor neuron cell profiles (arrows). These are lost in ALS (**F**). Note the preservation of motor neurons in Onuf's nucleus (white circles) despite the total loss of motor neurons innervating limb and axial muscles. [Immunocytochemistry for GFAP (**A**, **B**) and CD68 (**D**); Marchi impregnation method (**C**); Luxol Fast Blue/Cresyl Fast Violet (**E**, **F**).]

CST changes are usually most severe in the brainstem and upper spinal cord (Brownell et al., 1970) but can be present right throughout the extent of this axonal projection from the motor cortex to the lumbar spinal cord. Axonal degeneration in the distal corticospinal tract may not reflect neuronal loss from the motor cortex and there is usually no evidence of chromatolysis affecting Betz cell somata. The axonal loss is best demonstrated using immunocytochemistry against heavy or medium chain neurofilament proteins or an axonal silver stain. In man the cortex of BA4 contributes only a minority of the axons of the CST, estimated to be around 10% of the total fibers descending from the neocortex to make monosynaptic connections with bulbospinal lower motor neurons (Chou, 1995). The majority of corticospinal motor axons are derived from adjacent pre-motor cortex regions such as BA6 and it is probable that the disease process that affects Betz cells also affects other cortical pyramidal neurons in ALS. In primates and man the CST is concerned with fine motor control and the corticospinal projection to the cervical enlargement of the spinal cord is much greater than that to the thoracic and lumbosacral regions (Lemon and Griffiths, 2004). Below the cervical enlargement the CST is reduced in volume. Pathological changes in the corticospinal tracts can be demonstrated before myelin pallor is detectable in conventional stains or myelin degeneration demonstrable by the osmium impregnation method of Marchi (Smith et al., 1956) by immunocytochemistry for microglial activation (Fig. 5.3(D)). Using immunocytochemistry against CD68, a marker of microglial activation, it has been shown that at least half of cases who come to autopsy with a clinical diagnosis of progressive muscular atrophy, implying a pure lower motor neuron disorder throughout the course of the illness, have some degree of CST pathology (Ince et al., 2003). The implication of this observation in terms of the spectrum of disorders which make up "ALS/MND" is discussed below.

5.4.2.2. Lower motor neuron (LMN) pathology in ALS ALS principally affects spinal motor neurons of the ventral horn and brainstem motor neuron groups but there is sparing of the motor nucleus of Onufrowitz in the S2 spinal segment (Iwata and Hirano, 1978), which innervates skeletal muscles of the pelvic floor to control bladder and bowel sphincter function and has been shown to have typical motor neuron characteristics (Pullen et al., 1992) and the cranial motor nuclei of the oculomotor, trochlear and abducens nerves (Fig. 5.3(E, F)). The explanation for this selective resistance to degeneration in ALS has not been fully explained. Candidate factors include the absence or paucity of direct corticospinal monosynaptic innervation, differences in levels of calcium binding protein expression (Ince et al., 1993), size of neurons and differences in the neurophysiologic activity compared to most alpha motor neurons. None of these factors is firmly established to have a significant role.

Formal motor neuron counts in the spinal cord in ALS at autopsy show considerable variation between patients and at different spinal levels. These variations reflect the variable anatomical severity of the disease process and the individual factors which result in the fatal outcome in each individual's illness. However, typical counts for the lumbar spinal cord in ALS show an approximate loss of 50% of LMN at autopsy (Ince, 2000). Many of the remaining neurons show abnormal appearances, most frequently atrophic and basophilic changes. These have been interpreted as part of the spectrum of a programmed cell death pathway progressing from "axodendritic pruning" to cell death (Martin, 1999). In contrast true chromatolysis is rare, although changes described as chromatolysis are often mentioned in the ALS literature but may be representative of changes indistinguishable from normal aging. Widespread LMN chromatolysis, especially in the absence of the characteristic ubiquitylated inclusions of ALS, may be an indication of another pathology such as mitochondrial disease (Borthwick et al., 2006). Other cytopathologic features such as vacuolation are seldom seen and may also not represent specific pathology (Morrison et al., 1959; Shaw et al., 1997). Other pathological changes associated with lower motor neuron loss and corticospinal tract degeneration in the spinal ventral horn include loss of the presynaptic marker synaptophysin (Ince et al., 1995), MAP2 (Kikuchi et al., 1999) and astrocytic glutamate transporter subtypes (Fray et al., 1998). These changes are associated with diffuse and 'non-specific' astrocytic gliosis.

5.4.2.2.1. Neuronal inclusion bodies in ALS The key feature of lower motor neuron pathology in ALS is the presence of inclusion bodies within the soma and proximal dendrites. The classification and nomenclature of intraneuronal inclusions in ALS is unresolved and the literature includes a bewildering diversity of variations. Prior to 1989, the existence of ubiquitylated inclusions was not appreciated. These have emerged as the most frequent and almost universal neuronal lesion of sporadic ALS (Leigh et al., 1988; Lowe et al., 1989; Ince et al., 1998a). However the previous literature, and even much of that published since 1989, refers to a proportion of cases in which conventional stains demonstrate round inclusion bodies. These show various appearances described as basophilic or eosinophilic, other examples are amphophilic and show a peripheral halo so that they resemble Lewy bodies. This appearance has been dubbed "Lewy-like" or "Lewy-like hyaline" inclusions

(Murayama et al., 1989; Sasaki et al., 1998; Takehisa et al., 2001). It is our perspective that all these classes of inclusion body have either been shown to be ubiquitylated inclusions (see § 5.4.2.2.2), or else there is no mention of the use of ubiquitin immunocytochemistry to characterize the lesions. The pathological literature on inclusion bodies in ALS should now be regarded as having two distinct eras, i.e. before and after ubiquitin immunocytochemistry. All data from studies that did not use ubiquitin immunocytochemistry should be treated with caution and cannot be assimilated directly into the current literature or understanding. Even in the present era difficulties remain in the interpretation of reports in the literature because the panel of antibodies used to characterize the intraneuronal inclusions is incomplete or poorly illustrated.

5.4.2.2.2. Ubiquitylated inclusions (UBI) The UBI of ALS show a morphological spectrum extending from a few filamentous thread-like ubiquitylated profiles, through skeins of varying compactness (Fig. 5.4(A, B, C)), to more compact spherical bodies (Ince et al., 1998a) (Fig. 5.4(D, E)). The latter may show filamentous periphery. It is these compact lesions that seem to correspond to the eosinophilic, basophilic and "Lewy-like" inclusions reported in the literature on ALS. In sporadic ALS the prevalence of UBI is virtually 100% (Ince et al., 2003; Piao et al., 2003). This high frequency of UBI in clinically confirmed cases indicates that these lesions should be regarded as a necessary part of the pathologic diagnosis of ALS. The few cases in which they are not easily found include those with very severe neuronal loss and atrophy in end-stage disease. Other cases with a clinical diagnosis of ALS/MND in which UBI are not detected include examples of familial disease (unrelated to the SOD1 gene) and atypical cases (Shaw et al., 1991; Borthwick et al., 2006).

There is no evidence that the formation of these lesions involves any pathway concerned with the aggregation of tau, α-synuclein, neurofilament or tubulin (Mather et al., 1993). On that basis the designation 'Lewy body-like' inclusion is particularly unhelpful and has spawned misunderstanding among non-clinical scientists engaged in ALS research who may be less

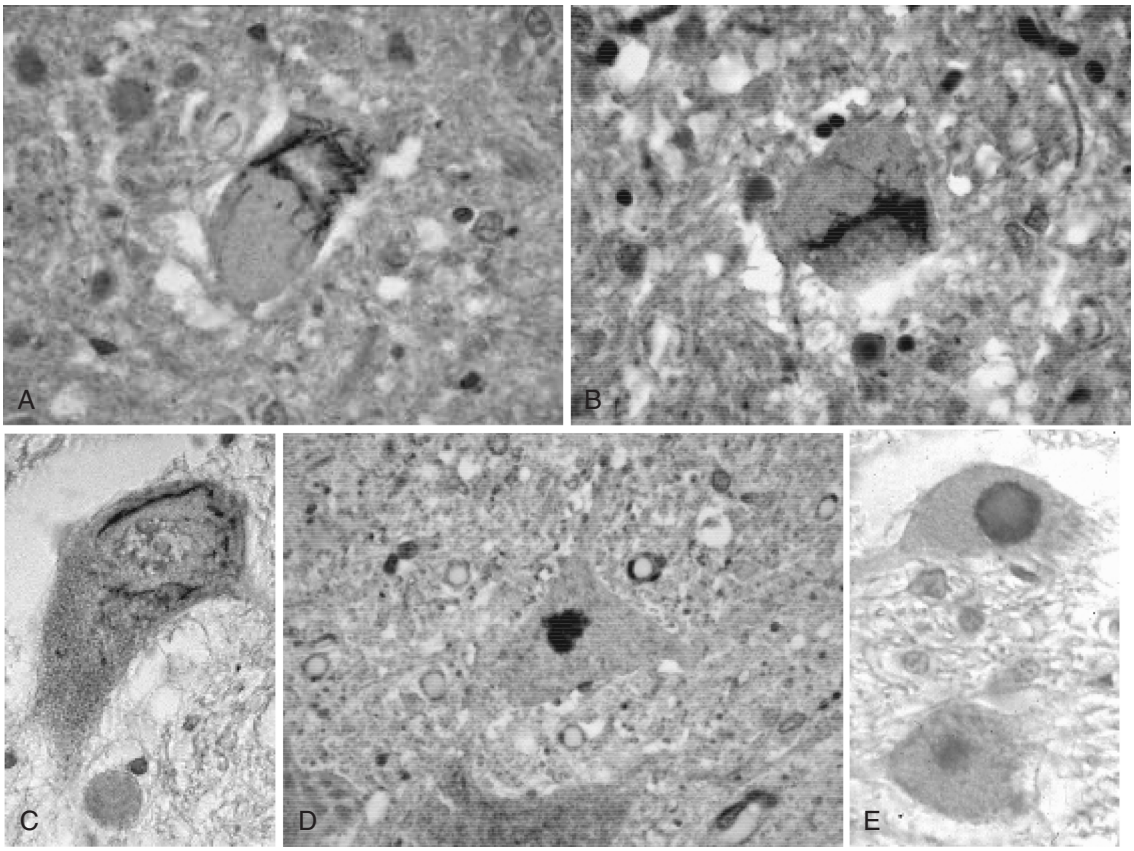

Fig. 5.4. Ubiquitylated skein-type inclusions (**A**, **B**, **C**) and compact ubiquitylated inclusions in spinal motor neurons (**D**, **E**) in ALS. [Immunocytochemistry for ubiquitin.]

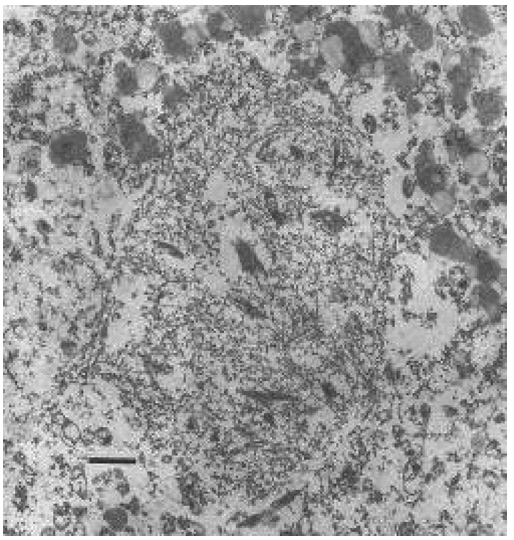

Fig. 5.5. Electron micrograph of a compact ubiquitylated inclusion within a spinal motor neuron shows a mixture of filaments and granular material. [Scale bar = 1 μm.]

aware of the subtleties and implications of the various inclusion bodies which characterize the spectrum of age-related neurodegenerative disorders. UBI are not detected by antibodies to any of the known molecular markers that characterize Alzheimer's disease, tauopathies, Parkinson's disease, other α-synucleinopathies or the trinucleotide-repeat expansion disorders. Without knowledge of the substrate(s) for ubiquitylation in ALS and related disorders they currently stand apart from the other major "families" of neurodegenerative disease. The EM appearance of both skein and compact inclusions (Fig. 5.5) consists of an accumulation of straight filaments of 15–20 μm diameter with abundant granular and amorphous material (Lowe, 1994).

5.4.2.2.3. Hyaline (neurofilament) conglomerate inclusions (HCI) These lesions occur in a much lower frequency of cases compared with UBI. They are especially associated with familial ALS in which pathogenic mutations of the *SOD1* gene are identified but do appear to arise infrequently in apparently sporadic cases. Clearly there is a pressing need to characterize such sporadic cases in terms of potential SOD1 mutations. However, not all SOD1 FALS cases have HCI pathology so that some have typical UBIs. The most consistent association with HCI is found in cases with the *SOD1* mutations A4V and I113T (Rouleau et al., 1996; Ince et al., 1998c). The HCI are larger and more prominent in conventional stains than UBIs, comprising irregular conglomerations of material with no internal structure on conventional light microscopy (Fig. 5.6(A, B)). They are detectable with silver stains in contrast to UBIs which are non-argyrophilic. This prominence in conventional stains allows some confidence in retrospective review of the "pre-ubiquitin" ALS literature confirming the view that they are very infrequently encountered in sporadic cases. Unfortunately the term 'hyaline' is also adopted as part of the nomenclature of the "Lewy-like hyaline" inclusions, as discussed above (§ 5.4.2.2.2) so that it is unclear whether "hyaline" inclusions in the previous literature were truly HCIs or, more probably, UBI. Perhaps the term "Neurofilament Conglomerate Inclusion" (NCI) would be the best nomenclature?

HCI are immunoreactive to a variety of antibodies directed at heavy and medium chain neurofilament protein but are not consistently stainable for neurofilament light (Kondo et al., 1986; Munoz et al., 1988; Kokubo et al., 1999). Antibodies to both phosphorylated and non-phosphorylated forms of neurofilament detect HCI (Fig. 5.6(C, D)) and there is no basis for the speculation that phosphorylation may be a key element in their pathogenesis (Rouleau et al., 1996). This latter study is an example of a literature report in which an inadequate panel of antisera was used and the data inappropriately over-interpreted. Staining with neurofilament antibodies demonstrates that affected neurons lose the normal neurofilament cytoskeleton from the remainder of the soma, and shows abnormal somatic neurofilament phosphorylation (Ince et al., 1998c). These observations suggest a generalized defect in neurofilament biology and intracellular distribution. A number of case reports of SOD1 gene associated FALS do not describe HCI but report the presence of classical, non-neurofilament staining UBI (Ince et al., 1996). The reasons why some SOD1 mutations are associated with HCI while others are not is unclear. Whether it reflects specific properties of the mutant SOD1 species present or is a reflection of some background genetic or environmental determinant is an interesting but unanswered question. Studies of sporadic ALS suggests that some degree of abnormal neurofilament phosphorylation may contribute to the cytopathology in most cases of ALS without the formation of discrete inclusion bodies (Hirano et al., 1984; Mannetto et al., 1988; Munoz et al., 1988; Sobue et al., 1990).

HCI should be regarded as separate structures from the "spheroids" and "globules" that are frequent in the spinal anterior horn in health and disease (Carpenter, 1968). These profiles are also neurofilament rich and located in the axons and dendrites of spinal LMN. Some authors claim they are more frequent in ALS, and they are often cited in support of the hypothesis of generalized changes in neurofilament metabolism and axonal transport in ALS. However, there is quantitative literature on the prevalence of spheroids which has failed to demonstrate any excess of spheroids in sporadic ALS (Leigh et al., 1989; Sobue et al., 1990).

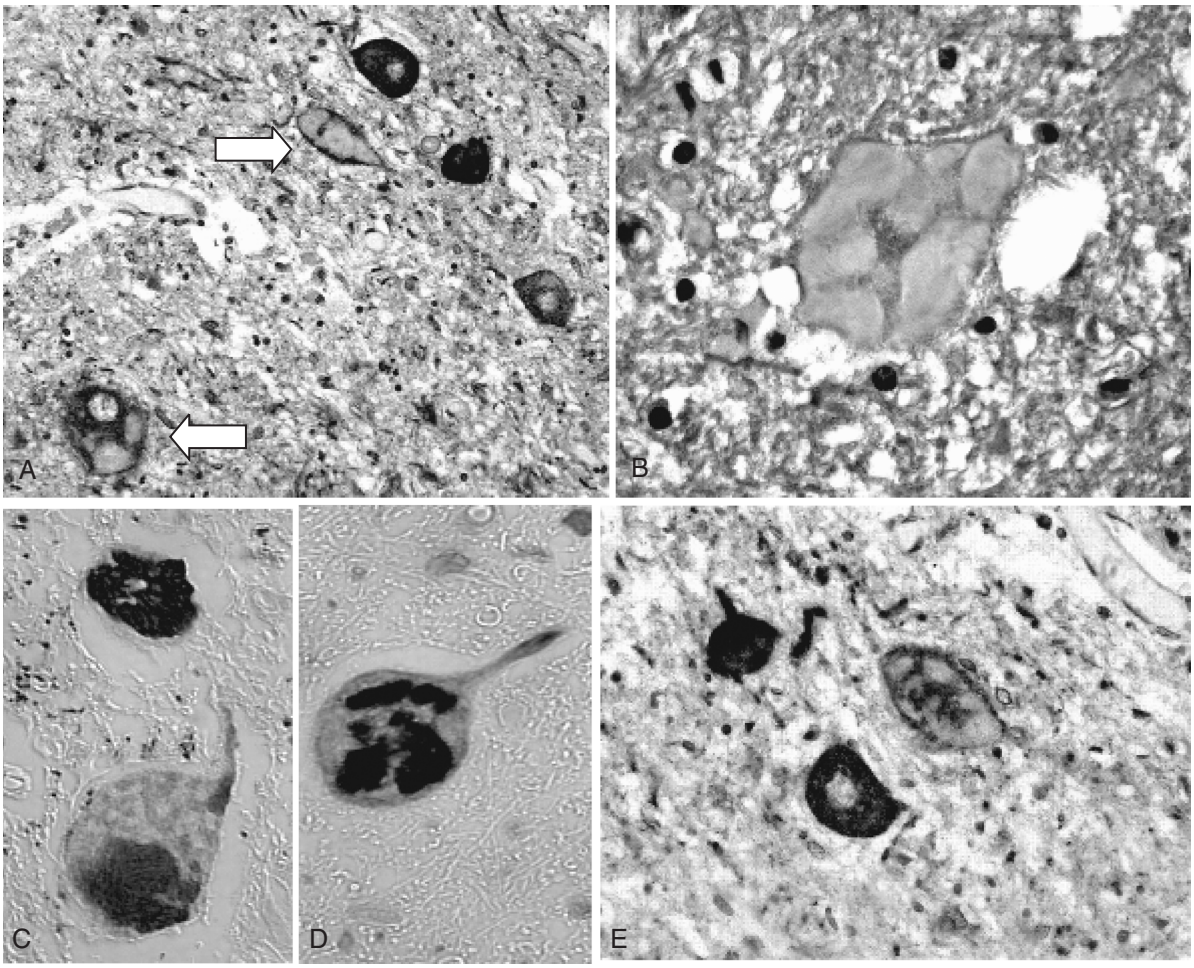

Fig. 5.6. Hyaline conglomerate inclusions in spinal motor neurons from cases with an SOD1 I113T mutation (arrows). The lesions show an amorphous texture (**A**, **B**) with multiple interlocking foci of aggregated material. This material stains strongly for both phosphorylated (**C**) and non-phosphorylated (**D**) neurofilament epitopes. Note the absence of diffuse background immunoreactivity for non-phosphorylated neurofilament in the soma of affected cells suggesting a profound disturbance of neurofilament regulation. These inclusions do not stain for SOD1 despite strong staining of the cytoplasm of adjacent normal motor neurons and in the unaffected cytoplasm of the neuron containing a conglomerate inclusion (**E**). [Haematoxylin & Eosin (**A**, **B**); Immunocytochemistry for SMI31 (**C**), SMI32 (**D**) and SOD1 (**E**).]

5.4.2.2.4. Bunina bodies These eosinophilic, paracrystalline bodies are present in lower motor neurons in many cases of ALS. In a large series of >100 cases the relative frequency was 86% (Piao et al., 2003). It is unclear whether this is the true prevalence in ALS or whether there are cases in which they are not readily demonstrated without a more extensive sampling protocol. This problem applies also to UBI to the extent that there is no consensus protocol which defines the number and location of spinal segmental levels that should be examined in the microscopical diagnosis of ALS or how many tissue sections should be screened at a particular spinal level.

Bunina bodies were first described in 1962 (Bunina, 1962) and have been shown to be immunoreactive to cystatin C a proteinase inhibitor with some selectivity for cathepsin B (Okamoto et al., 1993; Nycander et al., 1998). A quantitative study in ALS has suggested that there is a less robust relationship between the frequency of bunina bodies and lower motor neuron cell loss compared with UBI (van Welsem et al., 2002).

5.4.3. Extra-motor pathology in ALS and the spectrum of ALS-related disorders

Extramotor system pathology has been recognized to be a feature of ALS for many years (Brownell et al., 1970), although the extent and nature of such changes was unclear prior to the era of molecular pathology.

There is evidence in the literature that ALS patients may develop a severe multisystem degeneration either spontaneously (Machida et al., 1999) or in the context of prolonged ventilatory support (Hayashi and Kato, 1989; Mizutani et al., 1992; Hashizume et al., 1993; Kato et al., 1993a; Ono et al., 1997). Such changes particularly may affect the oculomotor nuclei, dentatorubropallidal system and the substantia nigra.

More recently a survey of CNS pathologies in ALS, including these non-motor system features, has been developed by immunocytochemical studies in the literature. The impact of this information for patients with typical ALS is limited, but the characterization of this pathology has profound implications in terms of our understanding of the relationship between ALS and other syndromes in which motor system degeneration is part of a more complex clinical phenotype. It also impacts on key concepts in pathogenetic research, especially "selective vulnerability" as discussed above.

The main method for defining extramotor pathology historically was gliosis and neuronal loss. Such changes are subjective and difficult to ascribe specifically to the effects of the degenerative process which underlies ALS. More recently the use of ubiquitin immunocytochemistry has introduced more precision so that neuronal lesions of the hippocampus, frontal neocortex and substantia nigra can be accurately attributed to ALS. However, even with ubiquitin, there are significant problems of interpretation. The normal brain in mid-life and in aging is associated with variable ubiquitin immunoreactivity that is assumed to be "physiologic" rather than a marker of pathology. This immunoreactivity includes structures such as "ubiquitin-dot profiles" which have been shown to be non-pathologic in many circumstances but are similar in morphology to the lesions in ALS (Papolla et al., 1989; Dickson et al., 1990; Migheli et al., 1992). In the diagnosis of classical ALS this problem of the non-specificity of ubiquitin immunoreactivity as a reagent for demonstrating neuronal inclusions is not a significant issue. This is because the lesions are present in a characteristic distribution – the soma and dendrites of spinobulbar lower motor neurons – and they have a characteristic morphology that is quite distinct from ubiquitin dot profiles in the brain. However, the diagnosis of extramotor pathology in ALS requires a more subjective appraisal. The most frequently affected regions, such as hippocampus, frontal cortex and substantia nigra (see below), usually contain significant age-related and non-specific ubiquitin immunoreactivity and these profiles resemble the ALS-associated pathology in these same regions. At the present time a new marker is emerging which also labels the ubiquitylated lesions of age-related neurodegenerative diseases (Zatloukal et al., 2002). This molecule is P62, a physiologically promiscuous transcription regulator, which has multiple functional domains including a ubiquitin-binding domain (Geetha and Wooten, 2002; Seibenhener et al., 2004). It has been reported to be a variable constituent of the intraneuronal inclusions that characterize Lewy-body diseases (Kuusisto et al., 2003), frontotemporal dementias (Furukawa et al., 2004), polyglutamine repeat tract expansion diseases (Nagaoka et al., 2004) and ALS-dementia (Nakano et al., 2004). The advantage of P62/Sequestosome 1 staining over ubiquitin for diagnostic purposes is that it does not stain age-related physiological ubiquitylated material, giving much cleaner results that are more straightforward to interpret. However, there are no literature reports at present which indicate the relative sensitivity of p62 staining or which indicate what proportion of pathological ubiquitylation is also reactive to p62 antibodies.

The extent and characteristics of these extra-motor features is highly variable and can include any or all of the following:

5.4.3.1. Spinocerebellar pathways

The spinocerebellar pathways lie in the dorsal and ventral spinal white matter and are derived from neurons situated in the associated spinal nuclei. For the dorsal spinocerebellar tract this is the ipsilateral thoracic nucleus of Clarke. The ventral tract has no clearly defined nucleus and is believed to arise from ipsilateral and contralateral neurons in the spinal posterior and lateral gray matter. Classical accounts of ALS pathology describe pallor of the whole anterior and lateral spinal white matter funiculi, so that the spinocerebellar tracts are affected in most cases of ALS (Swash et al., 1986). Degeneration of neurons in Clarke's nucleus is reported in terms of cell loss. However, the numerical data published appear to overstate the normal population of neurons observed in transverse sections of the spinal cord (Averback and Crocker, 1982). In contrast to this numerical data there is no evidence that the neurons of Clarke's column are susceptible to UBI formation so that, if neuronal loss does occur, the mechanism of cell death and its relationship to motor neuron loss in ALS, is unclear.

Myelin pallor attributable to axonal loss in the whole of the spinal anterolateral white matter is common in ALS (Oyanagi et al., 1995). The origin of these fibers in the brainstem tegmentum and from cervical spinal propioceptive pathways has been demonstrated and further indicates the multisystem nature of tract degeneration in ALS (Oyanagi et al., 1999).

5.4.3.2. Ascending sensory pathways

The ascending sensory pathways of the dorsal column are very commonly affected in ALS (Lawyer and Netsky, 1953; Castaigne et al., 1972; Iwata and Hirano, 1979;

Hudson, 1981). Clinically this has been confirmed using sensory evoked potentials (Radtke et al., 1986). The pathological changes affect the neurons innervating the lower limbs predominantly, possibly reflecting a general predilection for involvement of the longest axonal pathways in ALS. In the Japanese literature there is an emphasis on the prevalence of dorsal column pallor in familial ALS. The concept of "familial ALS with posterior column involvement" is often used to describe a specific subgroup of cases (Murayama et al., 1989). However other work suggests that posterior column myelin pallor is detectable at autopsy in up to 50% of all sporadic ALS cases and is in no way specific for familial or SOD1-related cases (Ince et al., 2003; Piao et al., 2003).

Further evidence for both central and peripheral sensory system involvement in ALS includes dorsal root ganglion cell loss (Kawamura et al., 1981), loss of sensory nerve axons (Dyck et al., 1975; Bradley et al., 1983) and thalamic gliosis (Brownell et al., 1970).

5.4.3.3. Substantia nigra

ALS is not an α-synucleinopathy but occasional cases in which both Parkinson's disease and ALS co-exist have been described (Williams et al., 1995). The co-existence of Parkinsonism and ALS as part of the Parkinsonism-dementia/ALS complex of Guam is well recognized but is probably not relevant to the issue of extramotor pathology in classical ALS. This judgment is made on the basis of review of the published literature on Guamanian ALS which shows little evidence for classical UBI pathology in spinal motor neurons in that disease and clearly describes a spinal neurofibrillary tangle disorder (Ince and Codd, 2005).

Many cases of ALS have substantia nigra neuronal loss at autopsy, especially in patients with combined ALS-dementia (Kato et al., 1993b). In some cases UBI pathology is present in the SN including cases with no clinical evidence of Parkinsonism or overt dementia syndrome (Al-Sarraj et al., 2002). This non-α-synucleinopathy nigral degeneration may be very severe and the absence of clinical evidence of Parkinsonism is likely to be a consequence of the coexistence of amyotrophy and spasticity. It is unclear how often nigral degeneration exceeds age-related changes because there are no good quantitative studies of the nigra in ALS. However it is the authors' impression that nigral degeneration is not uncommon in ALS and is not always clearly linked to 'ubiquitin-only' pathology.

5.4.3.4. Hippocampus and frontotemporal UBI pathology

The description of dentate gyrus granular cell inclusions in ALS-dementia was a seminal observation in the developing concept of ALS as a syndromic illness as part of a spectrum of disorders linked by a common pathogenesis. These lesions within dentate granule cells of the dentate fascia are immunochemically indistinguishable from UBIs and occur in cases of ALS in which UBIs are also present in the motor system (Okamoto et al., 1991; Wightman et al., 1992). Morphologically they comprise rounded and elliptical profiles together with more irregular profiles which resemble small skeins. Their presence in the hippocampus, and in the frontal and temporal neocortex, appears to provide a pathological substrate for dementia both in the context of ALS and in cases of frontotemporal dementia with no overt motor system signs (Caselli et al., 1993; Brun et al., 1994; Cooper et al., 1995; Jackson et al., 1996). Among the frontotemporal dementia disorders a new entity has emerged which has been given various names including "Motor Neuron Disease Inclusion Dementia" (MNDID). The shared 'ubiquitin-only' pathology across a spectrum from Progressive Muscular Atrophy through ALS and ALS-dementia to pure frontotemporal dementia suggests a spectrum of disease presenting syndromically on the basis of the most affected parts of the CNS (Ince et al., 1998a, 2003; Ince, 2000). It is important to note that these disorders are not pathologically or anatomically exclusive: MNDID cases have typical spinal UBI pathology at low frequency (Holton et al., 2002) and hippocampal dentate granular cell inclusions are present in up to a third of typical ALS patients (unpublished observation, PGI). There are also autopsy confirmed cases of "ubiquitin-only" pathology in which a typical frontotemporal dementia of "Pick's disease" type was present for more than a decade prior to the overt clinical expression of ALS (Tsuchiya et al., 2001)

Neuropsychological deficits and functional brain imaging changes have been identified in patients with ALS in whom no clinical dementia syndrome is present (Ludolph et al., 1992; Kew et al., 1993; Abrahams et al., 1996; Chari et al., 1996). Therefore, it is likely that subclinical frontal lobe dysfunction is common in ALS and is likely to correlate with the cerebral UBI pathology described above.

5.4.4. Cytopathology of familial ALS (FALS)

More than 100 pathogenic mutations in the *SOD1* gene are described that cause familial ALS. However, only a small proportion of these mutations is represented in the literature by an examination at autopsy. Regrettably among these existing case reports of SOD1 FALS individual reports are often incomplete in terms of the pathological information provided. It is therefore not yet possible to describe the full range of molecular pathological findings associated with SOD1 FALS.

Familial ALS may be related to *SOD1* gene mutations (SOD1 FALS) or non-SOD1 associated. In the latter the cases are represented in the literature as proven "SOD1-negative" cases, those with another defined genetic locus or Alsin gene mutation (Andersen, 2003) or as cases in which SOD1 and other screening was not available or not carried out. These uncharacterized cases predominate in the older literature and add an additional complexity in the interpretation of the pathological literature about FALS.

Most authors emphasize the similarity between non-SOD1 FALS, SOD1 FALS and sporadic ALS, although there is no autopsy data for Alsin-related FALS. Intraneuronal inclusion bodies of both UBI and HCI types have been described in SOD1 FALS (Ince et al., 1996, 1998c). It is unclear from the limited pathology data which specific mutations in the SOD1 gene are associated with UBIs or HCI. Within the limitations of this literature, and the need to subjectively interpret the incomplete characterization of inclusions in several reports, the following summary is offered: HCI have been described in three mutations: A4V (Chou et al., 1996; Rouleau et al., 1996; Ince et al., 1998c), I113T (Chou et al., 1996; Rouleau et al., 1996; Ince et al., 1998c; Kokubo et al., 1999) and H48Q (Shaw et al., 1997). In contrast, UBIs have been reported in association with the mutations A4T (Takahashi et al., 1994), E100G (Ince et al., 1996), L126S (Takehisa et al., 2001), H48Q (Shaw et al., 1997), D101N (Cervenakova et al., 2000), D101Y (Tan et al., 2004) and del125-126 (Kato et al., 1996; Kadekawa et al., 1997). Case reports of the mutations H46R (two cases examined) and V118L did not identify any inclusion bodies (Shimizu et al., 2000; Ohi et al., 2002; Arisato et al., 2003), possibly due to the limited tissue available for study and severe end stage disease.

The novel mutation G127insTGGG results in the formation of a truncated SOD1 species which has an aberrant and novel nonsense terminal sequence of five amino acids from Gly127 (Jonsson et al., 2004). This generates an epitope unique to the mutated form of SOD-1 and antibodies were raised against it. These show marked cytoplasmic accumulation of the mutant enzyme although quantification shows that the mutant enzyme comprises only 0.5% of the level of SOD-1 in control subjects. The colocalization of this immunoreactivity in relation to either neurofilament conglomerates or skeins was not demonstrated. However this report claims to illustrate ubiquitylated skein type lesions within motor neurons.

The mutations A4V and D101N are associated with relative preservation of the corticospinal tract which may reflect very rapidly progressive disease in which death supervenes through LMN respiratory failure prior to clinical expression of CST degeneration (Cudkowicz et al., 1998; Cervenakova et al., 2000).

The literature on SOD1 transgenic mice shows that SOD1 accumulates in affected tissues (Bruijn et al., 1997, 1998). There are a number of reports claiming that anti-SOD1 antibodies react with "Lewy-like hyaline inclusions" in human SOD1 FALS (Shibata et al., 1994, 1996, 1998; Chou et al., 1996; Sasaki et al., 1998; Watanabe et al., 2001). The morphology and immunoreactivity of these lesions as they are illustrated and described is different from UBI in that they are distinctly hyaline rather than fibrillary, rounded, and show a concentric pattern with a dense core. A single report suggests that they can be found in some sporadic patients (Matsumoto et al., 1996). The possibility that this morphology actually represents corpora amylacea has not been adequately addressed. In the authors' experience of autopsy tissue from three cases of I113T SOD1 FALS the neurofilament conglomerate inclusions are not immunoreactive to SOD1 (Fig. 3.6(E)).

Astrocytic hyaline inclusions are also described in both human SOD1 FALS and in animal models (Bruijn et al., 1997, 1998).

5.5. Hereditary spastic paraparesis

Hereditary spastic paraparesis (HSP), or Strümpell-Lorrain syndrome, is a group of genetically determined disorders whose primary clinical manifestation is slowly progressive spastic paraparesis (McDermott et al., 2000; Fink, 2002a). The disorder was first described by Strümpell in 1880, who also provided the first neuropathological description, demonstrating that the spastic paraparesis was due to degeneration of the corticospinal tract. The presence of complicated forms of HSP where spastic paraparesis may be accompanied by a variety of other neurological manifestations, such as epilepsy, dementia, ataxia, extrapyramidal involvement, deafness, optic atrophy, retinopathy or cataracts, indicates that pathology may not be confined to the motor system. Even in so-called pure HSP, older cases may develop sensory and urinary manifestations (Harding, 1981) whilst detailed testing reveals a high incidence of sub-clinical lesions affecting various central nervous systems (Tedeshi et al., 1991). The presence of extra-motor manifestations suggests that multisystem pathology may be present, even in pure HSP.

The genetic classification of HSP continues to evolve with the discovery of new linked loci. Most of the reported neuropathological studies on HSP are of cases that have not been characterized for the gene defect, so that further studies are needed in this area to relate neuropathological findings to specific gene defects. To date, 27 loci have been linked to HSP and 11 genes

identified (Figlewicz, 1999; Fink, 2002b, 2003; HUGO), including autosomal dominant, autosomal recessive and X-linked patterns of inheritance. Genes identified to date include spastin, hsp60, seipin, kinesin heavy chain, atlastin, NIPA1, paraplegin, spartin, maspardin, L1CAM and proteolipid protein. Some of these are considered in relation to neuropathology below. The varying gene functions suggest a variety of mechanisms by which corticospinal tract degeneration may occur. A common factor is that the comparative length of the axons of the corticospinal tract may render them vulnerable to a variety of mechanisms that impair axonal physiology.

5.5.1. Neuropathology

The cardinal neuropathological feature of HSP is myelin pallor and axonal loss from the lateral and, more variably, anterior corticospinal tracts (Schwarz, 1952; Schwarz and Liu, 1956; Behan and Maia, 1974; Sack et al., 1978). The changes are more marked distally, in thoracic and lumbar segments (Fig. 5.7(A, B)). The dorsal columns may also be involved, particularly fasciculus gracilis at cervical levels, again reflecting worse distal involvement. There is variable spinocerebellar involvement. The more severe involvement of distal parts of these long tracts has led to the suggestion of a dying-back axonpathy. This is supported by quantitative examination, which demonstrates relative distal loss of axons. This loss appears not to be size-selective, involving both large and small fibers (DeLuca et al., 2004). A study in spastin-related HSP has shown that axonal loss is accompanied by a microglial reaction (Wharton et al., 2003).

The cytopathology of neuronal cell bodies is less clear. Some studies have demonstrated a loss of Betz cells from the motor cortex (Schwarz and Liu, 1956; Ferrer et al., 1995), whilst others have demonstrated no loss (White et al., 2000) (Fig. 5.7(C)). All of these studies have been limited in terms of their methodology for this particular question. Lower motor neurons are generally said to be uninvolved (Schwarz, 1952; Behan and Maia, 1974; Sack et al., 1978), although lower motor

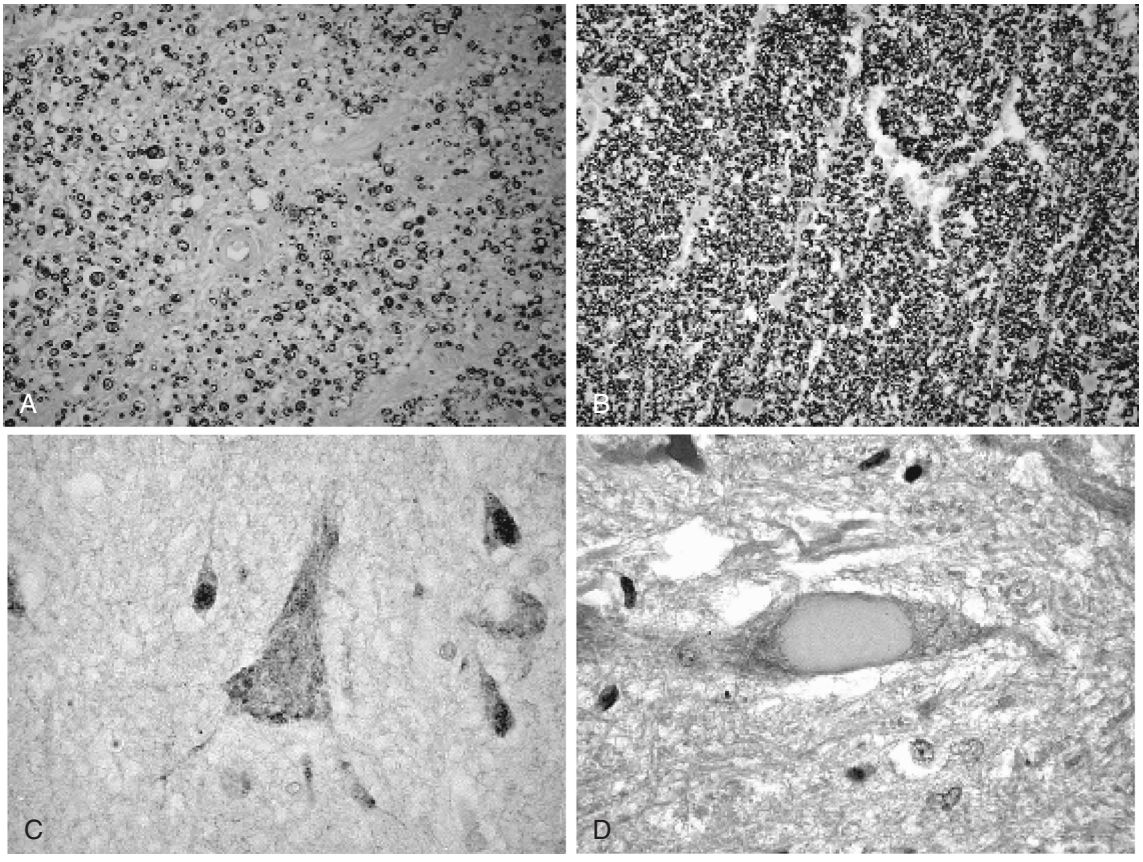

Fig. 5.7. In HSP the extent of axonal loss in the corticospinal tract (**A**) contrasts strongly with normal myelin in the dorsal column (**B**). Despite the axonal loss in the spinal cord and brainstem the motor cortex contains normal Betz cell profiles (**C**). Spinal lower motor neurons infrequently show abnormalities such as large cytoplasmic inclusions (**D**) [Luxol Fast Blue (**A**, **B**); Haematoxylin & Eosin (**C**, **D**).]

neuron cytopathology has recently been described in spastin mutation related cases (Wharton et al., 2003) (Fig. 5.7(D)).

Abnormalities have also been described out-with the pyramidal motor system. In a study of a case of HSP with dementia, atrophy of pre- and post-central, superior temporal and frontal gyri was noted, associated with decreased white matter, atrophy of corpus callosum and deep gray nuclei and depigmentation of substantia nigra. The cortex showed a loss of neurons and immunohistochemical studies demonstrated a decrease in calbindin D28K+ cells and parvalbumin reactive dendrites (Ferrer et al., 1995).

5.5.2. Neuropathology in relation to specific gene defects

HSP is a genetically heterogeneous group of disorders and different gene defects may be associated with different clinical phenotypes in addition to the core symptomatology. There are few detailed neuropathological studies on cases of HSP with defined gene defects. As biological studies continue to unravel the function of the genes whose mutation may lead to HSP phenotypes, it is becoming clear that there are a number of candidate cellular mechanisms for the production of corticospinal tract degeneration (reviewed in Casari and Rugarli, 2001; Reid, 2003). Further neuropathological studies of genetically defined cases will be required to define the pathological basis of clinical phenotypes and to test mutation-specific hypotheses of pathogenesis. The extent to which gene-specific neuropathological studies have been carried out and areas in which developments in pathogenesis studies might suggest neuropathological findings are discussed below.

Mutation in the gene for spastin at the SPG4 locus on chromosome 2p is the single most common autosomal dominant form of HSP. Spastin is a member of the AAA (ATPases associated with diverse cellular activities) group of proteins (Hazan et al., 1999). The AAA proteins are involved as 26S proteasome components, organelle biogenesis associated proteins, metalloproteases and cell-cycle regulators (Patel and Latterich, 1998), so that there are many potential candidate mechanisms for pathogenesis. Within the CNS, spastin is a neuronal protein, apparently not expressed in glia (Charvin et al., 2003; Wharton et al., 2003), but its detailed subcellular localization and function are unclear. Spastin has a nuclear localization signal (Hazan et al., 1999) and some studies have suggested nuclear expression (Charvin et al., 2003). However, other cell culture data suggest a cytoplasmic function and association with microtubules. A role in microtubule dynamics, perhaps similar to the microtubule severing protein katenin, has been suggested (Errico et al., 2002; McDermott et al., 2003). Neuropathological study using immunohistochemistry on autopsy cases has supported a predominantly cytoplasmic localization for spastin in neurons, particularly motor neurons, although localization may be nuclear in some neuronal groups (Wharton et al., 2003). A recent study has suggested that spastin localizes to regions rich in dynamic microtubules and interacts with centrosomal protein NA14. Spastin may have complex functions, dependent on cell type and cell-cycle state, but may have an important role in cytoskeletal dynamics (Errico et al., 2004). Mutation in spastin might therefore perturb microtubular and cytoskeletal function, perhaps affecting transport of organelles such as mitochondria. This would be expected to have a particular impact in the long axons of the corticospinal tracts. In addition to corticospinal axonal degeneration, hyaline inclusions have been demonstrated in lower motor neurons, although their significance and specificity are currently unclear. Altered staining patterns for cytoskeletal proteins and mitochondria have also been described. Such changes may lend support to the hypothesis that there is cytoskeletal dysfunction (Wharton et al., 2003). Cognitive impairment has been particularly associated with SPG4-mutation HSP cases (Webb et al., 1998; Byrne et al., 2000; McMonagle et al., 2000; Tallaksen et al., 2003). This appears to be age-related, correlating with disease severity, and of a subcortical pattern, but the pathological basis remains to be fully defined. Study of a case with dementia demonstrated extra-motor pathology, with neuronal depletion from the pyramidal sector of the hippocampus, tau-positive tangles but not plaques, and a-synuclein positive Lewy bodies (White et al., 2000). This tau pathology is not typical of known tauopathies (Wharton et al., 2003). These reports raise the possibility that HSP may be associated with more diffuse neurodegenerative changes in some SP4-mutation families.

Mutations at the SPG10 locus involve the kinesin heavy chain (KIF5A) gene. This protein is a molecular motor that is important for anterograde and retrograde axonal transport of organelles and macromolecules (Reid et al., 2002). The function of kinesin suggests a potential common mechanism with SPG4-related cases, with altered axonal transport resulting in axonal degeneration. Specific neuropathology studies have, however, not been reported, and the question of whether there is abnormal distribution of organelles or macromolecules in axons has not yet been addressed.

Mutation at the SPG3A locus, associated with an autosomal dominant form of HSP, involves the gene for atlastin, a GTPase with homology to members of the dynamin family. Although the function of atlastin is not fully defined, involvement in synaptic vesicle recycling, mitochondrial distribution and an association

with the cytoskeleton have been suggested (Fink, 2003; Reid, 2003). Mutation at the SPG20 locus, associated with Troyer syndrome, involves the gene for spartin (Ciccarelli et al., 2002). Spartin shows some sequence homology to spastin and may be involved in endosomal trafficking. MRI studies of cases of Troyer's syndrome have shown deep white abnormalities, probable brainstem atrophy and low T1 signal in the internal capsule in the region of the corticospinal tracts (Proukakis et al., 2004). Mutation at the SPG21 locus is associated with MAST syndrome. In these cases, spastic paraparesis is accompanied by dementia and other neurological abnormalities. The locus encodes maspardin, which may have a role in protein transport and sorting. MRI studies have shown a thin corpus callosum, gray matter atrophy and white matter abnormalities, suggesting that pathology is not confined to the motor system (Simpson et al., 2003). Neuropathology studies are again lacking in these disorders and the pathological correlate of the MRI findings is unclear.

Mutations at the SPG7 locus produce an autosomal recessive form of HSP with either a pure or complicated phenotype. The gene product, paraplegin, is localized to mitochondria. Muscle biopsy in these cases has demonstrated ragged red fibers, cytochrome oxidase negative fibers, peripheral accumulation of mitochondria and elevated succinate dehydrogenase activity. These changes are suggestive of a mitochondrial cytopathy and suggest that oxidative phosphorylation defects may be important in pathogenesis (Casari et al., 1998; McDermott et al., 2001). Such muscle pathology changes are not generalized in HSP; muscle biopsy has been reported as normal in chromosome 8q-linked HSP (Hedera et al., 1999) and there is no evidence of primary mitochondrial dysfunction in SPG4-related cases (Hedera et al., 2000). In a mouse model of paraplegin-related HSP, distal axonopathy was associated with early mitochondrial abnormalities suggesting that local failure of mitochondrial function may be important in axonal degeneration (Ferreirinha et al., 2004). An important pathogenic role for mitochondrial dysfunction is supported by the association of HSP with mutations in the gene for the mitochondrial chaperonin Hsp60 (Hansen et al., 2002).

Of interest recently has been the identification of the gene at the SPG17 locus, mutation of which is associated with Silver syndrome, a disorder that is allelic with a form of distal hereditary motor neuropathy. The BSCL-2 gene at this locus encodes seipin, a protein that is normally localized to the endoplasmic reticulum. Mutation affects the consensus sequence for N-glycosylation that is important for proper folding. In cell culture models, a change in expression pattern results with the protein becoming concentrated into foci resembling aggresomes (Windpassinger et al., 2004). This may therefore be a disorder of protein misfolding. How this relates to the development of the neuropathology is unclear. However, protein misfolding and aggresome (microtubule dependent inclusion body) formation are implicated in many of the more common neurodegenerative diseases (Kopito, 2000; Olanow et al., 2004). Neuropathological studies are required to determine whether this disorder is associated with pathological hallmarks of this group of disorders, including inclusion body formation and ubiquitin pathology.

The neuropathologies of the X-linked forms of HSP may be somewhat distinct from the other types in that they are likely to have a developmental basis. Mutations at the SPG1 locus involve the gene for L1CAM, a cell adhesion molecule and a member of the immunoglobulin gene superfamily. In addition to spastic paraparesis, complex and varying development abnormalities may be present. The MASA syndrome consists of mental retardation, aphasia, shuffling gait and adducted thumbs. L1CAM mutations are also associated with X-linked hydrocephalus and corticospinal tract abnormalities. These varying phenotypes form part of the CRASH syndrome (corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia, hydrocephalus) (Jouet et al., 1994; Vits et al., 1994; McDermott et al., 2000; Reid, 2003). L1CAM has important roles in mediating axon pathfinding and neurite outgrowth, so that SPG1-related HSP is likely due to impaired development of the corticospinal tract (Casari and Rugarli, 2001). Neuropathology studies have been limited. In cases of X-linked hydrocephalus, hypoplasia or aplasia of the corticospinal tract has been described, along with hypoplasia of the corpus callosum (Yamasaki et al., 1995). Functional studies have also demonstrated abnormality of corticospinal tract function, but, in a further autopsy case, most fibers of the corticospinal tract were found to have decussated normally (Dobson et al., 2001). Mutations at the SPG2 locus involve the proteolipid protein (PLP) gene (reviewed in McDermott et al., 2000; Casari and Rugarli, 2001; Reid, 2003), but the pathology of SPG2-related HSP is still more poorly defined. PLP is a major myelin protein and mutations at this locus also underlie the more severe phenotype of Pelizaeus–Merzbacher disease, a dysmyelinating CNS disease in which hypomyelination is associated with decreased numbers of mature oligodendrocytes (Saugier-Veber et al., 1994; Yool et al., 2000). PLP knockout mice develop axonal swellings secondary to impaired axonal transport. It has been suggested that the pathology of PLP-mutation related HSP is due to impaired axonal-glial interactions, with oligodendrocytes failing to provide local support for axons (Griffiths et al., 1998; Casari and Rugarli, 2001). Detailed observations of axonal and glial pathology in the corticospinal tracts in human autopsy cases of SPG2-related HSP however have not been reported.

5.6. Viral induced motor neuron pathology

5.6.1. Acute poliomyelitis

Poliomyelitis is an acute disease in which viral infection is targeted on lower motor neurons, producing flaccid paralysis. Often, inflammatory changes develop elsewhere within the brain. Poliomyelitis is due to infection with poliovirus, an enterovirus of the Picornaviridae family. Worldwide vaccination has markedly reduced the incidence of poliomyelitis, but disease may still occur in non-vaccinated areas, in inadequately vaccinated subgroups or in rare live vaccine-associated cases that may arise from reversion to neurovirulence (Blondel et al., 1998). Poliovirus, like other members of the enteroviruses, is an enteric pathogen transmitted via the fecal–oral route. Following replication in the alimentary tract, the virus may spread to the nervous system via the hematogenous route or via peripheral nerves, to cause nervous system disease in a minority of cases. The receptor for the virus, hPVR (CD155), has been identified as a member of the immunoglobulin gene superfamily (Mendelsohn et al., 1989; Ohka and Nomoto, 2001). The virus replicates within neurons, especially motor neurons. Soon after infection, motor neurons show changes of chromatolysis with disappearance of Nissl substance, perinuclear chromatin aggregation and subsequently undergo lysis. The cytopathological changes have been suggested to be indicative of death by apoptosis. Inclusion bodies are not prominent within the neurons. Neuronal death is accompanied by neuronophagia, characterized by the accumulation of microglia around dying neurons. This phase is short, with neuronophagia only observed for the first few days after infection. In addition to the neuronal pathology, chronic inflammatory infiltrates in the form of perivascular and meningeal lymphocytic infiltrates are seen. Pathological changes are most severe in spinal cord and medulla, where their patchy and asymmetrical distribution correlates with the pattern of clinical weakness. The pathology can, however, be widespread in the brain, particularly also affecting brainstem, hypothalamus and motor cortex. In contrast to other viral encephalitides, the cerebral cortex with the exception of motor cortex is rarely involved and, even in the latter, involvement is generally not severe (Bodian, 1949; Dalakas, 1995; Love and Wiley, 2002).

Other enteroviruses may also cause a poliomyelitis-like illness with a similar pathology. These include especially EV 71, but also Echoviruses and Coxsackie virus (Solomon and Willison, 2003). In addition, however, non-enteroviruses may in some cases target the motor neuron to produce a poliomyelitis type presentation, with flaccid paralysis. These include rabies, a Rhabdovirus from the Lyssavirus genus. In humans, the disease most commonly presents in the excited or furious form, but a paralytic presentation may occur in 20–30% of cases that may be confused initially with poliomyelitis or with Guillain-Barré syndrome (Hemachudha et al., 2002; Love and Wiley, 2002). The pathology is of a polioencephalomyelitis with neuronophagia. Negri bodies, eosinophilic inclusions in the neuronal cytoplasm characteristic of rabies infection, may be sparse in paralytic presentations (Mrak and Young, 1994; Love and Wiley, 2002). Infection by West Nile virus, an arbovirus of the Flavivirus family, has also been associated with a paralytic disease, with meningitis and encephalitis. Other members of this family, such as Japanese encephalitis virus, may also involve the anterior horns. The spinal cord pathology of West Nile Virus associated cases is reported as being similar to that of poliovirus poliomyelitis, with neuronal loss, neuronophagia, a microglial reaction and a perivascular chronic inflammatory cell infiltrate. Inflammatory changes may also occur in the brain and in dorsal root and sympathetic ganglia (Jeha et al., 2003).

5.6.2. Post-polio syndrome

PPS is the onset of new deterioration after a period of stability, often of decades, in individuals who have recovered from paralytic poliomyelitis. This has been attributed to on-going motor neuron degeneration. Its clinical features include general and muscular fatigue, new gradually progressive weakness, pain and muscle atrophy. Some individuals may also suffer from respiratory insufficiency, sleep abnormalities and dysphagia (Howard, 2005; Trojan and Cashman, 2005). In contrast to ALS, the rate of deterioration is slow (Dalakas, 1995). Neuropathological studies in the stable post-polio phase, without PPS, have demonstrated atrophy of the anterior horns of the spinal cord with neuronal loss and gliosis (Fig. 5.8(A)). An increase in neuronal lipofuscin with a loss of Nissl substance has been described and inclusions are not a feature. Perivascular, parenchymal and meningeal infiltrates have suggested on-going chronic inflammation in the stable post-polio state (Pezeshkpour and Dalakas, 1988; Kaminski et al., 1995). There is no evidence of corticospinal tract degeneration indicating that descending tract degeneration secondary to upper motor neuron loss is not a conspicuous feature (Fishman, 1987). The neuropathology of the spinal cord in an individual with PPS is essentially similar to that in the stable post-polio state with the exception of occasional chromatolytic neurons (Fig. 5.8(B)), suggesting that there may be on-going anterior horn cell pathology (Pezeshkpour and Dalakas, 1988). Axonal spheroids have also been noted, but these are common in the aging spinal cord. Ubiquitylated neuronal cytoplasmic inclusions, which are a diagnostic pathological feature

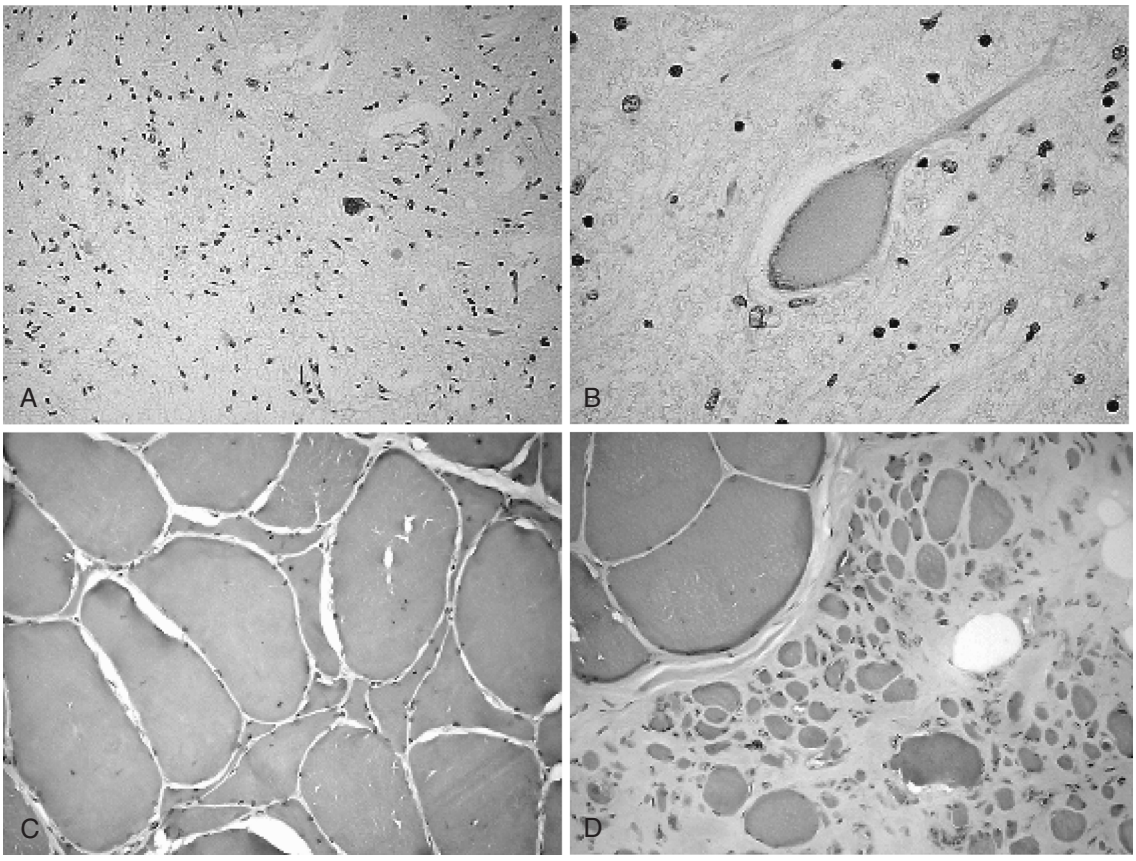

Fig. 5.8. There is marked depletion of motor neuron cell bodies from the ventral spinal gray matter in post-polio muscular atrophy (**A**). Surviving motor neurons may show chromatolysis (**B**). The skeletal muscle shows typical changes of small group atrophy and fascicular atrophy typical of active chronic muscle denervation (**C**, **D**). [Haematoxylin & Eosin.]

of ALS, are not seen in motor neurons from PPS cases (Ito and Hirano, 1994).

Muscle biopsies from PPS cases show fiber type grouping (large groups of fibers of the same histochemical fiber type) and groups of atrophic fibers, changes consistent with the previous denervation and reinnervation (Fig. 5.8(C, D)). Secondary myopathic changes may also be present (Dalakas et al., 1986; Dalakas, 1988, 1995). These changes may be present in muscles that had not been clinically involved during the original acute illness, indicating that denervation is more widespread than clinically apparent. Sub-clinical involvement of muscle is likely to underlie the involvement of muscle groups in PPS that had been previously unaffected during the acute illness (Trojan and Cashman, 2005). In addition to changes of chronic denervation, muscle in PPS (but not stable post-polio) shows isolated, angular esterase positive fibers (Dalakas et al., 1986; Dalakas, 1988) suggesting that there is new onset of denervation. Recent denervation is supported by the presence of normal sized fibers expressing NCAM (neural cell adhesion molecule) (Dalakas, 1995). This and electrophysiological data has led to the suggestion that PPS occurs due to new onset denervation. The model proposes that denervation and re-innervation due to acute poliomyelitis leads to enlarged motor units that are electrophysiologically unstable, with continuous remodeling and denervation. Neurons supplying enlarged motor units are proposed to be stressed and their ability to maintain distal axonal sprouts is impaired, leading to individual fiber (as opposed to whole motor unit) denervation (Dalakas, 1995; Trojan and Cashman, 2005).

These studies of muscle pathology have also described perimysial and perivascular lymphocytic infiltrates composed mostly of T-cells and expression of MHC class I on muscle fibers. This, and inflammation present in spinal cords, has suggested an immune component to the pathogenesis of PPS. Detection of cytokine production in the CNS of PPS patients may also support immune activation (Gonzalez et al., 2002). However, inflammation is also found in the cord in the stable post-polio state. To date the role if any of inflammatory mechanisms is unclear but it is possible that they contribute to some of the clinical features (Dalakas, 2002). One study

has also shown the presence of rimmed vacuoles and filamentous deposits staining for β-amyloid and ubiquitin in chronically denervated muscle from post-polio patients (Semino-Mora and Dalakas, 1998). These changes are reminiscent of those found in inclusion body myositis, but the significance of these findings for the development of PPS is uncertain at present.

5.7. Motor neuron degenerations associated with environmental toxins

The amyotrophic lateral sclerosis – parkinsonism dementia complex (ALS–PDC) seen on the island of Guam in the Pacific appears pathologically to be a tauopathy, characterized by the formation of neurofibrillary tangles. Variation in the anatomical distribution of pathology accounts for the varying clinical presentations, but the so-called Lytico and Bodig forms appear to have a common pathogenesis. In the Lytico form, with its ALS-like presentation, tangles are seen predominantly in the motor system, with tangles in spinal motor neurons. Whether skein or compact ubiquitylated inclusions are also present, as seen in ALS, is debatable, but as a tauopathy, the pathology is distinct from ALS. Biochemical studies have shown that all six isoforms of tau, both 3 and 4 repeat, are present, as in Alzheimer's disease. Although the etiology of ALS-PDC remains uncertain, epidemiological data suggests exposure to an environment toxin. The leading environmental hypothesis suggests a relationship to ingestion of foodstuffs derived from the cycad plant. These contain an amino acid, β-methylaminoalanine (BMAA) that has excitotoxic properties at glutamate receptors. This is derived from symbiotic cyanobacteria that colonize cycad roots. BMAA appears to be present at too low a concentration in the cycads to account for disease, but a recent hypothesis suggests that biomagnification of BMAA may occur through human consumption of indigenous fruit bats that contain high levels of BMAA.

There are two toxin-induced disorders that appear to target the upper motor neuron, producing spasticity with subacute onset. Neurolathyrism is caused by consumption of the chickling pea *Lathyrus sativus*, which contains an excitotoxic amino acid, β-N-Oxalyl amino L alanine (BOAA), that is a potent agonist at AMPA receptors. Autopsy studies are limited, but swelling of anterior horn motor neurons with reduced Nissl substance and Hirano bodies have been described. Degeneration of the corticospinal tract and also of the spinocerebellar pathways and fasciculus gracilis have been described and an early report suggested that loss of motor neurons occurs from the motor cortex. Neurocassavism (Konzo) is associated with consumption of the root crop cassava, *Manihot esculenta*. The neuropathology of this disorder remains to be defined (Ludolph and Spencer, 1996; Ravindranath, 2002).

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Chapter 6

Animal models of motor neuron death

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurological disorder that is characterized by the selective loss of motor neurons leading to progressive weakness, muscle atrophy with eventual paralysis and death within 5 years of clinical onset. Approximately 10% of ALS cases are familial, the remaining ALS cases being diagnosed as sporadic (90%). The discovery a decade ago of missense mutations in the gene coding for the Cu/Zn superoxide dismutase 1 (SOD1) in subsets of familial cases directed most ALS research to elucidating the mechanism of SOD1-mediated disease. Unraveling the mechanisms of toxicity of SOD1 mutants has been surprisingly difficult. Nonetheless, many neuronal death pathways have been unraveled through studies with transgenic mice expressing SOD1 mutants. Another key question to understanding the ALS problem is to what extent cytoskeletal abnormalities such as intermediate filament (IFs) accumulations, a hallmark of the disease, actively participate in the neurodegenerative mechanism. Again, transgenic mouse approaches have been used to clarify the role of IF proteins in motor neuron death but with complex results. In addition, there is growing evidence that genetic defects in components of the microtubule-based transport might be implicated in degeneration of motor neurons. Here, we will review the animal studies that contributed toward understanding the pathogenic pathways of motor neuron disease and the testing of therapeutic approaches with animal models of ALS.

6.1. Mice expressing ALS-linked SOD1 mutants

6.1.1. Toxicity unrelated to copper-mediated catalysis

Over 100 missense mutations have been discovered in the SOD1 gene that account for ~20% familial ALS cases (Rosen et al., 1993; for review see Cleveland, 1999; Julien, 2001). SOD1 is an abundant and ubiquitously expressed protein. Because of its normal function in catalyzing the conversion of superoxide anions to hydrogen peroxide, it was first thought that the toxicity of different SOD1 mutants could result from decreased free-radical scavenging activity. However, different SOD1 mutants showed a remarkable degree of variation with respect to enzymatic activity.

Mice expressing mutants $SOD1^{G93A}$ (glycine substituted to alanine at position 93) or $SOD1^{G37R}$ developed motor neuron disease despite elevation in SOD1 activity levels (Cleveland, 1999). In addition, SOD1 knockout mice did not develop motor neuron disease (Reaume et al.,1996). Therefore, the conclusion from these combined results was that the mutations in SOD1 provoke a gain of new toxic properties. Unlike transgenic mice overexpressing the wild-type SOD1, the mice bearing the $SOD1^{G93A}$, $SOD1^{G37R}$ or $SOD1^{G85R}$ mutants developed a motor neuron disease with many pathological changes reminiscent of human ALS (Gurney et al., 1994; Wong et al., 1994; Bruijn et al., 1997). As shown in Fig. 6.1, massive loss of motor axons occurs at end stage of disease in mice expressing mutant SOD1.

Elucidating the toxicity of SOD1 mutants has been difficult. Initially, studies have focused on aberrant copper-mediated catalysis as potential sources of toxicity. One hypothesis was that SOD1 mutations enhanced the ability of the enzyme to use hydrogen peroxide as substrate to generate toxic hydroxyl radicals that can damage cellular targets including DNA, protein and lipid membranes (Wiedau-Pazos et al., 1996). Another hypothesis suggested that the misfolding of SOD1 induced by mutations would allow the access of abnormal

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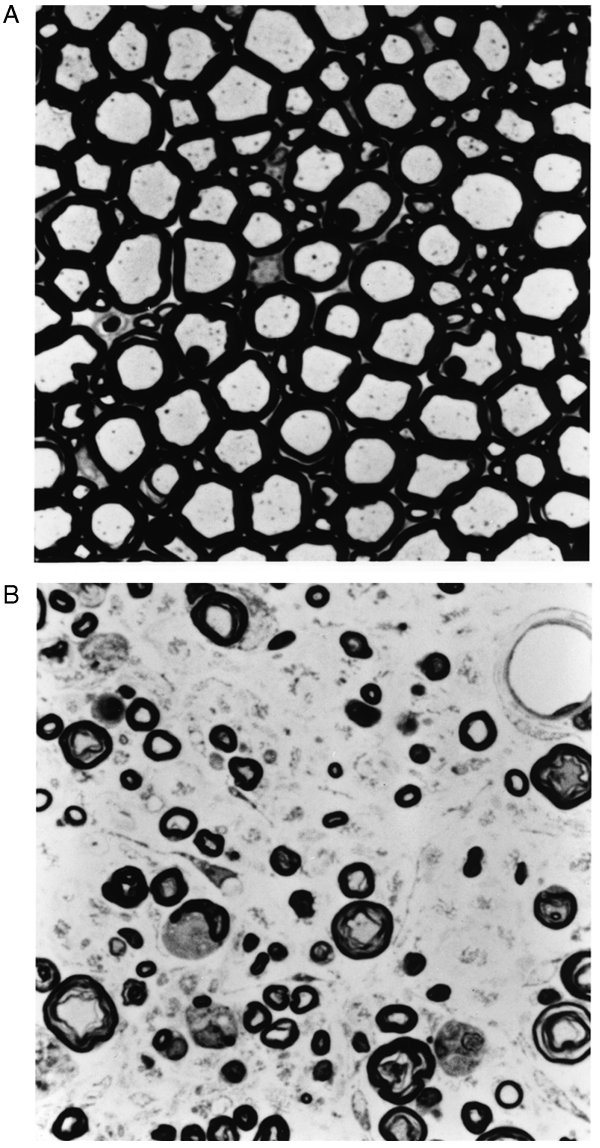

Fig. 6.1. Massive loss of motor axons in L5 ventral root from mice expressing mutant SOD1^{G37R}. Microscopy of L5 ventral root from normal mouse (**A**) and mouse expressing SOD1^{G37R} at end-stage of disease (**B**).

substrates such as peroxynitrite to the catalytic site leading to the nitration of tyrosine residues (Beckman et al., 1993). However, neither the peroxidase activity nor peroxynitrite hypotheses were supported by transgenic mouse studies. The absence of endogenous SOD1 or the addition of wild-type SOD1 did not affect disease progression in mice expressing mutant SOD1^{G85R} (Bruijn et al., 1998). Moreover, the gene knockout for the copper chaperone for SOD1 (CCS) that delivers copper to SOD1 catalytic site had no effect on disease progression in mutant SOD1 transgenic mice (Subramaniam et al., 2002). Finally, transgenic mice overexpressing a mutant form of SOD1 lacking two of the four histidine residues coordinating the binding of the Cu at the catalytic site still developed motor neurodegeneration despite a marked reduction in SOD1 activity (Wang et al., 2002b). Thus, the combined studies with genetically altered mice indicate that SOD1 mutants cause motor neuron disease through the gain of a new function that is independent of the enzymatic activity involving the copper catalytic site.

6.1.2. Complex pathways to motor neuron loss

Even though the most prevailing view is that the toxicity of SOD1 mutants is related to the propensity of misfolded protein mutants to form aggregates (Durham et al., 1997; Bruijn et al., 1998; Johnston et al., 2000; Subramaniam et al., 2002; Wang et al., 2002a), the toxicity of these protein aggregates is still poorly understood (Fig. 6.2). Deleterious effects could result from the co-sequestering of essential cellular components and from overwhelming the capacity of the protein folding chaperones (Bruening et al., 1999; Batulan et al., 2003) and/or of ubiquitin proteosome pathway to degrade important cellular regulatory factors (Urushitani et al., 2002). In cultured neurons, increasing levels of Hsp70 decreased formation of SOD1 mutant aggregates and toxicity (Bruening et al., 1999; Liu et al., 2005). However, increasing Hsp70 levels by ~10-fold did not affect disease pathology in mice expressing SOD1 mutants (Liu et al., 2005).

Studies on mice expressing mutant SOD1 suggest that the motor neuron death pathway is complex and that it involves multiple cascades of events including oxidative damage, excitotoxicity, alterations in calcium homeostasis, caspase activation, changes in levels of Bcl-2, mitochondrial defects (Liu et al., 2004; Pasinelli et al., 2004) and Fas transduction (Raoul et al., 2002). The axonal transport machinery is also affected through unknown mechanisms (Zhang et al., 1997; Williamson and Cleveland, 1999). Such transport defects may explain the presence of abnormal IF accumulations in human ALS cases with SOD1 mutations (Rouleau et al., 1996) and in transgenic mice expressing SOD1 mutants (Gurney et al., 1994; Tu et al., 1996).

Analysis of mice expressing mutant SOD1^{G37R} revealed a mislocalization and upregulation of Cdk5 activity in the spinal cord (Nguyen et al., 2001b). In SOD1^{G37R} mice, the Cdk5 deregulation is associated with an increase of p25 to p35 ratio and it is accompanied by the abnormal hyperphosphorylation of tau. In the SOD1^{G37R} mice, there is also an upregulation of Cdk4, another member of the Cdk family (Nguyen et al., 2003). Both Cdk5 and Cdk4 may be involved in apoptosis

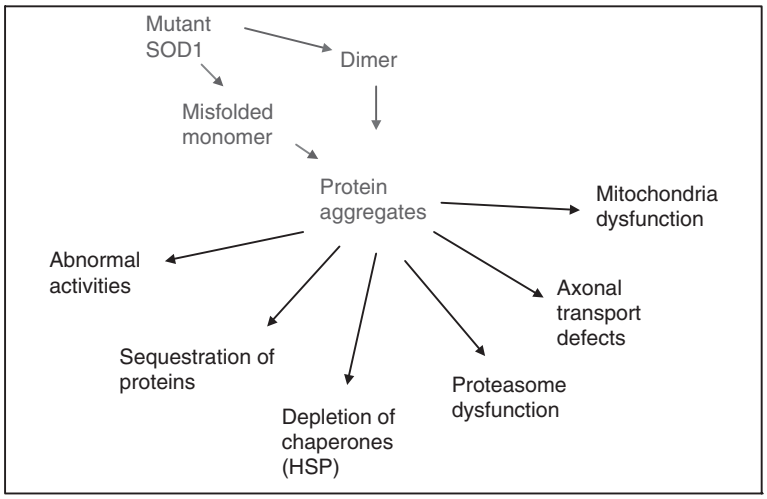

Fig. 6.2. Putative toxicities of misfolded SOD1 mutant proteins. The toxicity of misfolded SOD1 mutants is not fully understood. Multiple deleterious effects might result from the formation of protein aggregates.

and neurodegeneration. Expression of the p25/Cdk5 complex in cultured cortical neurons induced cytoskeletal abnormalities and apoptosis (Patrick et al., 1999; Lee et al., 2000). Moreover, the involvement of p25 as a trigger of neurodegeneration was supported by a study showing that overexpression of p25 in the CNS of transgenic mice caused hyperphosphorylation of tau and neurofilaments, cytoskeletal disruption and behavioral deficits (Ahlijanian et al., 2000; Bian et al., 2002). Phosphorylation of the retinoblastoma protein (Rb) by upregulation of Cdk4 can promote apoptosis through the dissociation of Rb from the Rb/E2F-1 transcription repressive complex and subsequent E2F1-dependent expression of apoptotic proteins (Knudsen and Wang, 1997; Park et al., 1998; Liu and Greene, 2001). The upregulation of nuclear Cdk4 and of phosphorylated Rb in motor neurons of $SOD1^{G37R}$ mice (line 29) was alleviated by overexpression of human NF-H (Nguyen et al., 2003). Note that a report by Takahashi and Kulkarni (2004) revealed no evidence of Cdk5 deregulation in the widely used $SOD1^{G93A}$ mice, a mouse line that exhibit higher transgene expression and more rapid disease onset than line 29 of $SOD1^{G37R}$ (onset at 100 days $SOD1^{G93A}$ instead of 340 days in $SOD1^{G37R}$). The knockout of p35 gene did not affect disease onset and progression in the $SOD1^{G93A}$ mice suggesting that pathogenesis is independent of Cdk5 in this mouse line of disease.

6.1.3. Involvement of non-neuronal cells in pathogenesis

Pathological analysis of $SOD1^{G93A}$ mice carried out at various ages at neuromuscular junctions, ventral root and spinal cord revealed that motor neuron pathology begins at the distal axon and progresses in a "dying back" pattern (Fischer et al., 2004). Thus, the $SOD1^{G93A}$ mice show end plate denervation a long time before ventral root axons loss and motor neuron loss. Significant degeneration of sensory axons has also been reported in this mouse model of ALS supporting the view that the disease is not solely a motor disorder (Fischer et al., 2005).

In addition to intrinsic motor neuron death pathways, there is now compelling evidence that non-neuronal cells might contribute to the pathogenic process in mice expressing SOD1 mutants. In transgenic mice or rats expressing mutant SOD1, there is a reduction in levels of astroglial glutamate transporter EAAT2 that may provoke a glutamate-induced excitotoxicity (Bruijn et al., 1997; Howland et al., 2002). Excess glutamate can cause neuronal death via abnormal activation of glutamate receptors, allowing Ca^{2+} entry into the cell and altering cytosolic free Ca^{2+} homeostasis. Moreover, microglial activation may be involved in the neurodegenerative process (Hall et al., 1998; Almer et al., 1999; Nguyen et al., 2001a, 2002a). Robust NF-κB activity and expression of proinflammatory cytokines and chemokines were detected by in situ hybridization within spinal cord in $SOD1^{G37R}$ mice (Nguyen et al., 2001a). The chronic induction of innate immunity by intraperitoneal injection of lipopolysaccharides (LPS) exacerbated disease by about 3 weeks in $SOD1^{G37R}$ mice (Nguyen et al., 2004), suggesting that inflammation may contribute to the neurodegenerative process. Conversely, an attenuation of neuroinflammation by minocycline or COX-2 inhibitors extended the longevity of ALS mice (Kriz et al., 2002; Drachman et al., 2002).

To unravel what cell types produce the deleterious effects leading to motor neuron death, transgenic mice

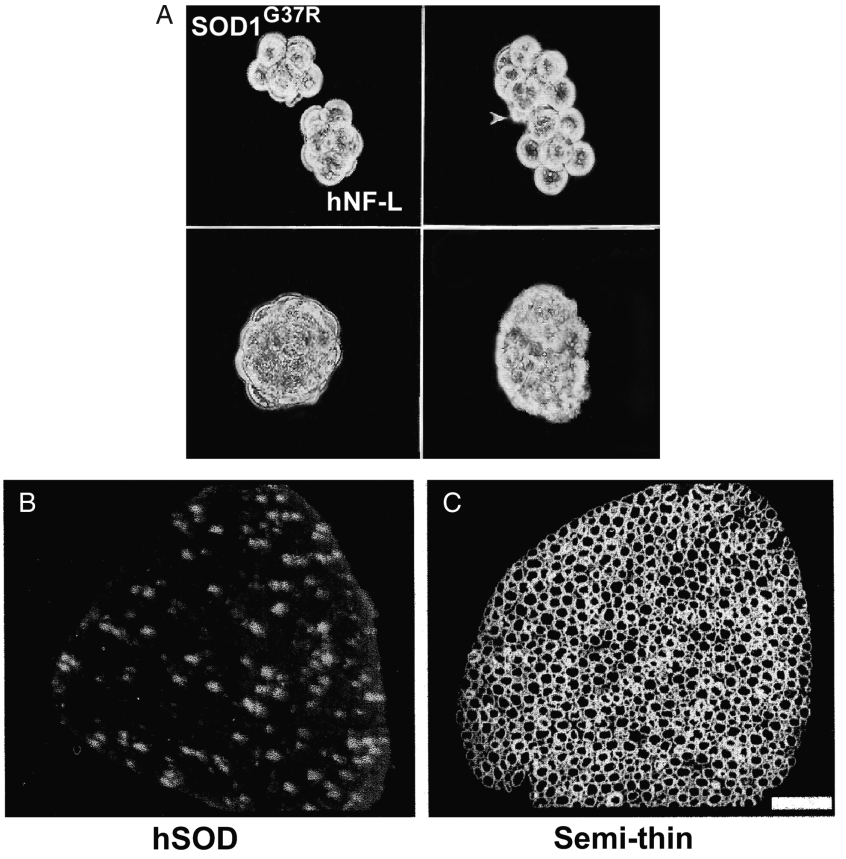

Fig. 6.3. No sign of motor axon degeneration in ventral root of a chimeric mouse expressing mutant SOD1^{G37R} in 30% of neurons. Chimeric mouse was produced by aggregation of embryos derived from SOD1^{G37R} and human NF-L transgenic mice (**A**). (**B**) A ventral root section of the L5 segment of SOD1^{G37R}/hNF-L chimeric mouse labeled for anti-human SOD1. Human SOD1 mutant was identified in approximately 30% of ventral root axons. Yet, no sign of neurodegeneration was revealed by axonal counting (978 axons) of a semi-thin section of the same root (**C**).

expressing SOD1 mutants under astrocyte- or neuronal-specific gene promoters have been generated. Expression of the SOD1^{G86R} mutation under the GFAP promoter produced astrocytosis but no motor neuron disease (Gong et al., 2000). Surprisingly, neuron-specific expression of SOD1 mutants with NF-L or Thy1 gene promoters in mice did not induce motor neuron disease (Pramatarova et al., 2001; Lino et al., 2002). However, the possibility remained that the level of transgene expression during aging was below the threshold necessary to provoke disease. This concern has subsequently been addressed by the generation of chimeric mice comprised of mixtures of normal and SOD1 mutant expressing cells (Clement et al., 2003). For example, Figure 6.3 shows microscopy of ventral root of a mouse chimera produced by the aggregation of a SOD1^{G37R} embryo with a human NF-L transgenic embryo. This SOD1^{G37R}/hNF-L chimera had 30% of the axons expressing the SOD1 mutant and yet there was no sign of neurodegeneration at 70 weeks old, which is 20 weeks beyond the age by which all germline SOD1^{G37R} mice from this line had died. This analysis supports the view that the toxicity of mutant SOD1 to motor neurons is not strictly cell autonomous. Moreover, the studies reported in a paper by Clement et al. (2003) demonstrated that degeneration can be delayed or eliminated when motor neurons expressing mutant SOD1 are surrounded by wild type non-neuronal cells.

6.2. Mice with intermediate filament disorganization

Neurofilament and peripherin proteins are two types of intermediate filaments (IFs) detected in the majority of axonal inclusion bodies, called spheroids, in motor neurons of ALS patients (Hirano et al., 1984; Corbo and Hays, 1992). Multiple factors can potentially cause the accumulation of IF proteins including deregulation of IF protein synthesis, proteolysis, defective axonal transport, abnormal phosphorylation and other protein modifications. Evidence for neurofilament involvement in disease came from the discovery of codon deletions or insertion in the KSP phosphorylation domain of the neurofilament NF-H gene in a small number of sporadic ALS patients (~1% cases) (Figlewicz et al., 1994; Tomkins et al., 1998; Al-Chalabi et al., 1999) and from the report of mutations in the rod domain of the NF-L gene in cases of Charcot-Marie-Tooth disease type 2 (Mersiyanova et al., 2000; De Jonghe et al., 2001).

Recently, a peripherin frameshift mutation has also been reported in a case of ALS (Gros-Louis et al., 2004).

6.2.1. Intermediate filament gene knockout

In the past decade, genetic manipulation in mice has been used to address the role of neurofilament and peripherin proteins in neuronal function and disease. Mice knockout for any of the neuronal IF proteins, i.e. neurofilament proteins, peripherin or α-internexin, do not develop gross developmental defects or motor neuron disease (Zhu et al., 1997, 1998; Elder et al., 1998; Rao et al., 1998; Jacomy et al., 1999, Levavasseur et al., 1999). Yet, IF deficiencies are not completely innocuous. The reduction in caliber of myelinated axons lacking NF-L was accompanied by 50% reduction in conduction velocity (Kriz et al., 2000b), a feature that would be very deleterious for large animal species. The NF-L null mice exhibited mild sensorimotor dysfunction and spatial deficits, but without overt signs of paresis (Dubois et al., 2005a). Moreover, altered cytochrome oxidase activity in numerous hindbrain regions has been detected in the NF-L null mice (Dubois et al., 2005b). Significant loss of motor axons has also been observed in the NF-L null mice (Zhu et al., 1997) and in double NF-M/NF-H knockout mice (Jacomy et al., 1999; Elder et al., 1999).

In either NF-M or NF-H null mice the velocity of transport of slow components in the axon was increased and alterations occurred in the neuronal cytoskeleton such as a higher abundance of microtubules. In peripherin knockout mice, the number and caliber of myelinated motor and sensory axons in the L5 roots remained unchanged but there was a substantial reduction (~34%) in the number of L5 unmyelinated sensory axons, demonstrating a requirement of peripherin for the proper development of a subset of sensory neurons (Lariviere et al., 2002).

6.2.2. Neurofilament overexpressors

The overexpression in mice of any of the three wild-type neurofilament subunits alone can provoke the accumulation of neurofilaments in neuronal cell bodies (Cote et al., 1993; Xu et al., 1993; Wong et al., 1995). For example, high-level expression of human NF-H proteins cause large perikaryal neurofilament accumulations (Fig. 6.4). The sequestration of neurofilaments in cell body resulted in atrophy of motor axons and altered axonal conductances but without motor neuron death even in 2-year old mice (Kriz et al., 2000a). Surprisingly, overexpressing NF-L in NF-H transgenic mice reduced the perikaryal swellings and rescued the motor neuron dysfunction illustrating again the importance of subunit stoichiometry for proper neurofilament assembly and transport (Meier et al., 1999). The proof that neurofilament abnormalities can induce neuronal death came from the expression of an assembly-disrupting NF-L transgene having a Leucine to Proline substitution near the end of the conserved rod domain (Lee et al., 1994). Mice expressing this NF-L mutant at only 50% of the endogenous NF-L level exhibited within 4 weeks after birth a massive loss of motor neurons. However, the exact mechanism of toxicity of mutant NF-L is not fully understood. Increasing the levels of bcl-2 did not

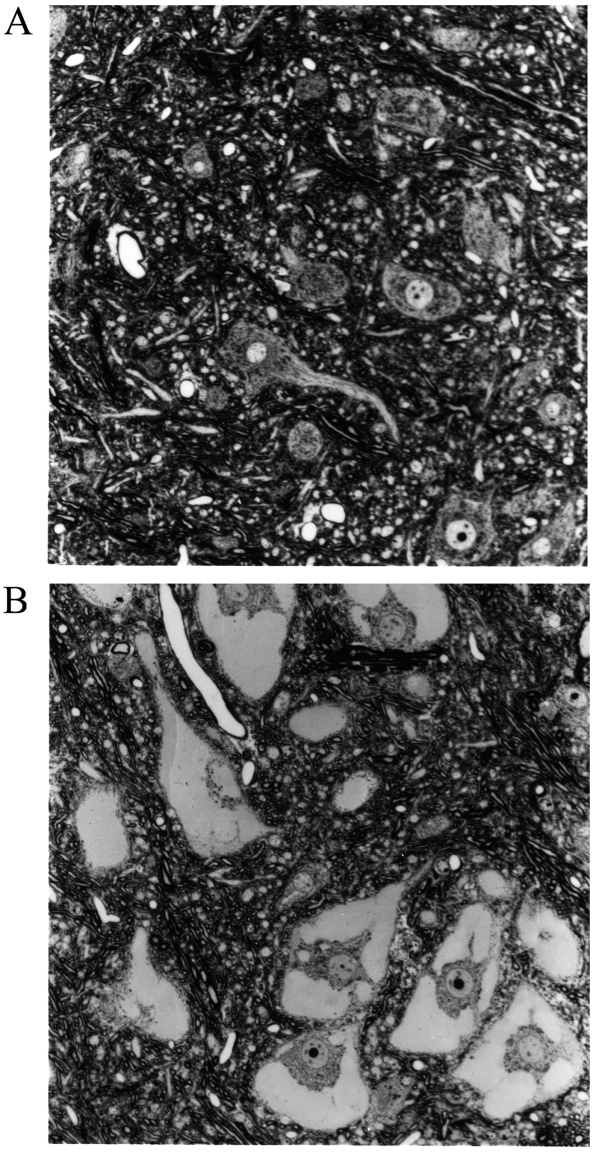

Fig. 6.4. Perikaryal neurofilament accumulations in mice overexpressing human NF-H. Overexpression of human NF-H transgene provokes the abnormal accumulation of neurofilaments in the perikaryon of spinal motor neurons (**B**). Normal spinal motor neurons are shown in (**A**).

protect the large motor neurons from the toxicity of mutant NF-L (Houseweart and Cleveland, 1999).

6.2.3. Peripherin abnormalities

The sustained overexpression of wild-type peripherin in mice caused the selective loss of motor neurons during aging (Beaulieu et al., 1999, 2000). The onset of neuronal death was precipitated by the absence of NF-L, as revealed by cross-breeding of peripherin transgenic mice with NF-L knockout mice. This context is reminiscent of the findings in ALS in which there is a reduction of NF-L mRNA levels in affected motor neurons (Wong et al., 2000). In addition, it induced formation of perikaryal and axonal IF inclusions resembling spheroids in motor neurons of human ALS. The toxicity of peripherin overexpression in mice may be related in part to the axonal localization of IF aggregates. This is supported by the rescue of peripherin-mediated disease in mice by the overexpression of NF-H transgene (Beaulieu and Julien, 2003). A plausible explanation is that perikaryal sequestering of excess peripherin protein reduces the formation of deleterious axonal IF accumulations. Other mechanisms may also contribute to the toxicity of peripherin overexpression. In vitro studies have shown that dorsal root ganglion (DRG) neurons from peripherin transgenic embryos die when grown in a proinflammatory CNS culture environment rich in activated microglia (Robertson et al., 2001), suggesting that peripherin aggregates might predispose neurons to deleterious effects of a proinflammatory environment. To investigate the role of peripherin in disease caused by SOD1 mutations, we generated by breeding procedure mice expressing $SOD1^{G37R}$ that lack peripherin or that overexpress peripherin (Lariviere et al., 2003). The excess or absence of peripherin did not affect the onset and progression of motor neuron disease in mutant SOD1 mice. Thus, it can be concluded that peripherin is not a key contributor of motor neuron degeneration associated with toxicity of mutant SOD1. Nevertheless, because mutations in SOD1 are responsible for only ~2% of all ALS cases, it remains possible that peripherin might contribute to motor neuron loss in ALS of other etiologies. Further support for the peripherin involvement in disease came from the findings of toxic peripherin splicing variant (Robertson et al., 2003) and from the discovery of a frameshift mutation in the peripherin gene of a human ALS case (Gros-Louis et al., 2004).

6.3. Mice with defects of microtubule-based transport

Axonal transport is essential to neurons because of the extreme polarity and size of these cells. In humans, spinal motor neurons may have axons of more than 1 m in length. Because the axon has no protein synthesis machinery, proteins must be synthesized in cell bodies and transported to nerve terminals through axonal transport. Membrane vesicles and mitochondria are transported by the fast transport system whereas cytoskeletal proteins like neurofilaments and microtubules move by the slow transport. Various molecular motors, which are multi-subunit ATPases members of the kinesin family and dynein, move cargos along microtubules in anterograde and retrograde directions, respectively. Axonal transport is crucial for maintaining neuronal functions. Impairment of axonal transport has recently emerged as a common factor in several neurodegenerative disorders. Mutations that disrupt either kinesin or the dynein complex cause impairment of axonal transport, blockade of membranous cargoes and axonal degeneration.

6.3.1. Kinesin knockout

The creation of mice heterozygotes for disruption of the kinesin KIF1B gene provided the proof that defects in axonal transport can provoke neurodegeneration (Zhao et al., 2001). These mice showed defect in transporting synaptic vesicle precursors and they suffer from progressive muscle weakness similar to human neuropathies. This discovery subsequently led to the identification of a loss-of-function mutation in the motor domain of the KIF1B gene in patients with Charcot-Marie-Tooth disease type 2A (Zhao et al., 2001). In addition, missense mutations in the conventional KIF5A are responsible for a hereditary form of spastic paraplegia (Reid et al., 2002) and disruption of KIF5A gene in mice was reported to cause neurofilament transport impairment (Xia et al., 2003).

6.3.2. Dynein disruption

Dynein is a molecular motor involved in retrograde axonal transport of organelles along microtubules. Dynein activity requires association with dynactin, a multiprotein complex that activates the motor function of dynein and participates in cargo attachment (Echeverri et al., 1996; King and Schroer, 2000). The overexpression of the p50 subunit of dynactin, dynamitin, disrupts the dynein/dynactin complex and thereby inhibits motor activity. Transgenic mice overexpressing dynamitin developed a late-onset and progressive motor neuron disease resembling ALS with neurofilamentous swellings in motor axons (LaMonte et al., 2002). Other mouse mutants, called *legs at odd angles (Loa)* and *cramping 1 (Cra1)* that arose by mutagenesis with N-ethyl-N-nitrosourea, were found to carry missense mutations the dynein heavy chain 1 gene (Hafezparast et al., 2003). The *Loa*

and *Cra1* mice bearing heterozygous dynein mutations develop progressive motor neuron degeneration due to impairment in retrograde transport. The notion that impairment of retrograde axonal transport may play causative role in pathogenesis is further supported by the discovery of missense mutations in the dynein-interacting protein dynactin/p150^{glued} cause a lower motor neuron disease in humans (Puls et al., 2003).

6.3.3. The pmn *and* wobbler *mice*

6.3.3.1. Progressive motor neuronopathy (pmn)

This autosomal recessive disease was discovered by spontaneous mutation in mice (Schmalbruch et al., 1991). Mice that are homozygous for the *pmn* mutation develop a progressive caudio-cranial degeneration of their motor axons from the age of 2 weeks and die 4–6 weeks after birth. Evidence for the importance of the axonal transport machinery in motor neuron disease came from the identification of the gene mutation responsible for the *pmn* in the mouse. Two groups identified the *pmn* mutation as a Trp to Gly substitution at the last residue of the tubulin-specific chaperone (Tbce) protein (Bommel et al., 2002; Martin et al., 2002). The Tbce is essential for proper tubulin assembly and for the maintenance of microtubules in motor axons. This suggests that altered function of tubulin cofactors might be implicated in human motor neuron diseases.

6.3.3.2. The wobbler

The wobbler mice originated from a spontaneous mutation that is transmitted by an autosomal recessive gene *wr* mapping to chromosome 11 (Kaupmann et al., 1992). However, the exact genetic defect has not yet been identified. A mutation analysis by Fuchs et al. (2002) did not reveal mutations or abnormal levels of mRNA in candidate genes located in the wobbler critical region. The wobbler mice have been extensively investigated as a model of motor neuron disease (Pioro and Mitsumoto, 1995). The symptoms can be recognized early in the postnatal period. The disease is associated with the degeneration and loss of spinal motor neurons. Pathology of cortical motor neurons has also been reported (Pioro et al., 1998). The wobbler phenotype is characterized by perikaryal vacuolar degeneration and swelling of motor neurons, astrogliosis and microglia activation. There is evidence of dysfunctional mitochondrial respiration in the wobbler with decreased activity of complex IV in a manner similar to what has been reported in the spinal cord of patients with sporadic ALS (Xu et al., 2001). Ubiquitin and hyperphosphorylated NF-H immunoreactivities have also been detected in cortical neurons of affected animals (Pioro et al., 1998). Moreover, increased expression of neurofilament NF-M protein has been observed in affected motor neuron in the wobbler mice (Pernas-Alonso et al., 2001). To what extent the NF-M overexpression contributes to pathogenesis remains unknown. In future, it would be of interest to use the NF-M knockout mice to address this question.

6.3.4. Tau overexpressors

Abnormalities of tau in human disease are known as tauopathies, including Alzheimer's disease (AD), frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP) and amyotrophic lateral sclerosis/Parkinsonism-dementia complex of Guam (Lee et al., 2001). Tau is a microtubule-associated protein involved in stabilization of microtubules. There are six tau isoforms that are derived from a single gene via alternative splicing of the primary gene transcript. Transgenic mice overexpressing the shortest tau isoform developed axonal degeneration of spinal neurons and motor weakness (Ishihara et al., 1999). These tau transgenic mice are characterized by the presence of filamentous aggregates of hyperphosphorylated tau not only in cortical and brainstem neurons but also in spinal neurons (Trojanowski et al., 2002). The inclusions contain 10- to 20-nm tau-positive straight filaments. Gliosis has been detected in the spinal cord with degeneration of axons in ventral roots. Neurofilaments are also associated with these aggregates, demonstrating that abnormalities in tau protein can directly affect neurofilaments. Breeding experiments with tau transgenic mice and NF-L knockout mice revealed an alleviation of tau-mediated disease by reducing the neurofilament content (Ishihara et al., 2001). This led to the hypothesis that neurofilaments may act as chaperones in the development of tau aggregates.

6.3.5. Mice with targeted disruption of hypoxia response element

Vascular endothelial growth factor is a cytokine crucial for angiogenesis. Targeted disruption of the hypoxia response element (HRE) sequence in the mouse vascular endothelial growth factor (VEGF) gene resulted in severe motor deficits at 5–7 months of age with pathological changes resembling ALS such as neurofilament accumulations in the spinal and brainstem motor neurons (Oosthuyse et al., 2001). The mechanism of disease is still unclear. It has been suggested that chronic vascular insufficiency and possibly a lack of VEGF neuroprotection might result in motor neuron degeneration. Subsequent studies found an association of some human haplotypes in the VEGF upstream promoter sequence with risk of ALS (Lambrechts et al., 2003; Terry et al., 2004).

6.3.6. Mice knockout for Als2

A few years ago, deletion mutations were discovered in coding exons of a new gene mapping to chromosome 2q33, *ALS2* coding for Alsin, from patients with an autosomal recessive form of juvenile ALS (JALS), primary lateral sclerosis and infantile-onset ascending hereditary spastic paralysis (IAHSP) (Hadano et al., 2001; Yang et al., 2001; Eymard-Pierre et al., 2002; Gros-Louis et al., 2003). The ALS2 gene is ubiquitously expressed. It encodes a protein having guanine nucleotide exchange factor (GEF) homology domains which are known to activate small guanosine triphosphatase (GTPase) belonging to the Ras superfamily. The RCC1-like, DH/PH and VPS9 domains are GEF for small GTPase Ran (Ras-related nuclear), Rho (Ras-homologous member) and Rab5 (Ras-related in brain 5), respectively. To investigate the function of *ALS2* and the mechanism of motor neuron disease caused by *ALS2* mutations, our laboratory generated a mouse knockout for *Als2* using a targeting vector by replacing exon 2 and part of exon 3 with a 1.1 kb Neo cassette (Kriz J, Millecamps S, Urushitani M, Zhu Q, Julien JP, unpublished results). The Alsin knockout mice are born with no obvious developmental defects and they are of normal size. However, in aging the Alsin knockout mice develop mild motor dysfunction as determined by rotarod test, modest decrease in grasping test and sometimes abnormal limb flexions when lifted by the tail. Obviously, the overt phenotypes in Als2 null mice are more subtle than anticipated from human ALS2 disorders. Nonetheless, electron microscopy (EM) provided evidence of pathological changes. In 18 month-old Als2 null mice, EM revealed a high number of degenerating axons in corticospinal tracts (Fig. 6.5). So, it is anticipated that the Alsin knockout mouse will provide a useful tool to

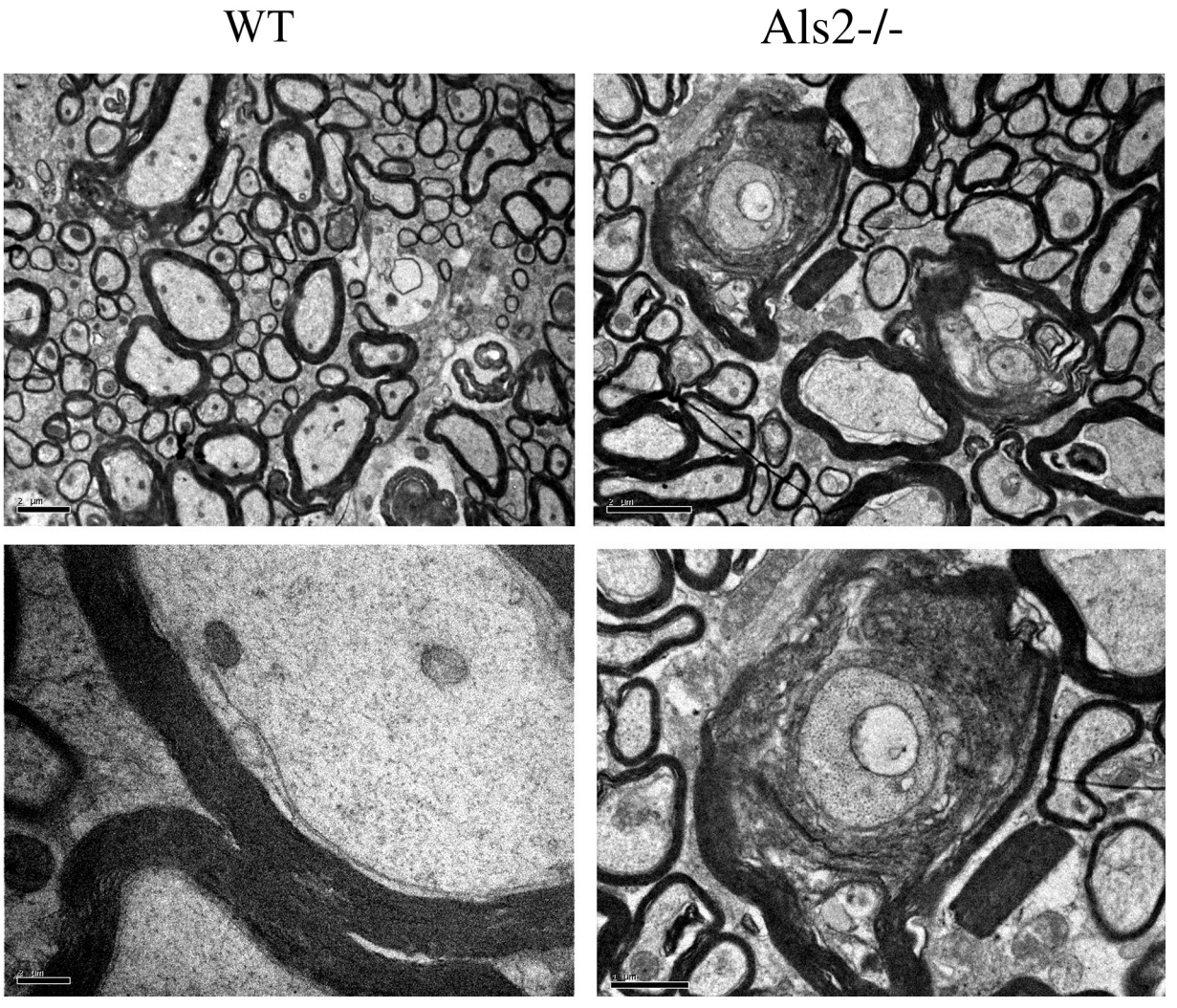

Fig. 6.5. Electron microscopy of degenerating axons in corticospinal tract of *Als2* null mice.

investigate the function of Alsin and to provide new insights on pathogenesis of human ALS2 and related disorders with involvement of long axonal tract degeneration such as HSPs.

6.3.7. Other animals with motor neuron disease

6.3.7.1. Transgenic rats overexpressing SOD1^{G93A}

Transgenic rats overexpressing mutant SOD1^{G93A} have been generated some years ago (Howland et al., 2002). The disease onset in the SOD1^{G93A} rats occurs at about 115 days and disease progression is very rapid with end stage within 11 days after onset. The pathological abnormalities include vacuoles and inclusions stained for SOD1, Hsp70, neurofilaments and ubiquitin. The loss of EAAT2 at end-stage disease is very pronounced in this model (over 90%). These transgenic rats should be a valuable animal model to carry out experimentation difficult to achieve in smaller ALS mice. The intracerebroventricular delivery of VEGF in the SOD1^{G93A} rat model led to extension of life span by 22 days (Storkebaum et al., 2005).

6.3.7.2. Hereditary canine spinal muscular atrophy (HCSMA)

The HCSMA is an autosomal dominant disease in Brittany spaniels. Affected heterozygous dogs develop motor neuron disease with onset at 6–24 months and some may survive for 7 years or more. The homozygous dogs develop a disease by 6–8 weeks that progresses to rapid paralysis at 3–4 months of age. To date, the disease gene for HCSMA remains unidentified. Dogs with HCSMA develop many clinical and pathological features of human motor neuron disease including neurofilamentous swellings in proximal axons of motor neurons axonal swellings (Cork et al., 1982). The abnormal accumulations of highly phosphorylated NF-H protein could result in part from the Cdk5 activity that was found to be increased in HCSMA dogs as compared to controls (Green et al., 1998). Sequencing of the NF-H cDNAs from normal and HCSMA dogs revealed no point mutations or deletions in the NF-H coding sequence from dogs with the disease suggesting that the NF-H gene is not the causative gene in this animal model (Green et al., 2005).

6.3.7.3. Equine motor neuron disease (EMND)

Horses with EMND were first diagnosed in 1990 in New York State. Affected horses exhibit striking weight loss attributed to neurogenic muscular atrophy. The disease is associated with abnormal gait and muscle tremors. Occasionally, the condition will stabilize but in most cases the condition deteriorates progressively requiring euthanasia. The finding of increased copper concentrations and low vitamin E in the spinal cord of EMND horses led to the hypothesis of oxidative injury involvement in this neurodegenerative disease (Polack et al., 2000).

6.4. Testing therapeutic strategies in mice expressing mutant SOD1

Presently, there is no effective pharmacological treatment for ALS. Transgenic animal models that exhibit many of the pathological changes in human ALS provide useful tools for drug testing (Table 6.1). Table 6.2 lists some of the genetic manipulations and drugs tested in mice expressing SOD1 mutants. Many of the pharmacological approaches tested so far have produced only modest beneficial effects. Vitamin E, gabapentin and salicylate had no effect on survival of SOD1^{G93A} mice (Gurney et al., 1996, 1998; Barneoud and Curet 1999). Riluzole, a glutamate antagonist and the only drug currently approved for ALS treatment, extended the life span of SOD1^{G93A} mice by 10–15 days without affecting disease onset. More neuroprotection was provided in SOD1^{G93A} mice by the intra-cerebroventricular administration of N-Benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (zVAD-fmk), a broad caspase inhibitor (Li et al., 2000). Celecoxib treatment significantly delayed the onset of weakness and weight loss and prolonged survival by 25% (Drachman et al., 2002).

Minocycline, a second-generation tetracycline with anti-inflammatory properties, has been shown to increase survival in at least three independent laboratories, using two different mutant SOD1 mouse lines and using various modes of drug delivery (Kriz et al., 2002; Van den Bosch et al., 2002; Zhu et al., 2002). Minocycline may confer neuroprotection by multiple pathways as it can reduce microglial activation, caspase-1, caspase-3, iNOS, p38 MAP kinase and mitochondrial cytochrome c release. Moreover, the addition of riluzole and nimodipine further enhanced the effect of minocycline on survival (Kriz et al., 2003). Minocycline is a clinically well tolerated drug. Based on minocycline studies with transgenic mouse models, there is an on-going human trial to test the effectiveness of minocycline in ALS.

In addition, there is a recent report that the beta-lactam antibiotic ceftriaxone can confer protection in mouse model of ALS probably through elevation of expression of glutamate transporter EAAT2 (also called GLT-1) (Rothstein et al., 2005). An elevation in EAAT2 would attenuate glutamate neurotoxicity. A long-term clinical trial is needed to test the efficacy of ceftriaxone in human ALS.

An approach for treatment of motor neuron disease that may be considered in the future would involve the

Table 6.1

Animal models with motor neuron dysfunction

Animal species	Pathological changes	References
Mouse		
Protein misfolding		
Mutant SOD1	Massive motor neuron death IF and SOD1 aggregates	Gurney et al., 1994 Wong et al., 1995
Synuclein mutant	Protein aggregates and motor dysfunction	Giasson et al., 2002 Bruijn et al., 1998
Intermediate filament disorganization		
hNF-H overexpressor	Perikaryal accumulations and axonal atrophy Altered conductivity but no neuronal loss	Cote et al., 1993 Kriz et al., 2000a
mutant NF-L	Perikaryal and axonal NF accumulations Trapped organelles and massive neuronal loss	Lee et al., 1994
NF-L–/–	Perikaryal accumulation of NF-M and NF-H Developmental loss of motor neurons of 20% Altered nerve conductivity Mild sensorimotor dysfunction	Zhu et al., 1997 Kriz et al., 2000b Dubois et al., 2005a
Peripherin overexpressor	Age-dependent IF aggregates in perikarya and axons Loss of motor neurons during aging	Beaulieu et al., 1999
Defects in microtubule-based transport		
Dynamitin overexpressor	Axonal IF swellings Motor neuron degeneration	LaMonte et al., 2002
KIF1B heterozygous knockout	Late-onset motor neuron degeneration	Zhao et al., 2001
KIF5A	Neurofilament transport defect	Xia et al., 2003
Dynein	Missense point mutations causing motor neuron disease	Hafezparast et al., 2003
pmn mouse	Early-onset motor neuron degeneration	Bommel et al., 2002; Martin et al., 2002
Short tau overexpressor	Perikaryal IF accumulations Degeneration of motor axons	Ishihara et al., 1999
P25 overexpressor	Abnormal tau phosphorylation and axonopathy	Bian et al., 2002
Others		
VEGF ΔHRE	Late-onset motor neuron degeneration	Oosthuyse et al., 2001
Als2 knockout	Late-onset degeneration of corticospinal axons	Our unpublished data
Rat SOD1^{G93A}	Massive motor neuron death	Howland et al., 2002
Dog HCSMA	Neurofilament accumulations	Green et al., 1998
Horse EMND	Vitamin E deficiency?	Polack et al., 2000

Table 6.2

Therapeutic interventions in animals expressing mutant SOD1

	ALS mouse	Increased life span	References
Pharmacological treatments			
Creatine (diet)	$SOD1^{G93A}$	20 days	Klivenyi et al., 1999
Riluzole (diet)	$SOD1^{G93A}$	10–15 days	Gurney et al., 1996
Gabapentin (diet)	$SOD1^{G93A}$	no effect	Gurney et al., 1996
Vitamin E (diet)	$SOD1^{G93A}$	no effect	Gurney et al., 1996
Lysine acetyl-salicylate (diet)	$SOD1^{G93A}$	no effect	Barneoud and Curet, 1999
Minocycline (diet)	$SOD1^{G37R}$	21–35 days	Zhu et al., 2002
	$SOD1^{G93A}$	11–21 days	Kriz et al., 2002; Van den Bosch et al., 2002
Cocktail of Minocycline-riluzole-nimodipine	$SOD1^{G37R}$	42 days	Kriz et al., 2003
Ceftriaxone	$SOD1^{G93A}$	10 days	Rothstein et al., 2005
Ginseng	$SOD1^{G93A}$	7 days	Jiang et al. 2000
zVAD-fmk (i.c.v.)	$SOD1^{G93A}$	27 days	Li et al., 2000
Copaxone	$SOD1^{G93A}$	50 days	Angelov et al., 2003
SOD1 injection	$SOD1^{G93A}$	24 days	Turner et al., 2005
Transgene overexpression			
Human NF-H	$SOD1^{G37R}$	2–5 months	Couillard-Despres et al., 1998
Mouse NF-H	$SOD1^{G93A}$	40 days	Kong and Xu, 2000
Mouse NF-L	$SOD1^{G93A}$	40 days	Kong and Xu, 2000
Bcl-2	$SOD1^{G93A}$	30–35 days	Kostic et al., 1997; Vukosavic et al., 1999
Dominant inhibitor of caspase-1	$SOD1^{G93A}$	27 days	Friedlander et al., 1997
Gene knockout			
NF-L–/–	$SOD1^{G85R}$	6 weeks	Williamson et al., 1998
	$SOD1^{G37R}$	10–15 weeks	Nguyen et al., 2000
Peripherin–/–	$SOD1^{G37R}$	No change	Lariviere et al., 2003
nNOS–/–	$SOD1^{G93A}$	No change	Facchinetti et al., 1999
Il-1beta–/–	$SOD1^{G37R}$	No change	Nguyen et al., 2001a
CCS–/–	$SOD1^{G85R}$	No change	Subramaniam et al., 2002
	$SOD1^{G37R}$		
	$SOD1^{G93A}$		
Viral gene therapy			
IGF1	$SOD1^{G93A}$	37 days	Kaspar et al., 2003
GDNF	$SOD1^{G93A}$	25 days	Wang et al., 2002c
VEGF	$SOD1^{G93A}$	40 days	Azzouz et al., 2004
Cardiotrophin	$SOD1^{G93A}$	27 days	Bordet et al., 2001
RNAi	$SOD1^{G93A}$	100 days	Miller et al., 2005; Ralph et al., 2005; Raoul et al., 2005
	ALS Rat		
Intraventricular injection			
VEGF	$SOD1^{G93A}$	22 days	Storkebaum et al., 2005

delivery of viral vectors to mediate expression of either growth factors such as GDNF, IGF-1 and VEGF (Wang et al., 2002c; Azzouz et al., 2004; Kaspar et al., 2003) or RNAi molecules (Miller et al., 2005; Ralph et al., 2005; Raoul et al., 2005) to target SOD1 mRNA for degradation. For instance, it is remarkable that SOD1 silencing with lentiviruses almost doubled the longevity of mice expressing $SOD1^{G93A}$ mutant (Ralph et al., 2005). Yet, mice are not humans and sometimes therapeutic approaches that confer benefits with mouse models might fail in a human trial. A good example is creatine, a compound believed to improve mitochondrial function. Creatine administered in drinking water was found to extend the longevity of mice expressing mutant SOD1 (Klivenyi et al., 1999; Zhang et al., 2003). However, a recent clinical trial of creatine was found to be ineffective in human ALS (Groeneveld et al., 2003).

6.5. Future directions

Recent studies with mouse models revealed that pathogenesis of motor neuron disease can be complex with involvement of neuronal and non-neuronal deleterious pathways. Despite important effort devoted in the past decade toward elucidating the mechanism of disease caused by SOD1 mutations, the neurodegeneration mechanism is still not fully understood. The SOD1 mutants cause disease through acquisition of toxicity. Yet, it is not resolved how SOD1 mutants can trigger through protein misfolding and perhaps aggregation some death pathways selectivity in neuronal subsets. In addition, evidence indicates that non-neuronal cells are involved in the disease process but the molecular mechanisms in various cell types that contribute to motor neuron death remain to be elucidated. In light of the emerging evidence for a crucial role of axonal transport in motor neuron disease, there is a need to clarify the cytoskeletal changes associated with human ALS. The abnormal IF accumulations, a hallmark of ALS, emerged as intrinsic factors that may affect the disease either negatively or positively. The mechanisms underlying the formation and neurotoxicity of IF accumulations are not fully understood. Disorganization of the IF network could result from a variety of primary causes including neurofilament gene mutations, deregulation of IF gene expression, post-translational modifications and axonal transport defects. Motor neuron loss has been observed in some transgenic mouse models exhibiting an axonal localization of IF swellings supporting the view of an 'axonal strangulation' disease model by which IF swellings can block axonal transport (Cleveland, 1999; Julien, 2001). Other toxic mechanisms may also be involved. For instance, in vitro culture studies suggest that peripherin aggregates can predispose neurons to apoptotic death induced by a proinflammatory CNS environment (Robertson et al., 2001). Here, the presence of IF aggregates may be viewed as a factor that lowers the threshold to neuronal death in a toxic environment. In contrast, perikaryal neurofilament accumulations are sometimes well tolerated and they can even confer protection. For example, NF-H overexpression alleviated the toxicity of mutant SOD1 in mice (Nguyen et al., 2001b).

A key role of axonal transport in pathogenesis of motor neuron disease was recently supported by the inhibition of molecular motors in transgenic mice. The disruption of the dynein-dependent retrograde transport caused motor neuron degeneration (LaMonte et al., 2002; Puls et al., 2003). Further proof that defects in the transport machinery can provoke neurodegeneration came from mice heterozygotes for disruption of the kinesin KIF1B gene (Zhao et al., 2001) and from the discovery of the *pmn* mouse gene that encodes a tubulin-specific chaperone (Tbce) protein (Bommel et al., 2002; Martin et al., 2002). Therefore, studies on the neuronal cytoskeleton and molecular motors of the microtubule-based transport might offer promising research avenues to understand the selective vulnerability of motor neurons to disease.

Presently, there is no drug available to stop motor neuron disease in human ALS or in mice models. In view of the complexity of the disease, a combination of different therapies acting in synergy will probably be needed for effective ALS treatment. New strategies might include a search for agents that can prevent the abnormal aggregation of proteins, mutant SOD1 or IF proteins. As more associated genes are discovered, new therapeutic approaches could potentially be derived. Gene therapy approaches involving the use of recombinant viruses offer a promising strategy for the delivery of genes to enhance motor neuron survival or to silence specific genes such as mutant SOD1. The next few years should also provide some perspective on the potential of neural stem cells to replace or to repair damaged neurons. Finally, a better understanding of ALS pathogenesis through the use of animal models and the development of efficient therapeutics will require the discovery of new genes and biomarkers associated with the disease, especially sporadic ALS.

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Chapter 7

Spinal muscular atrophies and hereditary motor neuropathies

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7.1. Introduction

By convention the use of the term spinal muscular atrophy (SMA) is reserved for disorders characterized by progressive muscle wasting due to loss of motor neurons in the ventral horns of the spinal cord. It is thus implicit in this definition that upper motor neuron (corticospinal tract) involvement is absent. Similarly the term SMA should not be used for conditions in which there is mixed motor and sensory loss. It will be immediately apparent that SMA could logically be used for the Progressive Muscular Atrophy variant of Motor Neuron Disease (see Leigh, Chapter 13, this volume). In most cases, the natural history of PMA (both with regard to age of onset and survival) is within the spectrum of disease progression (from the malignant to the slowly progressive over decades) seen in sporadic Amyotrophic Lateral Sclerosis (ALS) and the neuropathology is the same, having the characteristic hallmark of ubiquitinated inclusions. Therefore, while it may be difficult to make the distinction between adult-onset SMA and PMA in the early clinical stages, it is clear that they are separate etiopathological entities and the use of terms such as 'progressive Spinal Muscular Atrophy' to describe lower motor neuron forms of ALS/MND, while descriptively accurate, leads to confusion in the medical literature and only serves to alarm patients with the more typical slowly progressive forms of SMA described in this chapter. It should also be recognized that an overly restrictive definition of SMA may also lead to problems in categorizing lower motor neuron disorders in which pathological changes outside of the lower motor neuron occur as a minor feature.

A similar problem arises when considering whether the primary pathological process is in the motor neuron cell body in the ventral horn (a neuronopathy) or in the motor axon (a neuropathy). In the majority of cases there is insufficient knowledge to answer this question at present. Some authors have restricted the term SMA to those conditions in which it is assumed that the cell body harbors the degenerative process and therefore place the distal forms of lower motor neuron degeneration (see below) within the Charcot–Marie–Tooth neuropathies because of similar clinical manifestations such as peroneal muscular atrophy. As will become clear in this chapter, disorders of the lower motor neuron are gradually being redefined according to genetic causation and arguments about classification based on clinical criteria will become redundant, except in the sense that clinical features allow the targeting of genetic testing.

In describing the SMAs the question arises as to whether it is possible to provide a coherent and reproducible phenomenological classification that is of practical use in the clinic. Historically, where specific genetic tests have not been available, it has been helpful for the purpose of prognostication and research to describe different forms of SMA according to distribution. Increasingly, as more genes have been identified, it has become clear that there is considerable clinical overlap between and within specific genetic diseases. Notwithstanding this, the broad clinical classification of SMA currently in use remains helpful in focusing genetic testing and in counseling at-risk families. This scheme divides SMA by distribution (proximal, distal or regional), inheritance (autosomal recessive, autosomal dominant and sex-linked) and age of onset (infantile, childhood or adult) (Table 7.1). In addition to the considerable clinical and genetic heterogeneity observed in these disorders, the pattern of inheritance may be difficult to determine in the not infrequent cases of apparently 'sporadic' disease.

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Table 7.1

Genetic classification of spinal muscular atrophies

Inheritance	Age of onset	Distribution	Genetics	Gene function
Recessive	Birth to adult	Proximal	SMN	RNA/RNP metabolism
	Birth/infantile	Distal/diaphragm	IGHMBP2	?Transcriptional regulation
	Infantile to adult	Distal (LL>UL) diaphragm	Linked to 11q13	*
Dominant	Adult	Distal (LL>UL)	HSP B1 or B8	Protein chaperone
	Adult	Distal (UL>LL)	GARS	Glycyl tRNA synthetase
	Adult	Distal (UL=LL)	BSCL2	Endoplasmic reticulum
	Childhood	Distal	Senataxin	?RNA helicase
	Adult	Proximal>distal	VAPB	Vesicle trafficking/axonal function?
	Adult	Distal with vocal cord paresis	p150 dynactin	Retrograde axonal transport
	Adult	Distal with vocal paresis	Linked to 2p14	*
X-linked	Childhood	Distal (LL>UL)	Xq13.1-q21	*

The purpose of this chapter is to describe the various forms of inherited lower motor neuron degeneration and discuss what is currently known about the molecular basis of these disorders and the pathways underlying the specific susceptibility of motor neurons to degeneration.

7.2. Autosomal recessive proximal SMA

7.2.1. Clinical and genetic features

It is now clear that the three forms of childhood onset proximal SMA previously known as Werdnig–Hoffmann disease (Type I SMA), Intermediate SMA (Type II) and Kugelberg-Welander syndrome (Type III) are caused by mutations in the same gene, SMN1. The three forms are distinguished on grounds of severity but share common clinical features, being symmetrical, proximal and affecting lower limbs more than upper limbs. Facial muscles are spared, as is the diaphragm, an important distinction in the differential diagnosis of the floppy infant (Dubowitz, 1995). The clinical classification is based on the maximum motor milestone achieved. Collectively SMA Types I–III have an incidence of 1 in 6–10,000 live births (Pearn, 1978).

Type I SMA presents with infantile hypotonia during the first 6–12 months of life. The onset can be assumed to occur in some cases in utero because mothers of affected babies occasionally describe a reduction or cessation of fetal movements in the third trimester. In addition, some severely affected children have arthrogryphosis which indicates fetal hypotonia. The key feature of Type I SMA is that children do not achieve the ability to sit unaided. Survival without assisted ventilation is rare beyond 2 years, though increasing numbers of children are surviving for longer periods of time with the use of a tracheostomy, programmed physiotherapy and cough assist devices (Ioos et al., 2004). The differential diagnosis of Type I SMA has been made considerably easier due to the widespread availability of an accurate diagnostic test (van der Steege et al., 1995; Scheffer et al., 2001), but a number of other conditions which present as a floppy infant should be considered in the differential diagnosis. These include rarer forms of infantile onset SMA such as SMA with Respiratory Distress (described below), complex developmental syndromes such as cerebellar hypoplasia/SMA (Goutieres et al., 1977; Chou et al., 1990; Dubowitz et al., 1995), defects of mitochondrial function (Rubio-Gozalbo et al., 1999; Mancuso et al., 2002; Berger et al., 2003; Tarnopolsky et al., 2004) and others. For a detailed account the reader is referred to textbooks of pediatric neuromuscular disease (Dubowitz, 1995).

Type II SMA is defined by the child being able to sit but failing to achieve independent walking. The survival is heavily dependent on the degree of respiratory involvement, which is a combination of neuromuscular weakness and the development of kyphoscoliosis, which is almost universal in this type of SMA.

Individuals with Type III SMA develop weakness at a later and more variable stage and the key feature is that they are able to walk independently, even if only transiently. Zerres and Rudnik-Schoneborn (1995) have distinguished between those children with onset before (IIIa) and after (IIIb) the age of 3 years and shown that

this has prognostic implications. Some authors have used the term Type IV SMA to describe cases with onset in adulthood. Many of these do not have mutations in the SMN gene and a family history is often absent, suggesting that this is a more heterogeneous condition than the childhood form. However, given that there is a continuum of age of onset from infancy to middle age, SMN gene testing is relevant in all patients with proximal symmetrical SMA.

The strict clinical criteria described above were designed to group together patients with homogeneous clinical features that could be included in international collaborative efforts to identify genetic linkage. Initially "chronic" (Type II/III SMA) was linked to chromosome 5q13 (Brzustowicz et al., 1990), followed shortly afterwards by 'acute' (Type I) (Gilliam et al., 1990; Melki et al., 1990), indicating that these disorders were allelic. Positional cloning efforts were hampered by the evident degree of genomic instability in the SMA region of chromosome 5, a fact which is intimately related to the molecular pathogenesis described below. In 1995, Melki and colleagues identified deletions and gene conversion events in a novel gene that they called Survival of Motor Neurons (SMN) (Lefebvre et al., 1995). Mutations were identified in 97% of their patients and in the remaining few had intragenic missense mutations or small deletions. Initially, the neighboring Neuronal Apoptosis Inhibitory Protein (NAIP) gene was thought to be relevant, both because it is deleted in 60% of cases of SMA but also because its function is highly appropriate for a neurodegenerative disease (Roy et al., 1995). However, it is clear that SMA can occur in the absence of NAIP deletion and that multiple copies of this gene exist and probably render the deletion phenotypically neutral. The deletion of NAIP is best thought of as a marker which distinguishes cases in which SMN1 is deleted rather than gene converted (Campbell et al., 1997).

7.2.2. Molecular pathogenesis

The region of chromosome 5q to which these disorders were mapped harbors a high degree of genomic instability. The SMN1 gene lies within a 500 kb inverted duplication which arose about several million years ago in a common human-chimpanzee primate ancestor (Rochette et al., 2001). The two copies of chimp SMN are identical, but during human evolution there has been a sequence diversion between SMN1 and 2 resulting in a differential splicing pattern for SMN2 (Fig. 7.1). A translationally silent single nucleotide change in exon 7 results in the disruption of an exonic splice enhancer (the normal function of which is to provide a recognition sequence for the binding of the splicing activator SF2/ASF) leading to failure of exon 7 inclusion in 90% of transcripts from SMN2 (Lorson and Androphy, 2000; Cartegni and Krainer, 2002). The truncated protein that results from exon 7-deleted mRNA is unstable and has reduced functionality.

A carrier SMA chromosome can arise in one of three ways: (i) by deletion of the SMN1 gene (such deletions usually encompass deletion of neighboring genes such as NAIP, but these are thought to be functionally neutral),

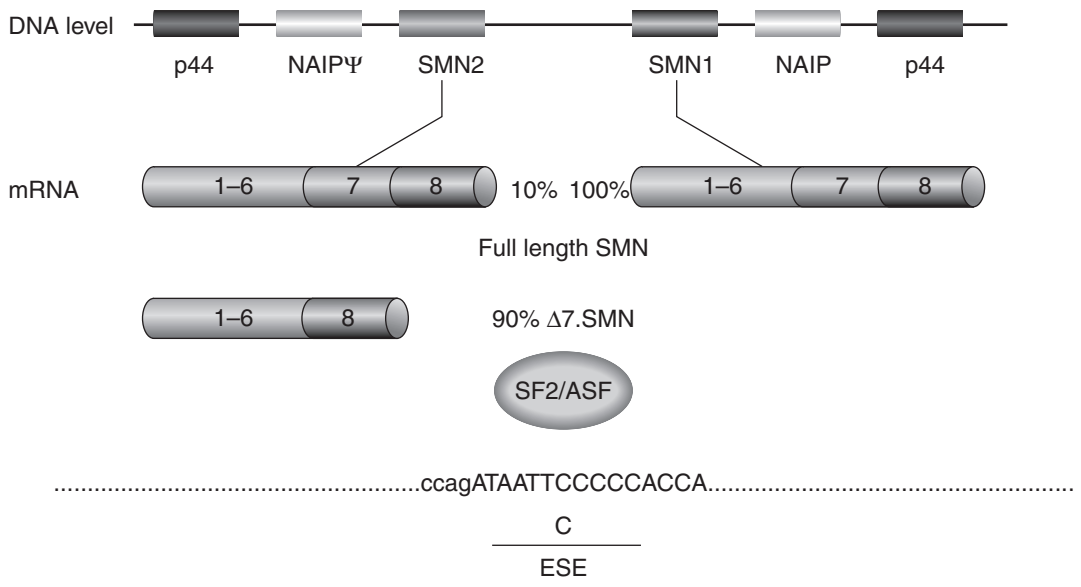

Fig. 7.1. The SMN gene is part of an inverted duplication spanning approximately 500 kb. SMN1, the ancestral gene, gives rise to a predominantly full length transcript. SMN2 contains a $t \rightarrow$ c nucleotide change which disrupts an Exonic Splice Enhancer (ESE) site, leading to failure of inclusion of exon 7 in 90% of transcripts.

(ii) by strand breakage of the SMN1 gene and subsequent repair on the SMN2 template, replacing the SMN1 gene with SMN2 (gene conversion) or (iii) by intragenic mutation (missense or small deletions) of specific coding sequences (Lefebvre et al., 1995; Hahnen et al., 1997; Talbot et al., 1997; Wirth, 2000). It will be evident that gene conversion events will result in two copies of SMN2 on a chromosome and that a patient with two such carrier chromosomes will carry four copies of SMN2, while a patient with two chromosomes with SMN1 deletion without gene conversion will have two copies of SMN2. In fact chromosomes have been identified in which SMN is present in multiple copies ranging from 0–6. Given that the SMN2 gene is transcribed and translated to produce approximately 10% of full length protein, the number of SMN2 copies is the critical factor in determining the amount of SMN protein that is ultimately present in cells. Immunodetectable SMN protein correlates well with disease severity (Lefebvre et al., 1997). SMA is thus a disease of SMN deficiency, not complete absence, which is lethal (Schrank et al., 1997). It is only through the evolutionary "accident" of duplication of the SMN region that the disease has occurred and no naturally occurring animal model of SMA can exist as no other organism has an SMN2 copy to provide rescue from embryonic lethality.

The SMN protein shows little homology to other proteins but contains some key functional domains which are also present in other proteins of similar function (Talbot et al., 1997, 1998). SMN is expressed at the RNA and protein level in all tissues at high levels, and complete inactivation in a mouse knockout model leads to failure of the embryo to implant, suggesting that it has an important "housekeeping" function in all cells (Schrank et al., 1997). Whether motor neuron degeneration arises because these cells have a greater requirement for this housekeeping role or because SMN has a motor neuron specific function has not yet been resolved, though accumulating evidence discussed below supports the latter hypothesis.

It has now been established that the SMN1 protein functions as a dimer within a large multiprotein complex (Gubitz et al., 2004). In cell lines and also in many whole tissues and primary cell cultures SMN stains both the nucleus, where it localizes discretely to intranuclear sub-organelles known as "Gemini of coiled bodies" (so-called "gems") and to the cytoplasm where it is diffusely localized. The other constituents of the SMN protein complex have been designated as gemin 2–6 (SMN being gemin 1).

SMN has been implicated in a number of cellular functions. The SMN complex plays a critical role in the maturation and assembly of the ribonucleoprotein complex (Wan et al., 2005). RNP is exported from the nucleus into the cytoplasm where SMN acts as a co-factor in the addition of Sm core proteins prior to the re-import of the Sm-RNP complex prior to its participation in splicing. SMN has also been implicated in other nuclear processes such as splicing itself and the control of transcription. It has been proposed that motor neurons may have a developmentally regulated requirement for elements of the ribonucleoprotein complex that exceeds that of other tissues.

In recent years the dogma that RNA is excluded from the axons, which are therefore incapable of protein synthesis, has been overturned (Koenig and Giuditta, 1999). Active transport of mRNA has been shown to occur in dendrites in the hippocampal neurons in response to post-synaptic signals and provides one mechanism for synaptic plasticity in processes such as long term potentiation (Eberwine et al., 2001). Similarly, SMN has been demonstrated in the axonal compartment of neurons in vivo and in the growth cone of primary neurons in culture (Fan and Simard, 2002; Rossoll et al., 2002). Exclusion of SMN from the axon leads to the failure of specific classes of mRNA, notably that for beta-actin, to be transcribed (Rossoll et al., 2003). Evidence from a *Drosophila* model of SMN deficiency, in which the fly survives to the larval stage because of the maternal contribution of SMN, suggests that the function of SMN in the axon may be to act as a translational repressor to allow specific mRNAs to arrive at the NMJ and be locally translated. Absence of SMN leads to failure of glutamate receptor clustering in flies (Chan et al., 2003).

In order to create a faithful mouse model of SMN deficiency it has been necessary to recapitulate the human genomic architecture in which SMN1 is deleted in the presence of multiple copies of SMN2 (not naturally present in the mouse). This has been achieved by crossing heterozygous SMN-null mice with mice in which human SMN2 has been transgenically inserted into the mouse genome (Monani et al., 2000). This results in several lines of mice with varying copy number of SMN and increasing severity with low copy number. In these mice NMJs show similar failure of normal architecture as that seen in the fruitfly model (Le et al., 2005). This supports the idea that SMN deficiency has a specific effect on peripheral motor neurons.

7.2.3. Treatment strategies

Supportive treatment for types I and II SMA includes respiratory assessment and nutritional support as appropriate, and for type II patients corrective spinal surgery for scoliosis.

Carrying out adequate clinical trials in childhood onset recessive SMA represents a major technical challenge. This is a disorder in which after an initial period of decline, which may be surprisingly rapid, there is a longer phase of slower or arrested decline. It remains unproven whether clinical rating scales or surrogate measures such as MUNE can adequately assess change in motor function in clinical trials (Crawford, 2004). Current attempts at molecular therapy of SMN deficiency can be summarized under three broad approaches.

Since every patient alive with SMA will carry at least one, and in all probability several, copies of SMN2, one approach would be to find compounds that dramatically up-regulate the transcription and translation of SMN2, with the intended effect of increasing the sum total of full length SMN protein. SMN is one of about 5% of human genes which are regulated epigenetically by histone acetylation (Kernochan et al., 2005). Histone deacetylase (HDAC) alters the conformation of histones to make transcription less likely. HDAC inhibitors such as phenylbutyrate, sodium valproate and others have been shown in pre-clinical models to increase the amount of total SMN (Chang et al., 2001; Sumner et al., 2003; Lunn et al., 2004). Clinical trials are now underway to see if this can ameliorate the disease in patients. Significant potential for toxicity, however, may limit the clinical usefulness of such drugs.

As discussed above, SMN2 is differentially transcribed to produce a predominant product lacking exon 7 and therefore significant functionality. If the splicing "behavior" of SMN2 could be altered to produce a greater amount of mRNA including exon 7 this would lead to an increase in full length protein expression. Evidence from mouse models suggests that ubiquitous over-expression of SMN2 (and thus the exon 7 deleted truncated transcript) has no deleterious effects (Le et al., 2005). Several groups have used tailed oligonucleotides which have recognition sequences for the exonic splice enhancer (ESE) sequence in exon 7 of SMN2 in order to promote inclusion of exon 7 in the final transcript (Cartegni and Krainer, 2003; Skordis et al., 2003). This suggests that this approach is both viable and potentially applicable to many other diseases in which control of splicing may be therapeutic. The main technical problem is one of delivery to the spinal cord.

Viral delivery of SMN1 to motor neurons has been attempted in mice deficient of SMN using lentiviral vectors. Initial results showed some promise but the number of animals used was small and larger experiments with more rigorous controls are required (Azzouz et al., 2004). In addition direct injection to enough muscles to provide retrograde transport to large populations of spinal motor neurons in humans is a technically different proposition from achieving the same in small animal work.

When most patients present with SMA (especially type I) there is usually very significant disability which has arisen in a sub-acute fashion. Thus, effective intervention is likely to require population screening at birth to identify who is going to develop the disease.

7.2.4. Spinal muscular atrophy with respiratory distress (SMARD)

One of the distinguishing features of proximal SMA type I due to SMN mutations is the lack of diaphragmatic involvement. In contrast, severe, early onset recessive infantile SMA with diaphragmatic weakness leading to eventration of bowel into the chest and respiratory failure is associated with a distal pattern of weakness and accounts for about 1% of cases of infantile SMA (Rudnik-Schoneborn et al., 1996). The majority of children with this severe phenotype die within 6 months of birth. Linkage to chromosome 11 was described in a number of families (Grohmann et al., 1999) and mutations identified in the IGHMBP2 gene (Grohmann et al., 2001). Subsequent mutation screening appears to have extended the phenotype to include patients with infantile hypomyelinating neuropathy, including at least one case in which there was little evidence of anterior horn cell loss at autopsy (Pitt et al., 2003). Intriguingly, given the function of SMN in RNA metabolism, IGHMBP2 is thought to function as a transcriptional activator and helicase. The *neuromuscular degeneration* mutant is due to mutations in the mouse orthologous gene (Cox et al., 1998). Immunolabeling indicates that IGHMBP2 has a widespread cellular distribution including axons (Grohmann et al., 2004). Ultrastructural studies and a potential response in the mouse model to treatment with NGF agonists support the notion that SMARD may be primarily a disorder of the peripheral nerve (Diers et al., 2005; Ruiz et al., 2005).

7.2.5. Other recessive forms of SMA

Slowly progressive childhood onset distal SMA was reported in early population based surveys of SMA (Pearn and Hudgson, 1979). A single large consanguineous family from Lebanon has been linked to chromosome 11q, but is genetically distinct from SMARD (Viollet et al., 2002). The age of onset is variable but typically in the first 2 years of life with slow progression. A proportion of patients develop diaphragmatic weakness over decades.

A single Brazilian pedigree of European genetic heritage has been linked to Xq13.1-q21 (Takata et al., 2004).

Males affected by this disorder develop childhood (1–10 years) onset distal lower limb foot deformity and weakness with very slow progression and maintenance of ambulation into later life.

7.3. Dominantly inherited SMA

7.3.1. Introduction

Distal SMAs or Hereditary Motor Neuronopathies are a range of conditions characterized by slowly progressive distal wasting and weakness in the absence of clinical or electrophysiological evidence of sensory involvement. Motor conduction velocity is normal. The commonest presentation is classical peroneal muscular atrophy with pes cavus and foot deformity. Age of onset, while variable, is generally from the 2nd to 6th decade. Other distinct syndromes have been described in which there is a predominance for hand over foot weakness, associated vocal cord involvement, mixed proximal and distal SMA or additional features such as pyramidal tract involvement. In the pre-molecular era, Harding (1993) produced a useful clinical classification of distal hereditary motor neuronopathies, which with some reservations is still clinically useful. However, as will be described below, for each new gene that is identified there is a range of phenotypes and age of onset. The question arises as to whether these disorders should be considered as motor axonal neuropathies or anterior horn cell degenerations. There are no autopsy studies of patients with these disorders to support either theory, and animal models are awaited. However, given that these disorders appear to represent age dependent pure motor neuron degeneration, with minimal or no sensory involvement, elucidation of their pathogenesis is of considerable interest to those working on all forms of motor neuron disease. For practical purposes, the terms distal SMA and distal HMN should be considered synonymous.

7.3.2. Autosomal dominant SMA due to an identified genetic mutation

7.3.2.1. Distal SMA due to mutations in small heat shock proteins B1 and B8

Heat shock proteins are molecular chaperones which broadly function to process misfolded proteins so that they can be sequestrated or eliminated to prevent damage to the cell. Neurons, being post-mitotic and long-lived, are specifically vulnerable to accumulation of aberrant forms of misfolded protein. Several classes of hsp are recognized, based on molecular size. Mutations in genes coding for specific members of each of these groups has now been associated with a range of diseases including Hereditary Spastic Paraparesis (hsp 60), cataract (alpha-crystallin) and distal SMA (small heat shock proteins 22 and 27) (Muchowski and Wacker, 2005).

Linkage to chromosome 12 was identified in a series of families with distal HMN (Timmerman et al., 1996). Recently, missense mutations in the small heat shock protein B8 (previously known as hsp 22) were identified in this and a number of other families with distal HMN (Irobi et al., 2004b). Mutations were also found in the related small hsp B1 (hsp 27) gene in several European dHMN families including one from our clinic (Fig. 7.2) in which affected individuals have a pure lower motor neuron syndrome but carry the same (S135F) mutation as a Russian family with CMT2F, which is characterized by distal HMN with additional mild distal sensory loss (Evgravof et al., 2004). As yet, only a small number of individuals with mutations in these genes have been identified, making precise delineation of the clinical phenotype difficult. However, the disorder appears in the 2nd or 4th decade with weakness of toe extension and ankle dorsiflexion and then progresses proximally to involve knee and hip over a 5–10 year period before significantly threatening ambulation in most patients. The upper limbs are usually less severely affected. Reflexes are reduced and then progressively lost. There are no distinguishing features which separate dHMN patients with hsp mutations clinically from those without mutations. We have screened a large panel of patients with various forms of inherited and sporadic lower motor neuron degeneration which has revealed that these genes are not a common cause of the distal SMA phenotype.

The mechanism whereby mutations in the small heat shock proteins lead to motor neuron degeneration is not yet established. A number of roles have been identified for hspB1 which include both anti-apoptotic functions and interactions with various components of the cytoskeleton (Perng et al., 1999; Bruey et al., 2000; Charette et al., 2000). Members of the small hsp family are defined by a low molecular weight and the presence of a highly conserved region known as the a-crystallin domain (Sun and MacRae, 2005), which contains a cysteine residue known to be essential for binding cytochrome c and hence crucial in preventing the subsequent activation of apoptosis via the formation of the apoptosome. In experiments in which primary neurons in culture have been transfected with mutant hsp B1 we have found that the axonal cytoskeleton is disrupted, with aggregation of neurofilament and also wild-type hsp B1 being prevented from entering the axon. The length of axons in comparison to control cells is also reduced, suggesting that mutant hsp affects axonal transport and subsequent growth.

A number of heat-shock proteins including hspB1 have been demonstrated to be upregulated in various

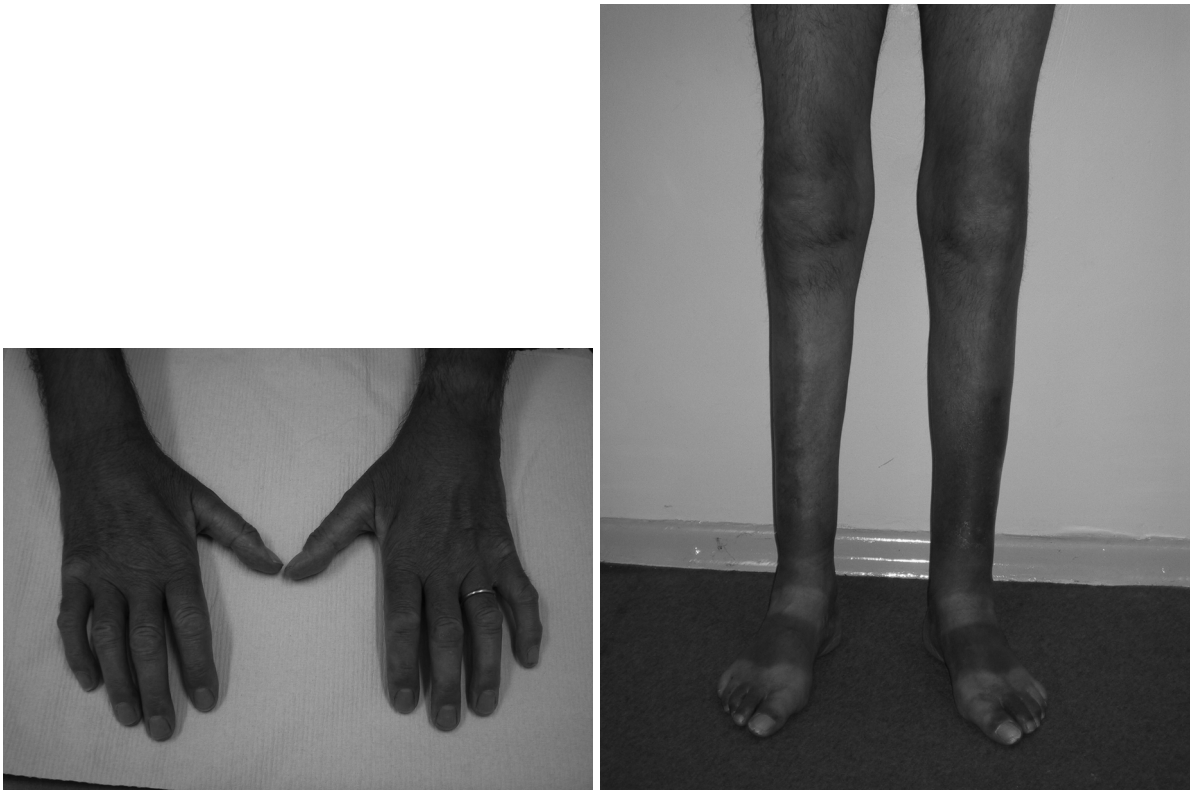

Fig. 7.2. Distal hereditary motor neuropathy due to a S135F mutation in the alpha-crystallin binding domain of the small heat shock protein hsp 27 (hspB1) [photographs courtesy of Dr David Hilton-Jones].

neurodegenerative diseases and hspB1 has also been shown to be essential for the survival of both sensory and motor neurons (Benn et al., 2002).

In the G93A SOD1 mouse model of familial ALS, hsp 27/B1 is downregulated immediately prior to the onset of neuronal degeneration (Maatkamp et al., 2004). Collectively these findings suggest that the small heat shock proteins are logical therapeutic targets in motor neuron diseases.

7.3.3. Distal SMA due to mutations in glycyl tRNA synthetase

Families have been described in which distal SMA predominantly affects the upper limbs. These have been given the label dHMN V in the Harding (1993) classification and are also referred to as "spinal Charcot-Marie Tooth Disease" (CMT2D). These conditions probably represent the same entity where the underlying pathology is a neurogenic pattern of muscle wasting, presumed to be due to a primary neuronopathy, with minimal or no sensory involvement. Both sporadic and familial cases of dSMA V have been observed (Meadows and Marsden, 1969; McLeod and Prineas, 1971). Christodoulou et al. (1995) described a large Bulgarian family as having weakness and wasting which was more prominent in the distal upper limbs, especially the thenar muscles and first dorsal interossei, and mapped the gene to chromosome 7p. The disease commenced with hand involvement at a mean age of 17 years and, in 40% of patients, symptoms subsequently developed in the feet within about 2 years. In one branch of the family, mild pyramidal features and, rarely, extensor plantar responses were observed. The progression of the disease was very slow, with patients still ambulant at the age of 64 years. Clinically, CMT 2D patients are very similar but more often have sensory involvement and this disorder was mapped by linkage to the same genetic locus on chromosome 7p (Ionasescu et al., 1996) and has subsequently been found to be allelic to dSMA V. The gene for this disorder has been identified as GARS, which encodes glycyl-tRNA synthetase (Antonellis et al., 2003). Reported mutations have been clustered around residues of the catalytic core of the enzyme and would be expected to affect the

enzyme kinetics, although this remains to be formally demonstrated. We have recently identified a novel mutation (G598A) in a highly conserved region of the anticodon binding domain which is important for discriminating the correct tRNA to ligate with glycine, and which in functional assays completely abolished the synthetase activity of the enzyme. Interestingly, this mutation occurred in a child with very early onset and widespread motor neuron degeneration who had never walked. Thus it is likely that the phenotypic spectrum associated with GARS mutations will be further extended.

Glycyl-tRNA synthetase (GlyRS) is an essential enzyme that ensures the fidelity of the genetic code by charging specific tRNA species with glycine. As with SMN-related SMA, the question arises as to why a gene with a housekeeping function gives rise to such a specific form of cell loss. For example, mis-incorporation of amino acids into glycine-rich protein sequences may be the key mechanism and as such mutation affecting the anti-codon binding could be particularly critical. It is also reasonable to expect that the mitochondrial form of Gly-RS will be similarly affected by the G598A mutation and, indeed, it is well established that motor neurons are vulnerable to degeneration through mitochondrial specific dysfunction (Menzies et al., 2002).

7.3.4. SMA due to mutations in the VAPB gene

An apparently novel form of motor neurone disorder, though bearing some resemblance to previously described forms of SMA, was reported from Brazil and mapped to chromosome 20 (Nishimura et al., 2004a). A single mutation was subsequently identified in the Vesicle Associated Membrane Protein-Associated Protein B (VAPB) (Nishimura et al., 2004b) in six Brazilian families with a common ancestral haplotype. The clinical phenotype associated with this disorder appears to be quite variable and to include pyramidal tract signs in some patients, allowing the authors to use the designation ALS8 for this disorder. The rate of progression is highly variable but onset is consistently between 25–40 years. Some patients have a slowly progressive lower motor neuron syndrome with fasciculations and cramps, others have respiratory and bulbar involvement with a reduction in vital capacity and death from respiratory failure within a decade of onset. A mixed proximal and distal pattern is observed. The one family with slowly progressive late onset SMA in which we have identified a mutation in VAPB has striking scapuloperoneal, eye closure and abdominal wall muscle weakness.

Vesicle associated membrane proteins form intracellular complexes promoting the fusion and transport of cytoplasmic vesicles. VAPB localizes to the Golgi and ER and mutant protein expressed in cells forms aggregates. An association with microtubules implies that specific motor neurone degeneration may reflect a failure of axonal transport.

7.3.4.1. Distal SMA due to mutations in BSCL2: the Silver Syndrome and overlap with hereditary spastic paraparesis

What has been referred to as the 'Silver Syndrome' consists of amyotrophy of the hands in association with spastic paraplegia (Silver, 1966). It is transmitted as an autosomal dominant trait and has been classified with the hereditary spastic paraplegias as SPG17. The gene for this disorder was identified as BSCL2 which encodes the protein 'seipin' (Windpassinger et al., 2004) and in recessive families where there are inactivating mutations leads to the complex developmental disorder Bernadelli-Seip Congenital Lipodystrophy. Two mutations in BSCL2 have been identified, N88S (Fig. 7.3) and S90L, both of which are predicted to affect glycosylation of the protein, which is a constituent of the endoplasmic reticulum. Overexpression of the mutant protein leads to cytoplasmic aggregation. However, it is now clear that individual families carrying BSCL2 mutations may contain individuals with the classical Silver phenotype or distal SMA with upper limb predominance (Chen et al., 2004; Irobi et al., 2004a). In a family that we have studied in which affected individuals have the S90L mutation in BSCL2, several members developed amyotrophy of the hands followed with a mean of 10–15 years by the development of extensor plantar responses and progressive spastic paraparesis, leading to loss of ambulation by the fifth decade in one individual who had been diagnosed as having multiple sclerosis in the pre-MRI era. The other individuals have been examined in the 8th and 9th decade on several occasions and have no evidence of pyramidal tract involvement. The explanation for this marked interfamilial heterogeneity is unexplained but might suggest the operation of a modifier gene.

7.3.4.2. Distal hereditary motor neuronopathy due to mutations in senataxin

Another form of dHMN in which pyramidal tract signs are variably reported has been designated ALS4 and is due to heterozygous mutations in the senataxin gene (Chen et al., 2004). Onset is with symmetrical lower limb motor dysfunction in adolescence or early adulthood, with slow progression and normal lifespan. Homozygous inactivating mutations in the senataxin gene lead to the early onset recessive disorder Ataxia with Oculomotor Apraxia 2 (AOA2). A link with the pathogenesis of other forms of motor neuron degeneration

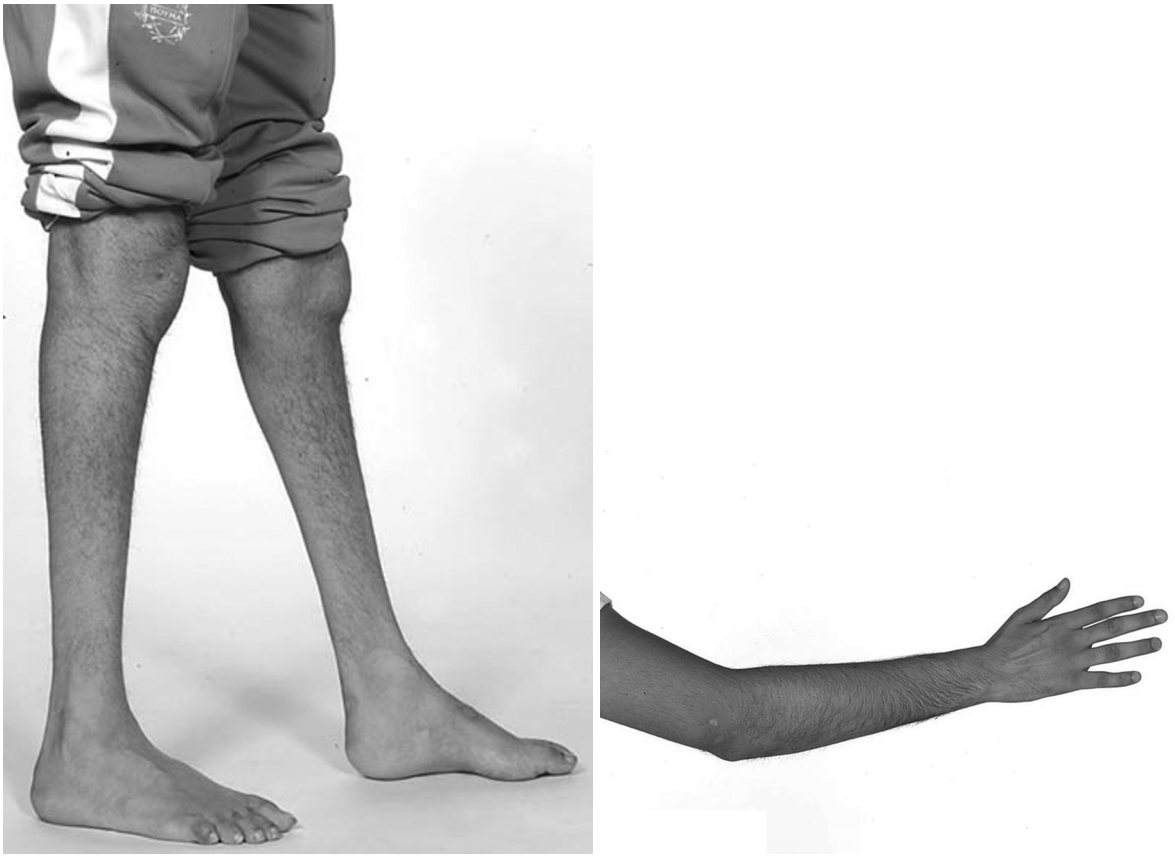

Fig. 7.3. Distal amyotrophy of the hands and feet associated with the N88S mutation in the BSCL2 gene (Silver Syndrome).

is the fact that senataxin shows homology to DNA/RNA helicases including IGHMBP2.

7.4. SMA with vocal cord paresis

Several families have been described in which distal motor neuropathy is associated with vocal cord paralysis leading to hoarseness and stridor (Young and Harper, 1980; Boltshauser et al., 1989; Pridmore et al., 1992). At least two separate genetic loci exist and a G59S mutation has been identified in one family in the p150 subunit of dynactin (McEntagart et al., 2001; Puls et al., 2003). The age of onset in this family is from 28–44 with stridor or distal upper limb weakness and wasting. A number of patients required surgery to the vocal cords but none of the patients lost ambulation. At autopsy there is evidence of ballooning of motor neurons and aggregation of immunoreactive p150 (Puls et al., 2005). In contrast, the families linked to chromosome 2p14, including the original one described by Young and Harper (1980), present with distal weakness in adolescence and less extensive bulbar involvement.

The complex of dynein and dynactin mediates retrograde axonal transport and is critical for axonal integrity. The *loa* mouse mutant results in motor neuron degeneration due to a mutation in the cytoplasmic dynein heavy chain (Hafezparast et al., 2003). Together these findings underline the importance of axonal transport as a key area of vulnerability for motor neurons.

7.5. Conclusions

The identification of the genes responsible for various forms of spinal muscular atrophy in recent years has transformed our understanding of the key areas of vulnerability in lower motor neuron function and promises to open up new areas for therapeutic endeavor. A completely unexpected finding has been that mutations in several genes with an apparent housekeeping function in RNA metabolism lead to selective loss of motor neurons

(Anderson and Talbot, 2003). It is likely that transport of mRNA into the axon and local translation, potentially in response to molecular cues derived from muscle, may turn out to be a mechanism for maintaining neuromuscular integrity both in development and maturity. It is less surprising that defects in protein trafficking in neurons, particularly in the axonal compartment, lead to motor neuron loss.

It is clear from genetic screening that most cases of childhood onset proximal SMA are accounted for by inactivating mutations in the SMN gene. However, the other genes for dominantly and recessively inherited SMA/HMN individually account for very few cases. A number of other linkages and causative genes remain to be established, which collectively will enhance our understanding of motor neuron degeneration.

The considerable inconsistency in nomenclature with slowly progressive hereditary motor neuronopathies with occasional pyramidal tract involvement being considered variously as forms of ALS, SMA or HSP will ultimately be consigned to a historical footnote as individual disorders are defined by genetic mutation.

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Chapter 8

Spinobulbar muscular atrophy (Kennedy's disease)

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The slowly progressing motor neuronopathy, spinobulbar muscular atrophy (SBMA), is a rare inherited disease but the discovery of its genetic cause in 1991 was a milestone in neurogenetics. Not only was the genetic defect responsible for SBMA a novel type of mutation, namely the expansion of a translated trinucleotide repeat tract, but it was also the first mutation linked to an adult onset motor neuron disease.

SBMA was first described in 1968 by William Kennedy and his colleagues who reported clinical and pathological observations on 11 members from two families affected with proximal muscular atrophy (Kennedy et al., 1968). Because of the authors its original description, SBMA is also known as Kennedy's disease. The disease has an X-linked pattern of inheritance and involves spinal and bulbar musculature and has a prevalence of about 1 in 50,000 live male births. The onset is usually in the third to fifth decade of life, but can have a juvenile onset, between 8 and 15 years of age (Echaniz-Laguna et al., 2005). Males only are affected and the disease is usually accompanied by signs of androgen insensitivity, particularly gynecomastia. Female carriers are either asymptomatic or display only mild signs of motor dysfunction. Histopathological examination demonstrates a marked loss of anterior horn cells in the spinal cord and atrophy of muscle fibers secondary to the loss of motor neurons. An MRI study has also revealed a significant atrophy of the upper spinal cord (Sperfeld et al., 2005).

8.1. Genetics of spinobulbar muscular atrophy

8.1.1. Androgen receptor gene mutations

The genetic cause of SBMA was identified in 1991 by Albert La Spada and Kenneth Fischbeck as the expansion of a polymorphic CAG repeat sequence in the first exon of the gene encoding the androgen receptor (AR), on the X chromosome, at Xq11-12 (La Spada et al., 1991). In the normal population, the AR gene has a number of CAG repeats between 12 and 36. A disease phenotype develops after 40 repeats and expansions of up to 62 repeats have been reported. The CAG repeat sequence is translated into an expanded polyglutamine (polyQ) stretch in the N-terminal domain of the AR protein (Fig. 8.1). The AR is a ligand-activated transcription factor and has the typical structure of steroid receptors and mediates the physiological action of androgens in male sexual differentiation and spermatogenesis.

Known mutations of the AR gene, other than CAG repeat expansions, are truncations or missense mutations that affect the function of the AR as a steroid hormone receptor and are associated to androgen insensitivity syndromes, such as testicular feminization (McPhaul et al., 1991; Griffin, 1992). Individuals with testicular feminization have a complete insensitivity to androgens and develop phenotypically as females. However, this condition is not associated with motor symptoms, supporting the notion that CAG repeat expansions in the AR gene linked to SBMA are gain-of-function mutations. Some forms of prostate cancer are associated with somatic mutations of the AR gene (Bentel and Tilley, 1996) and short CAG repeats represent a risk factor for prostate cancer (Nelson and Witte, 2002).

SBMA was the first neurogenetic disease linked to expansions of a polymorphic CAG repeat sequence in the open reading frame of the causative gene. Nine such diseases have now been identified including Huntington's disease and several types of spinocerebellar ataxias (SCA) (Table 8.1). With the exception of

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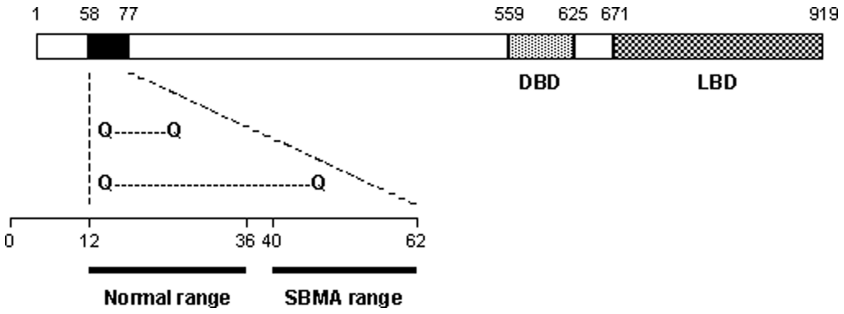

Fig. 8.1. Structure of the functional domains of the human androgen receptor, showing the location of the polymorphic polyQ stretch. DBD: DNA-binding domain; LDB: ligand-binding domain.

SBMA, all CAG repeat expansions diseases are autosomal dominant neurodegenerative disorders that affect different parts of the nervous system. The disease genes encode seemingly unrelated proteins, all containing a polyQ tract. PolyQ tracts are found in a wide range of proteins, with diverse functions, but predominantly in transcriptional regulators. In addition to SBMA, the only other example of a CAG repeat expansion disorder for which the function of the gene product is known is spinocerebellar ataxia, type 6 (SCA6) that is caused by a polyQ expansion in the α_{1A} subunit of the voltage-sensitive calcium channel (Zhuchenko et al., 1997). In the majority of CAG repeat expansion diseases, an increase above approximately 35 in the number of repeats defines the threshold between normal and disease phenotypes (Zoghbi and Orr, 2000). SCA6 is unusual within CAG repeat expansion disorders in that a pathogenic process develops from approximately 20 repeats.

8.1.2. CAG repeat expansion size and disease progression

8.1.2.1. Anticipation

Expanded CAG repeats exhibit somatic and germ line instability and expand or contract after successive generations. In SBMA, CAG repeat length changes have been observed in about 25% of transmissions and occur more frequently through male than female transmission. In addition, expansions occur more often than contractions (La Spada et al., 1992). A variation from 46 to 53 repeats has been observed in a single family over four generations (Biancalana et al., 1992). Instability of CAG repeats may involve human-specific factors as a transgene with a moderate expansion (45 repeats) showed no changes in transgenic mice during transmission across at least four generations (Bingham et al., 1995). The need for human-specific factors was confirmed by the intergenerational instability of the AR gene in transgenic mice generated using

Table 8.1

Main characteristics of neurodegenerative diseases caused by translated CAG repeat expansions in the disease gene

Disease	Chromosome	Protein	kDa	Normal repeats	Expanded repeats
Huntington's	4	Huntingtin	348	6–35	36–121
DRPLA	12	Atrophin-1	124	3–36	49–88
SBMA	X	Androgen receptor	110	9–36	40–62
SCA-1	6	Ataxin-1	87	6–39	40–83
SCA-2	12	Ataxin-2	90	14–32	33–77
SCA-3/MJD	14	Ataxin-3	42	12–40	55–86
SCA-6	19	α1A-Ca^{2+} channel	255	4–18	21–33
SCA-7	3	Ataxin-7	95	4–35	38–306
SCA-17	6	TATA-binding protein	49	29–42	47–55

DRPLA: dentatorubropallidoluysian atrophy; SBMA: spinobulbar muscular atrophy; SCA: spinocerebellar ataxia; MJD: Machado-Joseph disease.

a yeast artificial chromosome containing the entire human AR gene with 45 CAG repeats (La Spada et al., 1998).

8.1.2.2. Correlation between repeat expansion size and phenotype

A positive correlation exists in SBMA between the size of the CAG repeat expansion in the AR gene, the severity of the disease and the extent of androgen insensitivity signs (Doyu et al., 1992; Igarashi et al., 1992; La Spada et al., 1992). Conversely, repeat number correlates negatively with the age of onset. More generally, irrespective of the disease, analysis of repeat size and symptoms in patients carrying CAG repeat expansions has clearly demonstrated an inverse relationship between the size of the repeat expansion and the age of onset (Zoghbi and Orr, 2000). In addition, other factors, probably both environmental and genetic, are likely to contribute to disease onset (Li et al., 2003).

8.2. Clinical aspects of spinobulbar muscular atrophy

8.2.1. Phenotype of affected males

The usual onset of SBMA is in early adulthood, but the first clinical signs can appear as early as in adolescence, often in the form of muscle cramps (Sperfeld et al., 2002). Proximal muscle weakness and atrophy develop later followed by bulbar signs at a later stage. Bulbar signs include weakness of facial muscles, with frequent fasciculations, and difficulties with speech, articulation and swallowing. Twitching movements of the chin with pursing of the lips are common and characteristic. Deep tendon reflexes are depressed and frequently absent. Postural tremor of the upper limbs is common. Despite the marked reduction or absence of sensory nerve action potentials (see § 8.3.1), sensory symptoms and signs are usually absent.

A comprehensive study of endocrine features in 22 SBMA patients showed partial androgen resistance in more than 80% of the patients, with gynecomastia being the most prominent sign. Gynecomastia was post pubertal but appeared before muscular weakness in most cases. Thirteen patients had alteration of testicular exocrine function. Hormonal profile of partial androgen resistance was present in 86% of the patients, with an elevated testosterone level in 68%. Androgen insensitivity seems to appear later in life but is apparent before the development of motor and other neurological signs (Dejager et al., 2002).

8.2.2. Phenotype of female carriers

8.2.2.1. Heterozygous female carriers

Only males carrying the SBMA mutation develop a clear motor phenotype, although female carriers of the mutation, either heterozygous or the very rare homozygous carriers, show subclinical motor signs. In a study of eight heterozygous female carriers, only one was symptomatic, the other individuals were neurologically normal, but had frequent muscle cramps, mild muscle weakness and neurogenic changes on electromyography indicative of mild chronic denervation (Sobue et al., 1993; Ishihara et al., 2001).

8.2.2.2. Homozygous female carriers

An important breakthrough in the understanding of the pathogenesis of SBMA came from the description of two sisters homozygous for the SBMA mutation (Schmidt et al., 2002). The two individuals were aged 34 and 42, with a number of CAG repeats for each allele of the AR gene of 46/47 and 47/48, respectively. On clinical examination, both women were found to be neurologically normal, but showed occasional muscle cramps and twitches and slight hand tremor. Electromyograms studies showed evidence of mild motor axonal loss in the sternocleidomastoid muscle in one of the sisters. Several important conclusions can be drawn from this study. First, CAG expansion in the AR gene cannot be considered as a recessive mutation *sensus stricto*, as two copies of the mutant gene do not cause disease. Secondly, the absence of a phenotype in heterozygous female carriers cannot be due to random or skewed X-inactivation. Consequently, the development of the disease in males requires male-specific factors. Androgens are the obvious candidates and the importance of high levels of circulating androgens in the pathogenesis of SMBA has been demonstrated in animal models of the disease, as detailed below (§ 8.6.2).

8.3. Electrophysiology

The definitive diagnosis of SBMA depends on genetic testing. However, patients with this disease are frequently first seen in amyotrophic lateral sclerosis (ALS) or neuromuscular clinics and often thought to have ALS. Electrophysiology is an easy screening method with a constellation of features, which, in the right clinical context, are very helpful. The presence of diffuse, slowly progressive lower motor neuron disease, with chronic neurogenic changes, in a male patient with absent or depressed deep tendon reflexes, in whom sensory nerve action potentials are small or absent, makes SBMA very likely. Fasciculation is prominent with a predilection for perioral muscles, and the presence of postural tremor and gynecomastia should make a clinical diagnosis straightforward.

8.3.1. Motor and sensory conduction studies

Motor conduction studies are usually normal apart from modest reduction of the compound muscle action

potential (CMAP) amplitude (Sobue et al., 1989; Olney et al., 1991; Trojaborg and Wulff, 1994; Ferrante and Wilbourn, 1997). F-wave studies have not been reported in SBMA. Sensory nerve action potentials are invariably reduced in amplitude and may be absent (Trojaborg and Wulff, 1994; Ferrante and Wilbourn, 1997). Somatosensory evoked potentials from both upper and lower limb stimulation have also been reported to be abnormal and brainstem acoustic-evoked potentials show an increase in wave I latency (Polo et al., 1996). However, whether this is simply a reflection of peripheral nerve fiber disease, or genuinely represents a disturbance of central sensory pathways is not clear. Repetitive nerve stimulation is normal in SBMA but neuromuscular jitter may be increased, even in the face of normal strength. This has been regarded as one explanation for fatigue in SBMA (Meriggioli and Rowin, 2003). Although involvement of large myelinated sensory fibers in the spinal nerves of SBMA patients is well established, little is known about the involvement of small sensory neurons and trigeminal nerves. Recently laser-evoked potentials (LEPs) were studied in six unrelated patients with SBMA; five of these patients underwent trigeminal reflex recordings and three also had sural nerve biopsies. LEPs were markedly abnormal, indicating a dysfunction in pain pathways (Antonini et al., 2000). It was hypothesized that, given the sparing of small fibers in the sural nerve specimens, dysfunction in spinothalamic cells, possibly due to an abnormal representation of the ARs, might account for the abnormal LEPs. Except for the jaw-jerk, all the trigeminal reflexes were markedly abnormal. Since the afferents for the jaw-jerk have their cell body within the central nervous system instead of the ganglion, the selective sparing of the jaw-jerk indicates a trigeminal ganglionopathy (Antonini et al., 2000).

8.3.2. Needle electromyography

Electromyography investigations show widespread chronic partial denervation with evidence of reinnervation characterized by long duration, large amplitude motor unit action potentials (Harding et al., 1982; Olney et al., 1991). The motor units, although large, are usually simple and stable, reflecting a chronic process. This motor unit morphology helps distinguish SBMA from ALS. Trojaborg and Wulff (1994) noted a profound loss of motor units in most muscle studies, even in muscles with normal or only mildly reduced strength. This was based on a reduced or incomplete interference of motor units on maximum voluntary contraction. Motor unit estimates have not been reported in SBMA. Denervation with fibrillation and positive sharp waves may be present but is relatively infrequent. Fasciculation is common and diffuse in SBMA; however, there is a remarkable predilection for fasciculation to occur in perioral muscles, which may also be seen and recorded in otherwise asymptomatic carriers (Huang et al., 1998). Clinically, similar abnormal twitching of the cheeks and perioral muscles are seen in olivopontocerebellar atrophy (OPCA). However, they are induced by facial movements and with the muscles at rest, EMG of the orbicularis oris and risorius muscles reveals myokymic discharges in the absence of visible movements (Lou et al., 1994). With voluntary contraction, the EMG shows synchronous discharges in the orbicularis oris and risorius muscles ipsilaterally associated with visible twitching. The duration of the EMG bursts was 10–75 ms with a frequency of 8–25 Hz, suggesting that the abnormal twitching is consistent with a myoclonic disorder (Lou et al., 1994). There are differences in the fasciculation of SBMA compared with ALS. Complex fasciculations are common in patients with ALS and sometimes consist of two or more components that occur independently but also in combination (Hirota et al., 2000). In ALS, complex fasciculations occur about 5% of the time whereas in SBMA they occur with a frequency of less than 1%. There is also a difference in the mean firing frequency of the fasciculations, being about 25 per minute in ALS, but significantly lower in SBMA, approximately 3 per minute (Hirota et al., 2000).

8.3.3. Upper motor neuron studies

There are no clinical signs suggesting upper motor neuron involvement in SBMA and transcranial magnetic stimulation has shown the central motor pathways to be normal (Eisen, 2001). This has been further confirmed using peristimulus time histograms which can assess the function of the descending motor volley of a select group of corticomotoneurons (Weber and Eisen, 1999). However, Shaw et al. (1998) reported that immunostaining for macrophage markers showed evidence for subtle corticospinal tract pathology in two cases of SBMA.

8.4. Histopathology of spinobulbar muscular atrophy

8.4.1. Pattern of neurodegeneration

Following the findings reported in the original description of SBMA by Kennedy et al. (1968), several studies have combined clinical and histopathological descriptions, including the two comprehensive papers by Harding et al. (1982) and Sobue et al. (1989), on 10 and nine affected individuals, respectively. Pathologically, SBMA is characterized by the selective loss of lower

motor neurons including anterior horn cells. Some brain stem motor neurons innervating facial and bulbar muscles are also lost. Muscle biopsies demonstrate chronic denervation. Sural nerve biopsies show evidence of demyelination and axonal atrophy with a decrease in large myelinated fibers; small myelinated and unmyelinated fibers are not affected. Some mild gliosis is also observed. Demyelination and distal axonopathy of dorsal root ganglion neurons is evident and is accompanied by general neuronal atrophy, but neuronal loss is limited (Li et al., 1995). This is consistent with the results of electrophysiological investigations of primary sensory neuron involvement in SBMA (see § 8.3.1).

8.4.2. Inclusions bodies

Proteins with long polyQ sequences fail to fold correctly and, as a result, are prone to aggregation into high molecular weight insoluble protein complexes. This was originally predicted from the structure of the polyQ chain that would oligomerize through the formation of "polar zippers" (Perutz et al., 1994). Neuronal intranuclear inclusions of huntingtin, the product of the Huntington's disease gene, were later discovered in mouse models of Huntington's disease (Davies et al., 1997) and in the brain of Huntington's disease patients (DiFiglia et al., 1997).

In post-mortem studies of SBMA patients, AR immunocytochemistry reveals the presence of inclusion bodies in the nuclei of about 10% of the surviving neurons in spinal and brainstem motor neurons (Li et al., 1998a) (Fig. 8.2). Although inclusions are essentially nuclear, immunocytochemistry with the 1C2 antibody, specific for long polyQ sequences, shows the presence of inclusions in the cytoplasm of some neuronal types, including motor neurons of the anterior horn and dorsal root ganglion neurons, where they are particularly prominent (Adachi et al., 2005). Unlike nuclear inclusions, cytoplasmic inclusions do not stain for ubiquitin. Inclusions are 1–5 μm in diameter and there is usually a single inclusion per cell, but cells with two or three inclusions are occasionally seen. In electron microscopy, inclusions appear to be composed of dense AR-positive material, without a limiting membrane (Li et al., 1998b). Inclusions stain for antibodies to the N-terminus of the AR, but not with C-terminus-specific antibodies. This suggests that the AR is proteolyzed or that its C-terminus is masked, either by a specific conformation or by interaction with other proteins (Li et al., 1998a,b). Nuclear AR-immunoreactive inclusions in SBMA are not restricted to motor neurons as they were also observed in non-neuronal tissues, in particular in scrotal skin epidermal cells, but not in muscle (Li et al., 1998b). The distribution of AR inclusions suggests that their mechanism of formation is not directly related to the pathogenic process, but rather to the abnormal structure of the expanded protein.

An interesting point regarding intraneuronal inclusions in SBMA, as well as in all CAG repeat expansion disorders, is that not only do they contain the protein product of the disease gene, but they are also immunoreactive for ubiquitin. Ubiquitin is a small peptide (8 kDa) implicated in the degradation of abnormal and rapidly turned-over proteins. Branched chains of ubiquitin attach to lysine residues in the target protein through

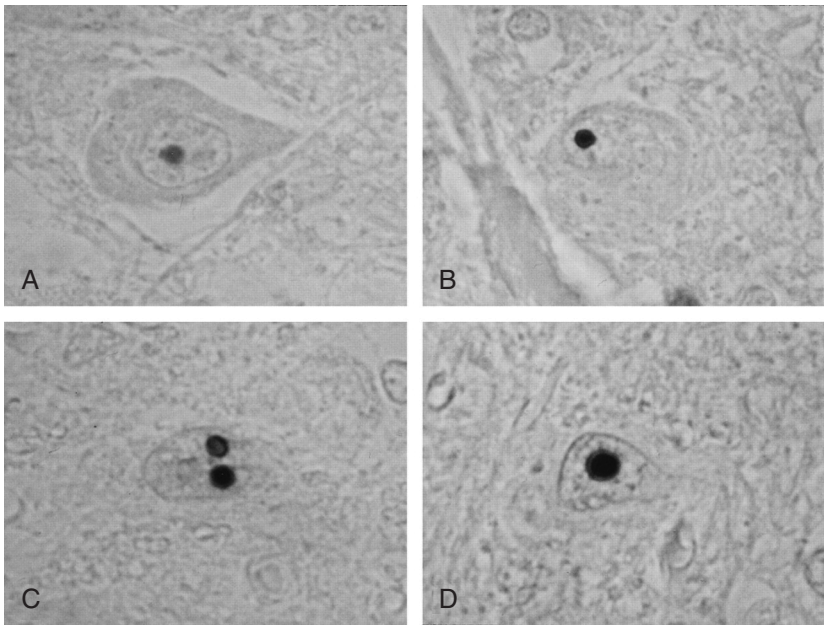

Fig. 8.2. For full color figure, see plate section. Immunohistochemistry of the AR protein in nuclear inclusions in the central nervous system of SBMA patients. AR staining is observed in motor neurons of the medulla oblongata (**A** and **B**) and pons (**C** and **D**). **A** and **B** are stained with PG-21 and **C** and **D** are stained with AR(N-20). Magnification: **A** to **D**, 600×. (Reproduced from Li et al. (1998b) with permission from the American Society for Investigative Pathology.)

isopeptide bonds and multiubiquitinated proteins are then degraded by a large multicatalytic protease, the 26S proteasome (Hochstrasser, 1996). Of note, AR inclusions in transfected cells (Stenoien et al., 1999) or transgenic mice (Abel et al., 2001) stain positively for proteasome components. Moreover, aggregation of misfolded proteins is likely to be a factor of general importance in neuronal cell death, as ubiquitinated inclusions are a hallmark of neurodegenerative diseases, including not only CAG repeat-expansion diseases, but also Alzheimer's disease, Parkinson's disease and ALS (Gallo and Anderton, 1989). Although inclusion bodies of the AR are a hallmark of SBMA, a recent study on 11 autopsied patients has shown that mutant AR accumulates in a CAG repeat length-dependent manner in a diffuse pattern, not only in the nucleus but also in the cytoplasm, in particular in the Golgi apparatus in neurons, and in a number of non-neuronal tissues (Adachi et al., 2005).

8.5. The androgen receptor in spinobulbar muscular atrophy

8.5.1. General properties of the androgen receptor

The AR is a ligand-activated transcription factor, with a structure and mechanism of action common to all members of the nuclear steroid receptor superfamily (MacLean et al., 1997). The AR has a molecular weight of approximately 110 kDa, depending upon the number of glutamine residues. The natural ligands of the AR are testosterone or its more potent derivative 5α-dihydrotestosterone. In the absence of ligand the AR is localized in the cytoplasm, forming a complex with chaperones. Chaperones interacting with the AR include the heat-shock proteins, HSP70, HSP90 and HSP56; these proteins maintain the receptor in a conformation optimal for binding of the ligand. After ligand interaction with the ligand binding domain (LDB) in the C-terminus of the molecule, the AR is released from the chaperone complex and translocates to the nucleus. In the nucleus, the AR forms a homodimer and binds to androgen response elements upstream of specific genes through the two zinc fingers of the DNA binding domain (DBD). The DNA and ligand binding domains are separated by a hinge region that contains the nuclear localization signal. The N-terminus of the AR, containing the polyQ sequence, regulates transactivational activity (Fig. 8.1).

8.5.2. Properties of polyglutamine-expanded androgen receptor

The precise role of the polyQ sequence in the activity of the AR is not clear, but long sequences, within the pathogenic range of SBMA decrease transactivational activity by up to 40% (Mhatre et al., 1993; Chamberlain et al., 1994; Kazemi-Esfarjani et al., 1995; Butler et al., 1998). This is likely to explain the mild signs of androgen insensitivity observed in the disease. Consistent with a lower transactivational activity, changes in the expression of a number of androgen-regulated genes has been reported in motor neuron × neuroblastoma hybrid cells expressing an expanded AR, as compared to cells expressing a normal form of the protein (Lieberman et al., 2002). Binding studies on genital skin fibroblasts from SBMA patients also revealed a decrease in the binding affinity of androgens for the AR (MacLean et al., 1995). As for all steroid receptors, the activity of the AR is regulated by a large number of co-activators and co-repressors, including members of the steroid receptor co-activator (SRC) family (for a review see Heinlein and Chang, 2002). One of the AR co-activators is the small GTPase, Ran (also referred to as ARA24). ARA24 is the only known co-activator that interacts with the AR in a polyQ length-dependent manner (Hsiao et al., 1999). Expansion of the polyQ sequence leads to a decrease in the affinity of ARA24 for the AR and in a reduction in its activity in promoting transactivation.

8.5.3. The androgen receptor in motor neurons

The AR has a widespread pattern of expression and is expressed in the nervous system. Within the nervous system, the AR is present in several neuronal populations, but is particularly abundant in motor neurons (Sar and Stumpf, 1977; Yu and McGinnis, 1986; Simerly et al., 1990; Menard and Harlan, 1993). Tubulin, the structural component of microtubules, appears to be a primary target for androgen regulation in motor neurons. Androgens promote motor axon regeneration after axotomy (Yu and Srinivasan, 1981; Kujawa et al., 1989, 1991; Jones, 1994) and this is correlated with a selective up-regulation of β_{II}-tubulin, the most abundant tubulin isoform in neurons (Jones and Oblinger, 1994). Similarly, tubulin is up-regulated by androgens in cultured neuroblastoma cells (Butler et al., 2001).

Within responsive tissues, testosterone is converted into 5α-dihydrotestosterone, by the enzyme, 5α-reductase. The two isoforms of 5α-reductase, type 1 and type 2, are encoded by different genes, display little homology and have different kinetic properties. Type 2 5α-reductase has a higher affinity for testosterone than type 1 and is specifically expressed in androgen-responsive tissues. RT-PCR and in situ hybridization analyses of rat spinal cord has shown that the two isoforms of 5α-reductase are present in the spinal cord,

but type 2 is exclusively expressed in anterior horn cells (Pozzi et al., 2003). Thus, the combination of high levels of AR and expression of type 2 5α-reductase can partly explain the selectivity of polyQ-expanded AR toxicity toward lower motor neurons.

8.6. The basis for gender specificity in spinobulbar muscular atrophy

An unique characteristic of SBMA in the context of CAG repeat disorders is that males only are affected. The simplest explanation for the absence of a phenotype in heterozygous female carriers would be that random X-inactivation would leave approximately half of the motor neuron population expressing a normal AR. This would be insufficient for the development of a full disease phenotype. An analysis of the methylation status of the AR gene in seven female carriers with mild motor symptoms demonstrated random inactivation of the affected allele. By contrast, in the same study, an asymptomatic carrier displayed highly skewed inactivation of the mutant allele (Ishihara et al., 2001). However, the importance of X-inactivation has been ruled out by the clinical description of two homozygous female carriers with phenotype similar to heterozygous carriers (see § 8.2.2.2). Thus, a likely explanation for the gender specificity in SBMA is the difference in the level of circulating androgens. For instance, the concentration of serum androgens in adult men is 3–10 μg l^{-1} (10–30 nM), whereas it is 0.1–1 μg l^{-1} (0.3–3 nM) in women.

8.6.1. Male-specific phenotype in transgenic mouse models of spinobulbar muscular atrophy

Several lines of transgenic mice have been generated that express a full-length human AR with expanded polyQ repeats under the control of an heterologous promoter (Katsuno et al., 2002; Chevalier-Larsen et al., 2004). Male animals develop a typical phenotype, with progressive muscle wasting and nuclear inclusions. The symptoms appear as early as 4 weeks of age and an average 50% mortality is approximately 100 days. By contrast, female animals show only a mild motor phenotype, with minimal nuclear localization of the AR. On the other hand, mice expressing an AR with a number of glutamine repeats in the normal range do not exhibit any neurological signs reminiscent of SBMA. Motor neuron loss can be minimal in some of these animals, suggesting that neuronal dysfunction in SBMA is not dependent on cell death. Transgenic mice harboring a human AR yeast artificial chromosome (YAC) with 20 or 100 CAG repeats have been generated (Sopher et al., 2004). The transgene has a normal expression pattern in these animals and mice expressing the AR with 100 CAG repeats develop a typical progressive neuromuscular phenotype and exhibit extensive motor neuron degeneration. The male-specific pathology of SBMA is reproduced in YAC transgenic mice as females are only mildly affected, being generally smaller, but they do not display a full disease phenotype.

8.6.2. Androgen requirement for motor neuron toxicity in spinobulbar muscular atrophy

Animal models of SBMA have now provided a clear demonstration for the requirement for high levels of serum androgens for the development of a full disease phenotype. The phenotype in male transgenic mice expressing expanded AR is dramatically reduced after surgical castration (Katsuno et al., 2002; Chevalier-Larsen et al., 2004). Conversely, disease in females is exacerbated by testosterone administration (Katsuno et al., 2002). Similarly, testosterone causes a marked degeneration of photoreceptor neurons in *Drosophila* expressing expanded AR (Takeyama et al., 2002). Treatment of flies with AR antagonists that promote nuclear translocation but not transactivation induces a similar phenotype as androgens. Thus, androgens exert toxicity in SBMA by promoting nuclear localization of the AR, independently of an action on androgen-regulated genes. Androgens also promote the formation of nuclear or cytoplasmic inclusions, as demonstrated in neuronal or non-neuronal cells expressing full-length expanded AR (Stenoien et al., 1999; Darrington et al., 2002; Walcott and Merry, 2002). Thus, the development of a pathological phenotype in SBMA clearly depends on high levels of circulating androgens and, conversely, low levels of androgens are likely to be the main explanation for the absence of an overt pathology in female carriers of the SBMA mutation.

8.6.3. Possible protective effects of estrogens in female carriers

Estrogens protect neurons from a number of toxic insults, such as β-amyloid toxicity, oxidative stress or glutamate excitotoxicity. Estrogens also protect neurons from cell death in animal models of ischemia and of Parkinson's disease (Green and Simpkins, 2000). Treatment of cultured neuroblastoma cells expressing polyQ-expanded AR with micromolar concentrations of 17β- or 17α-estradiol prevented AR aggregation (Darrington et al., 2003). This protective effect of estrogens is likely to occur through a non-genomic mechanism possibly involving estrogen binding to the AR. Hence, estrogens, possibly combined with other factors, may

contribute, to some extent, to neuroprotection in female carriers of the SBMA mutation.

8.7. Pathogenic mechanisms involved in spinobulbar muscular atrophy

8.7.1. Transcriptional abnormalities

Nuclear localization of polyQ-expanded AR is required for the development of toxicity. For instance, expanded AR constructs lacking the ligand binding domain, that are constitutively nuclear, induce neurodegeneration when expressed in photoreceptor cells in *Drosophila*, conversely constructs lacking a nuclear localization signal do not exert toxicity, even in the liganded form (Takeyama et al., 2002). More generally, nuclear localization is an essential aspect of the toxicity of most disease-causing polyQ containing proteins (Klement et al., 1998; Saudou et al., 1998). Microarray expression profiling in motor neuron × neuroblastoma hybrid cells has shown that expanded AR can activate androgen responsive genes in the absence of ligand, most probably due to abnormal interaction with co-regulators (Lieberman et al., 2002). Abnormal regulation of specific neuronal transcripts is an early event in cellular and animal models of other CAG repeat disorders (Lin et al., 2000; Luthi-Carter et al., 2000; Sugars and Rubinsztein, 2003). In the nucleus, polyQ-expanded proteins, including the AR, interfere with the transcription machinery by interacting with transcriptional regulators and compromising their functions. An important transcriptional regulator that has its activity inhibited by interaction with polyQ-expanded AR is CREB-binding protein (CBP), a co-activator of the cAMP response element binding protein (CREB) (McCampbell et al., 2000). CBP is a linker protein between CREB and general transcription factors and also has a histone acetyltransferase activity. CBP-mediated histone acetylation facilitates the accessibility of DNA to the transcription machinery. CBP contains a polyQ tract, which contributes to its affinity for expanded AR. CBP binds to normal and expanded versions of the AR and enhances androgen-dependent transcription. CBP co-localizes with intranuclear inclusions formed by the AR in transfected cells, and this results in a decrease in CBP-dependent transcription and in a reduction in histone acetylation (McCampbell et al., 2000). The pathways activated by cAMP-response elements are implicated in neuronal survival and overexpression of CBP also ameliorate cell death resulting from expression of expanded AR (McCampbell et al., 2000). More generally, recruitment of CBP into inclusion bodies and subsequent reduction of its histone acetyltransferase activity is a consistent feature of polyQ expansion disorders (Steffan et al., 2000; Nucifora et al., 2001).

8.7.2. Role of androgen receptor aggregation in toxicity

Long polyQ sequences in the AR result in its misfolding, as indicated by the propensity of expanded AR to aggregate into inclusion bodies that are hallmarks of SBMA, however, whether protein aggregation is a cause or a consequence of the pathological process is still a matter of controversy. For instance, AR aggregation in transfected cells does not correlate with cell death (Simeoni et al., 2000). By following in real time the fate of individual cultured neurons transfected with expanded huntingtin, Arrasate et al. (2004) have shown, in a very elegant study, that neurons containing inclusion bodies had a higher probability of survival than neurons in which huntingtin remained diffuse.

Proper folding of proteins after translation, refolding of misfolded proteins and their targeting for degradation by the proteasome is mediated by molecular chaperones that include the heat shock protein, HSP70 and the co-chaperones HDJ-1(HSP40) and HDJ-2/HSDJ. A large number of studies have shown that inclusion bodies formed by polyQ-expanded proteins recruit chaperones, moreover overexpression of HDJ-1 or HDJ-2/HSDJ reduces aggregation and inclusion formation. This was first demonstrated for ataxin-1, the SCA1 protein, in transfected cells in a landmark paper by Cummings et al. (1998). Similarly, inclusions formed by expanded AR in transfected HeLa cells are positive for HSP70 and HDJ-2/HSDJ and overexpression of HSJ-2/HSDJ reduces AR aggregation (Stenoien et al., 1999). In vivo, crossing SBMA transgenic mice with HSP70-overexpressing mice ameliorates the motor phenotype and this is correlated with a reduction in the nuclear level of the AR as well as with a reduction in the level of its aggregated form (Adachi et al., 2003). Reduction of AR levels by HSP70 is probably due to its targeting to the proteasome. Indeed, combined overexpression of HDJ-1 and HSP70 in transfected cells expressing an AR fragment enhances its degradation by the proteasome (Bailey et al., 2002). The recruitment of chaperones into inclusion bodies may make them unavailable to perform their housekeeping functions. A failed attempt by motor neurons to dispose of abnormal proteins may saturate the proteasome system and interfere with its physiological function in the catabolism of rapidly turned-over proteins.

8.7.3. Impairment of axonal transport

Most motor neuron diseases, such as ALS, display abnormalities of the axonal cytoskeleton. For instance, neurofilaments, the intermediate filament type of neurons, accumulate in the perikarya and proximal axons

of affected neurons (Leigh et al., 1989; Julien and Mushynski, 1998; Bruijn et al., 2004). Neurofilament accumulations have been observed in some cases of SBMA (Wilde et al., 1987; Sobue et al., 1989), but are not a consistent occurrence. Interestingly, a missense mutation in the protein, p150^{Glued}, has recently been discovered in a motor neuron disease with selective lower motor neuron involvement very similar to SBMA (Puls et al., 2003). p150^{Glued} is a component of the dynactin complex that mediates the binding of the retrograde transport protein, dynein, to a variety of cargo structures including membranes, chromosomes and microtubules. Degeneration of ventral horn motor neurons in individuals with the p150^{Glued} mutation is associated with inclusion bodies immunoreactive for dynactin and dynein (Puls et al., 2005). More generally, alteration of retrograde axonal transport seems to be a consistent feature of most forms of motor neuron disease. For instance, progressive motor neuron degeneration in the Legs at Odd Angle (*loa*) mutant mouse is caused by dominant mutations in the dynein heavy chain gene (Hafezparast et al., 2003). Conversely, inhibition of dynein function by overexpression of dynamitin, that results in the dissociation of the dynein-dynactin complex, in transgenic mice results in a late-onset progressive motor neuron degeneration displaying similarities with ALS (LaMonte et al., 2002). Thus, a primary defect in retrograde organelle transport can cause selective motor neuron degeneration.

Impairment of axonal transport also appears to be a general feature of polyQ expansion diseases. Indeed, expression of polyQ-expanded huntingtin or ataxin-3, the SCA-3 protein, in *Drosophila* causes axonal transport defects, characterized by organelle accumulations, but without affecting cell viability (Gunawardena et al., 2003). PolyQ-expanded proteins might interfere directly with the binding of motor proteins to microtubules; alternatively inclusion bodies formed in axons by polyQ-expanded proteins can physically block transport. Such neuritic inclusions have been observed in cultured neuronal cells expressing expanded AR (Darrington et al., 2002; Piccioni et al., 2002). Due to the length of their axons motor neurons are likely to be especially vulnerable to changes affecting intracellular transport, important for neurotrophic factor signaling (Salehi et al., 2003; Delcroix et al., 2003).

The various possible pathways involved in motor neuron dysfunction in SBMA are summarized in Figure 8.3.

8.8. Possible therapeutic approaches for spinobulbar muscular atrophy

8.8.1. Early trials of androgen therapy

Complete loss of androgen response, as in androgen insensitivity syndromes, is not associated with motor impairment. However, it was hypothesized at an early stage that the reduction of the transactivational activity of the AR resulting from expansion of the polyQ tract could contribute to motor neuron dysfunction. Indeed, androgens have a trophic effect on motor neurons of the sexually dimorphic spinal nucleus of the bulbocavernosus. In non-sexually dimorphic motor neurons, androgen treatment promotes the rate of axonal regeneration by up to 30% after axotomy of the facial or sciatic nerves (Jones, 1994). This concept led to several clinical trials of androgens in SBMA patients. In a first study a genetically confirmed SBMA patient was treated with nandrolone-decanoate for 6 months, but no improvement was observed (Danek et al., 1994). In another study, long term (6 to 18 months) oral administration of high doses (25–37.5 mg daily) of testosterone in two affected brothers led to some muscle

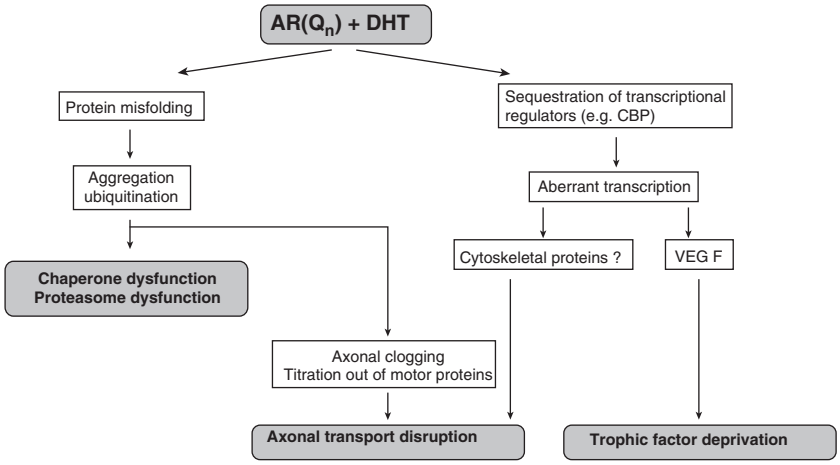

Fig. 8.3. Possible pathways involved in motor neuron dysfunction in spinobulbar muscular atrophy. DHT: 5α-dihydrotestosterone; T: testosterone.

work output improvement, when combined with exercising in one of the brothers (Goldenberg and Bradley, 1996). Since, as discussed above, testosterone, in fact, promotes the neurotoxic effects of the AR, the improvement observed in this patient is likely to be due to a direct effect of testosterone on muscle strength rather than to a protective effect on motor neurons.

8.8.2. Reduction of circulating androgens

The role of androgens in the pathogenesis of SBMA, clearly demonstrated in animal models, is consistent with the clinical trials with androgens that failed to provide conclusive improvement. Conversely, from a therapeutic standpoint, classical anti-androgens would be of little benefit in SBMA since most of them induce nuclear translocation of the AR. To circumvent this problem, Katsuno et al. (2003) treated the transgenic mouse model of SBMA they developed earlier with the lutenizing hormone-releasing hormone (LHRH) agonist, leuprorelin, that inhibits production of testosterone by the testis. Leuprorelin-treated mice showed improvement of their motor functions. Reversal of motor dysfunction was correlated with a decrease in nuclear accumulation of the AR. As predicted, treatment of the animals with the anti-androgen, flutamide, promoted nuclear translocation of the AR, with no effect on the motor phenotype. Leuprorelin is commonly used in the treatment of prostate cancer with a safety which is well documented, and thus could be a promising candidate in the treatment of SBMA.

8.8.3. Histone deacetylase inhibitors

As outlined above (§ 8.7.1), expression of polyQ-expanded proteins in cellular or animal systems leads to a reduction in the histone acetyltransferase activity of CBP. The partial loss of CBP histone acetyltransferase activity can be counterbalanced by treatment with histone deacetylase (HDAC) inhibitors. Treatment of transfected cells with the histone deacetylase inhibitors, trichostatin (TSA) or suberoylanilide hydroxamic acid (SAHA), reduces the toxicity caused by long polyQ sequences (McCampbell et al., 2001). HDAC inhibitors have a similar effect in vivo. For instance, HDAC inhibitors protect photoreceptor neurons from degeneration in *Drosophila* expressing the first exon of the huntingtin gene with an expanded CAG repeat stretch (Steffan et al., 2001). In a transgenic mouse model of Huntington's disease orally administered SAHA has been shown to cross the blood–brain barrier and to improve motor symptoms (Hockly et al., 2003). An HDAC inhibitor less toxic than SAHA, sodium butyrate supplied in drinking water to a transgenic mouse model of SBMA, improves the neurological phenotype and increased survival rates, but only a narrow range of dosage, 4 g l^{-1}, being the most effective (Minamiyama et al., 2004). Thus, sodium butyrate might have some therapeutic benefit in SBMA.

8.8.4. Gene therapy

8.8.4.1. Vascular endothelial growth factor

Deficiency in vascular endothelial growth factor (VEGF) might be a common feature of motor neuron disorders. The original evidence for such a deficiency came from the discovery that mice lacking the hypoxia response element in the promoter of the VEGF gene developed a motor neuron disease phenotype (Oosthuyse et al., 2001). Furthermore, specific haplotypes in the VEGF gene have been found to be associated with an increased likelihood of developing ALS (Lambrechts et al., 2003). In the context of SBMA, a quantitative analysis of RNA levels for different isoforms of VEGF in a transgenic mouse model of SBMA has revealed a clear reduction of VEGF164 in the spinal cord of presymptomatic animals (Sopher et al., 2004). The promoter of the VEGF gene contains a CBP-regulated element and reduction of VEGF164 in transgenic mice is probably mediated by the increased binding of expanded AR to CBP (Sopher et al., 2004). Thus, a gene therapy approach for VEGF delivery to motor neurons could be of therapeutic benefit for SBMA. In support of such a possibility, delivery of VEGF to motor neurons by intramuscular injection of rabies G-pseudotyped lentiviruses in transgenic mice expressing the familial ALS G93A Cu/Zn superoxide dismutase (SOD1) mutation results in a dramatic increase of 30% in the life expectancy of the animals (Azzouz et al., 2004b). Lentiviral vectors can transduce non-dividing cells, including neurons in vivo and integrate the transgene into the host cell genome hence maintaining long term expression. Incorporation of the rabies virus G glycoprotein, that binds to dynein, allows retrograde transport from the neuromuscular junction to the cell body (Azzouz et al., 2004a,b).

8.8.4.2. RNA interference

Downregulation of the transcript encoded by the disease gene using RNA interference (RNAi) is a promising approach for the treatment for diseases caused by dominant mutations and has been shown to be effective for CAG repeat expansion disorders. For instance, downregulation of ataxin-1 in the cerebellum of a transgenic mouse model of spinocerebellar ataxia, type 1 has been achieved by injection of recombinant adeno-associated virus vectors expressing short hairpin RNAs. This treatment elicited improved motor coordination and abolished intranuclear inclusions of ataxin-1 in

Purkinje cells (Xia et al., 2004). Similarly, an RNAi strategy improved the phenotype of a Huntington's disease transgenic mouse model (Harper et al., 2005). Furthermore, down regulation of mutant SOD1 by RNAi after intramuscular or intraspinal injection of lentivirus vectors in transgenic mice expressing the G93A SOD1 mutation results in improved motor performance and delay in the onset of the disease (Ralph et al., 2005; Raoul et al., 2005). Downregulation of the AR in models of SBMA has not been reported to date but the above evidence makes it a therapeutic strategy worth exploring.

8.9. Concluding remarks

From a therapeutic standpoint, the most significant advance in the understanding of the etiology of SBMA has been the demonstration of androgen requirement for the development of a motor neuron disease phenotype, hence lowering serum androgens is a promising therapeutic strategy. On the other hand, SBMA is both a polyQ expansion disease and a motor neuron disease and, as such, displays some of the typical pathogenic features from these two classes of diseases. Consequently, therapies developed for diseases such as Huntington's disease, for example HDAC inhibitors, or for ALS, for example VEGF delivery, could be of benefit for SBMA patients.

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Chapter 9

GM_2 gangliosidoses

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9.1. Introduction

The GM_2 gangliosidoses are a group of recessively inherited disorders in which deficiency of a lysosomal enzyme, hexosaminidase A, leads to abnormal intracellular accumulation of lipids in neurons and glia. The classic infantile form of the disorder, Tay-Sachs disease, is by far the most common form of GM_2-gangliosidosis. It was first recognized in 1881 by the British ophthalmologist, Waren Tay. The pathology of the condition was described by the American neurologist, Bernard Sachs, about 10 years later. Children with this disease, after a few months of apparently normal development, lose their head control, become hypotonic and apathetic and fail to progress, develop seizures and cortical blindness. Physical signs include an exaggerated startle reaction, spasticity, rigidity and quadriplegia. On fundoscopy cherry-red macula is seen. The head size grows disproportionately, probably due to the accumulation of the ganglioside within neurons. These children usually die of intercurrent infection and inanition before 5 or 6 years of age.

It is now apparent that in some cases the ganglioside accumulation may have a later onset. Patients with the so-called juvenile form of the illness typically develop signs and symptoms in early childhood and die within about 10 years. Other patients with hexosaminidase deficiency follow an even less malignant clinical course and can live into adulthood. People who develop this late form may manifest the first symptoms in their 20s or 30s and can survive for several decades. In all these cases, the disorder is generally designated late onset GM_2 gangliosidosis ($LOGM_2G$). The distinction into juvenile, early adult and late adult forms is arbitrary and intermediate cases occur, even in the same family. As opposed to the early infantile form, Tay-Sachs disease, which is quite homogeneous, $LOGM_2G$ shows a wide clinical heterogeneity with a diversity of neurological and psychiatric manifestations, affecting the cerebellum and its connections, the pyramidal and lower motor neurons and the autonomic nervous system. Extrapyramidal manifestations such as Parkinsonism, dystonia or choreoathetosis might occur. Other manifestations include peripheral neuropathy, autonomic dysfunction, psychosis and dementia. Examples of this heterogeneity are included in Table 9.1.

9.1.1. Pathology

The gross pathology is very similar in Tay-Sachs disease, $LOGM_2G$, with its various genetic-biochemical manifestations, as well as in Sandhoff disease, except that in Sandhoff disease there is also involvement of visceral organs. The most pronounced cellular change is the presence of swollen neurons with storage material in lysosomes throughout the nervous system. The storage material may increase cell size, resulting in the typical ballooned neurons, and it also may give rise to unique morphological abnormalities, seen mainly in electron microscopy. "Meganeurites" are formed which are associated with aberrant dendritic, neuritic and synaptic growth. Characteristic inclusions are the so-called membranous cytoplasmic bodies.

It is interesting that neurons are targeted in the absence of hexosaminidase, since gangliosides occur in all cells throughout the body. Even more remarkable is the unexplained selectivity of certain neuronal populations, such as the pyramidal cells in the cortex or the anterior horn cells. It is possible that higher production rates of substrate occur in certain cells, that the expression of residual enzyme in specific cell populations differs or that a limited ability of the mutated residual enzyme to function persists in some neurons in spite of the ganglioside accumulation.

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Table 9.1

Neuropsychiatric syndromes associated with GM_2 gangliosidosis

	Reference
Tay-Sachs disease	
Parkinsonism	Inzelberg and Korczyn, 1994
Motor neuron disease	Johnson et al., 1982
Dystonia	Meek, 1984
Spinocerebellar degeneration	Rapin et al., 1976
Cerebellar degeneration	Johnson et al., 1977
Seizures	O'Neill et al., 1978
Dementia	O'Neill et al., 1978
Sensory neuropathy	Barnes et al., 1991
Internuclear ophthalmoplegia	Barnes et al., 1991
Autonomic neuropathy	
Spinal muscular atrophy	Johnson et al., 1982

9.1.2. Biochemistry

Gangliosides are glycosphingolipids consisting of a hydrophobic acylsphingosine (previously termed ceramide) and a hydrophilic oligosaccharide chain bearing one or more *N*-acetylneuraminic acid (sialic acid) residues. Gangliosides are important components of the outer leaflet of plasma membranes of most animal cells. In animals, the highest ganglioside content is found in the gray matter of the brain, particularly in neuronal plasma membranes and in synaptic regions. However, glial cells, such as oligodendrocytes and astrocytes also contain substantial amounts of gangliosides. In the body gangliosides undergo a slow metabolism, being hydrolyzed by enzymes called hexosaminidases. In the adult human brain, at least 12 different gangliosides have been identified, four of which, G_{M1}, G_{D1a}, G_{D1b} and G_{T1b}, account for more than 90% of the total. In ganglioside G_{M2}, the oligosaccharide chain consists of a trisaccharide (gangliotriaose), to which sialic acid is bound. In the normal process of catabolism, GM_2 gangliosides are removed from the membrane by a so-called activator protein. The ganglioside-protein activator complex is transported to the lysosome where it is degraded by hexosaminidases. Accumulation of the ganglioside may thus result from an abnormality of either the enzyme or the activator protein.

Not surprisingly, neuronal function is compromised as the residual enzyme accumulates within lysosomes, but the relationship between the neuronal changes and the clinical manifestations of the disease remains to be elucidated. Siegel and Walkley (1994) have demonstrated a correlation between the amount of GM_2 ganglioside accumulation and the extent of ectopic dendritic growth in cortical pyramidal neurons in a number of neuronal storage diseases. Other data suggest that abnormalities in the processing of GM_2 gangliosides may be a final common pathway for degeneration, regardless of the primary metabolic defect.

Gangliosides are potent inhibitors of protein kinase C, important in the transduction of neurotransmitter signals, suggesting a possible link between the abnormal accumulation of gangliosides and neuronal dysfunction.

There are two isoenzymes of β-hexosaminidase, termed Hex A and Hex B, but only Hex A can act on the ganglioside GM_2. Genetic defects in any of three genes may lead to GM_2 gangliosidosis: *HEXA*, which encodes the β-subunit of Hex A; *HEXB*, which encodes the β-subunit of both Hex A and Hex B; and *GM2A*, which encodes the GM_2 activator. There are three forms of GM_2 gangliosidosis: (a) Tay-Sachs disease and its adult variants, resulting from mutations of the *HEXA* gene, are associated with deficient activity of Hex A but normal Hex B; (b) Sandhoff disease and variants, resulting from mutations of the *HEXB* gene, are associated with deficient activity of both Hex A and Hex B; and (c) GM_2 activator deficiency, due to mutations of the *GM2A* gene, is characterized by normal Hex A and Hex B proteins but inability to form a functional activator-ganglioside complex. There are also pseudo-deficient or clinically benign mutations characterized by biochemical defects of Hex A as seen in vitro but maintained functional enzymatic activity toward ganglioside GM_2.

Mutations affecting the α and β chains of Hex A or the activator protein cause the glycolipid to accumulate. In Tay-Sachs disease, β hexosaminidase activity is lost completely, leading to a relatively quick accumulation of the ganglioside. A reduction of enzymatic activity to 10–15% of normal (rather than complete absence as seen in Tay-Sachs disease), leads to a much slower accumulation and thus a later age of disease onset and a more protracted course.

Tay-Sachs disease is associated with severe mutations resulting in formation of no, or highly unstable, mRNA and thus a total absence of the alpha subunit protein coded by that mRNA. In the adult variant of the illness, mutations within the protein coding regions lead to stable mRNA and production of the corresponding protein subunits, but the subunits are defective. Many of the identified HEXA mutations, such as deletions, frameshifts and splice mutations, are "null" because they prevent the expression of α-subunits altogether. However, some point mutations that result in

amino acid substitutions are also functionally null because the resulting Hex A is inactive. A poor correlation exists between the mutation site and the clinical phenotype of the disease and the exact relationship between the mutation site, mRNA synthesis, hexosaminidase activity and clinical phenotype is still unclear.

9.2. Genetic heterogeneity

The classical mutation in HEX A responsible for GM_2 gangliosidosis responsible for Tay-Sachs disease is particularly common among Ashkenazi Jews. The disease is inherited recessively and those carrying this mutation in both alleles have never survived beyond infancy. However, several other different mutations have been identified in the same gene that may cause disease if present on both alleles, which will result in a later age of onset and different clinical presentations. In all cases, the dysfunction is limited to the central nervous system and affects one system preferentially, although throughout the progression of the disease additional systems may be affected (Table 9.1). Among the systems involved, motor neuron dysfunction is prominent.

As mentioned earlier, a disease only occurs if mutations are present on both alleles of the HEX A gene. Heterozygotes are clinically normal; they can be identified through biochemical examinations and more definitely by genetic analysis. Patients with late onset disease are frequently homozygous for a given mutation, particularly if they derive from consanguineous marriages. On the other hand, many other subjects are compound heterozygotes, i.e. carry different mutations on the two HEX A alleles which nevertheless have a cumulative effect on the enzyme activity, resulting in subnormal activity and thus ganglioside accumulation. This is particularly true in Ashkenazi Jewish patients. The Ashkenazi Jewish population has a high prevalence of the mutated HEX A gene responsible for Tay-Sachs disease and an additional, but different, mutation on the HEX A gene inherited from the other parent will lead to a compound heterozygote state.

To date, several dozen mutations have been identified in HEXA as responsible for $LOGM_2G$. These mutations are diverse in nature. They include several small and also large deletions, single and multiple nucleotide insertions, many missense and nonsense mutations and several mutations in or nearby intron/exon junctions that lead to abnormal splicing. These mutations are scattered throughout the gene, with a large cluster of mutations in exon 5.

Correlations between genotype and phenotype are straightforward if a mutation is common and therefore found in the homozygous state or in compound heterozygosity with other alleles clearly associated with an early-onset phenotype. The determination of phenotypes associated with uncommon mutations is more difficult if the patient has a delayed-onset form of disease, particularly since as mentioned above in addition to the deleterious HEXA mutations, polymorphisms with no apparent effect on Hex A activity have also been identified.

Environmental, or other genetic factors, may be involved since different phenotypes may also be seen within a given family. Cases with a Kugelberg–Welander phenotype and others with a more complex symptomatology can be seen in the same family, as reported by Argov and Navon (1984), Navon and Proia (1989) and Mitsumoto et al. (1985). Insight into the mechanism underlying this sensitivity of some neurons (or resistance of others) is of great interest and importance.

$LOGM_2G$ is more variable in age of onset and severity (Paw et al., 1990; Trop et al., 1992; Akli et al., 1993). The Gly269Ser mutation (G269A) is the most common mutation in $LOGM_2G$ patients and it is clearly associated with a chronic phenotype in either the homozygous or compound heterozygous forms (Navon et al., 1990). Several other mutations have been described in $LOGM_2G$, but always occur in people carrying the G269A mutation (compound form) (Navon et al., 1997).

9.2.1. Clinical phenotypes

Several clinical phenotypes have been described in patients carrying HEXA mutations. These include mostly cases with cerebellar or spinocerebellar syndromes (Rapin et al., 1976; Grabowski et al., 1980; Johnson et al., 1982), adult onset dementia (O'Neill et al., 1978), Parkinsonian syndrome (Inzelberg and Korczyn, 1994) and motor neuron syndromes. It could be assumed that over time all patients will develop a full clinical syndrome consisting of cerebellar, pyramidal, lower motor neurons, Parkinsonian, autonomic and cognitive dysfunction. However, data on this evolution is scarce and the question why different nerve elements are sensitive to the enzyme defect in different individuals is yet unknown. Also, unaffected individuals were described who had abnormally low hexosaminidase activity. These asymptomatic cases could in reality be presymptomatic, but some may be truly asymptomatic. This raises the issue whether abnormally low hexosaminidase activity may be coincidental in some cases rather than causative. Obviously neuropathological examination is required in some cases to decide whether typical ganglioside accumulation occurs which can account for the clinical phenotype.

In most cases of $LOGM_2G$ the clinical syndrome is complex. Patients may develop early stuttering in

childhood, then go on to develop cerebellar dysfunction. Motor neuron problems typically develop later, to be followed by dementia and sometimes psychosis. Autonomic nervous system involvement may also occur but can be difficult to detect if other features are predominant (Salman et al., 2001). However, there is marked clinical heterogeneity as is also seen in other genetic diseases, even in those with a single point mutation (Chapman et al., 1993).

The phenotype of motor neuron disease in adult hexosaminidase deficiency was first reported by Kaback et al. (1978) and more extensively by Johnson et al. (1982) and additional cases were described by Mitsumoto et al. (1985). However, most cases had additional neurological manifestations and a pure ALS-like syndrome is extremely rare (Johnson et al., 1982; Karni et al., 1988; Parboosingli et al., 1997).

Muscle weakness develops insidiously during the second decade or later. It may be heralded by muscle cramps and twitching. The weakness is first noticed in proximal muscles, although atrophy in hand muscles can be seen early and is prominent in some cases. Fasciculations may be sparse or widespread. The proximal weakness causes difficulties in rising from a low chair or a squatting position and Gower's sign may appear. Gait becomes waddling and – depending on the extent of upper motor neuron involvement – also spastic. The posture may be lordotic. Tendon reflexes are usually preserved, sometimes even brisk, but sometimes lost in the ankles. Clonus may be seen. Although clinically diagnosed as abnormal when in their teens or later, an even earlier involvement, manifested by high pedal arches and slight dysarthria, may occur.

At this stage the clinical diagnosis is frequently progressive spinal atrophy, Kugelberg-Welander disease or – if the upper motor neuron signs are obvious – juvenile amyotrophic lateral sclerosis (ALS). However, closer examination may demonstrate evidence of more widespread involvement, such as cognitive decline, extrapyramidal or cerebellar dysfunction or, rarely, autonomic problems. These, even if mild, should alert the clinician to the possibility of a multisystem degeneration.

Electromyography (EMG) and CT of the limbs and paraspinal muscles (Streifler et al., 1995) frequently identify muscle denervation or atrophy in patients with $LOGM_2G$, even if this goes unnoticed by the clinician.

The EMG demonstrates diffuse denervation and innervation with evidence of spontaneous activity, reduced recruitment pattern and high amplitude, polyphasic, prolonged motor unit potentials.

The discovery of adult patients presenting with both amyotrophic lateral sclerosis (ALS) and Hex A deficiency has suggested an enzymatic etiology in some forms of ALS (Drory et al., 2003).

The exact frequency of $LOGM_2G$ in general, and of ALS-like picture in particular, are unknown. Like many rare diseases, cases are misdiagnosed or remain undiagnosed. Even when the correct diagnosis is made, only a few cases are reported and these may not necessarily be the typical ones. Even when a series of cases are reported, emphasis is frequently on a given phenotype, e.g. movement disorder, and a full neurological and neurophysiological examination is frequently missing and the future evolution is not reported. However, the frequency of pure ALS picture as a manifestation of $LOGM_2G$ must be very rare. In a study in New York, which included 52 patients with atypical ALS (Jews and non-Jews), four had partial HexA deficiency (Gudesblatt et al., 1988). In that study, none of 50 patients with typical ALS had anormal HexA activity and the same finding was reported in 17 Greek ALS patients (Michelakakis et al., 1995). Similar results were reported by Drory et al. (2003) in a larger series of 115 patients with typical ALS.

9.2.1.1. Diagnosis

At the onset of their disease, some of the patients may carry diverse diagnoses such as juvenile amyotrophic lateral sclerosis or spinal muscular atrophy, but more detailed examination often reveals evidence for a multisystem involvement or this may be seen at follow-up.

The diagnostic challenges appear when confronted with a young adult presenting with a motor neuron disease, particularly affecting lower motor neurons.

Brain imaging, computed tomography and particularly MRI, demonstrate most frequently cerebellar atrophy (Fig. 9.1). This process affects mainly the vermis and occurs even in patients lacking any clinical evidence of cerebellar dysfunction (Streifler et al., 1993). On the other hand, the cerebral cortex usually appears normal, even if cognitive changes or psychosis are seen. The normal appearance of the cerebral cortex might be misleading since the intraneuronal lipid accumulation, causing swelling of neurons, may masquerade abnormalities. This remarkable picture of normally-appearing cerebral cortex with atrophy of the midline cerebellar structures occurring in an adult person with a neurodegenerative disease (including a motor neuron disease) should alert the physician to the possibility of $LOGM_2G$. Similar changes can be seen in other neurodegenerative diseases, such as Creutzfeldt-Jakob disease, the very slow progression rules out the latter diagnosis (Korczyn, 1991). Mugikura and colleagues described changes in the white matter and in the basal ganglia in Tay-Sachs disease. The anatomic changes may appear late during the course of the disease (Mugikura et al., 1996). Severe changes in phosphorous metabolism were also described in the

cerebral cortex and white matter regions lacking morphological abnormalities using MR spectroscopy (Felderhoff-Mueser et al., 2001).

The electroencephalogram is typically normal.

Electromyography shows a decreased number of motor units, which may be increased in size, as evidence of denervation and reinnervation. These may be widespread and occur in muscles that are clinically thought to be normal.

Muscle biopsy shows the expected atrophy with loss of type 2 fibers and group 1 fiber grouping.

On clinical examination, the suspicion of $LOGM_2G$ should be raised in a patient with adult-onset motor neuron disease with lower motor signs if there are other neurological manifestations, such as Parkinsonism, dystonia, ataxia or psychosis. In particular, the existence of severe dysarthria, disproportional to the muscular atrophy in the rest of the body and particularly the tongue and other bulbar structures, should raise suspicion. Macular cherry red spots, which are typical for Tay-Sachs disease, are not apparent in $LOGM_2G$. However, detailed neurophysiological examination of the retinas, such as by ERG or visual evoked responses, have not so far been reported.

$LOGM_2G$ is a purely neurological disease and therefore does not involve the peripheral organs (of course, visceral organomegaly is typical in Sandhoff disease). However, neurons all over the body accumulate gangliosides and it is easiest to biopsy them from the appendix or rectal submucosa, where the typical changes can be seen (Rubin et al., 1988). Again, it would be useful to have a picture of the changes found in this type of biopsy.

A still easier method is to use the peripheral blood, looking for the enzymatic defect. Genetic studies can also be done and are of special value if a mutation has already been identified in that family. The most rewarding genetic examination is to look for the G269A mutation that can occur on one allele or both. However, many other mutations have been identified, and it is impractical to sequence for all. In selected populations, such as Ashkenazi Jews or French Canadians, a compound heterozygote situation is quite common where the common Tay-Sachs mutations occur on one parental chromosome and another mutation, e.g. G269A, is inherited from the other parent (both parents, who are heterozygotes, are of course asymptomatic carriers).

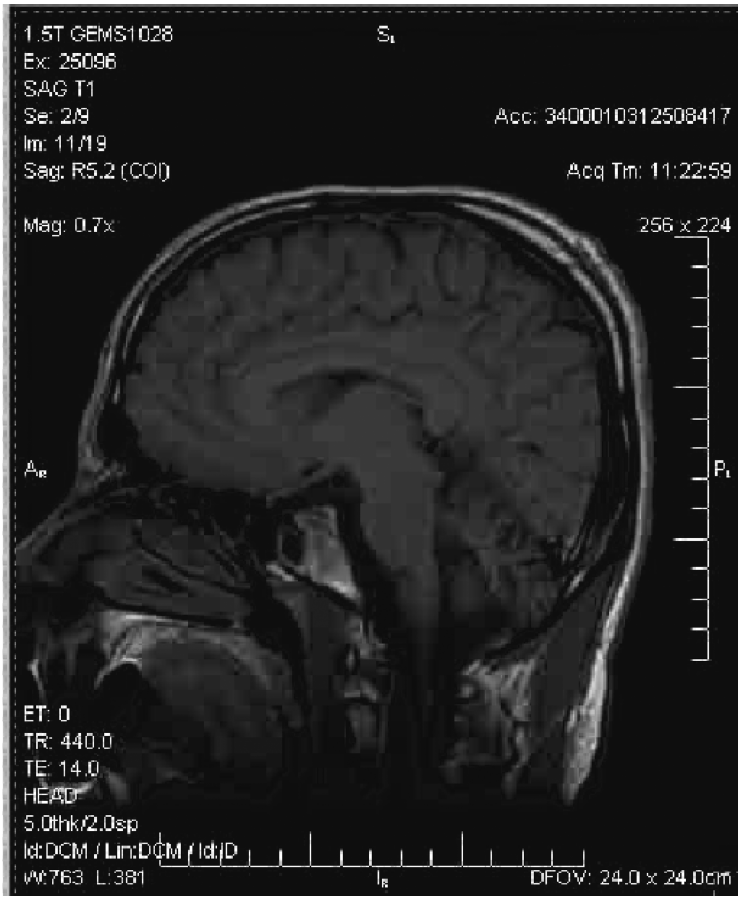

Fig. 9.1. MRI showing cerebellar atrophy.

9.2.1.2. Therapy

The therapy of the ALS-like picture in $LOGM_2G$ is supportive, similar to that in other cases with early onset ALS, and is covered elsewhere in this volume.

This terrible neurodegenerative disease affecting young people calls for the development of novel methods of treatment. One such method could be stem cell therapy, with the main rationale being to replace dying neurons in an organotypic appropriate manner. Lost neurons need to be replaced to allow for the re-establishment of a functional neuronal pathway. Potential sources to fulfill these goals include several kinds of stem cells, originating in embryos, fetuses or adult stem cells. Stem cells could be harvested from the patient, thus overcoming the need for future immunosuppression. However, since these cells carry the same mutations, they will have to be genetically engineered and the abnormal genes should be replaced by normal ones.

The introduction of enzyme replacement therapy (ERT) for Gaucher disease patients was an indisputable milestone in the treatment of inherited metabolic diseases, not only because treating the cause of the disease became a reality, but mainly because of its success. Whether the same success can be duplicated in $LOGM_2G$ is unclear. A particular problem is the blood–brain barrier, since in Gancher disease it is mainly the peripheral manifestations which respond.

Both methods are theoretically applicable to $LOGM_2G$ with its various presentations. However, because of the proximity of the lower motor neurons to the cerebrospinal fluid, they can be more accessible than higher neurons. Also, and very importantly, the simple structure of the lower motor neurons where specific connections to target cells are less critical and can be modified (as they are during the regeneration process in motor neuron disease patients in general), provide a possible advantage.

9.3. Summary

$LOGM_2G$ results from the defective activity of the lyosomal enzyme β-hexosaminidase A. Continued accumulation of undegraded substrate results in pathology in the central nervous system. The disease is progressive and disease dynamics may vary throughout life.

Clinically, the disease variants present a remarkable spectrum of phenotypes ranging from the lethal form to a slowly progressive disease type.

Genotype/phenotype correlations are imperfect. Homozygosity for the L444P genotype is almost always associated with the infantile form.

The pathological mechanism of the central nervous system damage is still not fully understood. Neuronal loss and neurodegeneration have been reported, as well as gray matter and white matter involvement, leading to multisystem expression.

Recently, the possibilities of using stem cells to replace damaged neurons or enzyme replacement therapy have been suggested for several neurodegenerative diseases. The anterior horn cells can theoretically be a target for this procedure. In fact, using stem cells engineered to carry a normal HEXA gene is potentially more likely to benefit $LOGM_2G$ cases than patients with other forms of motor neuron disease, since in ALS the degenerative process will continue and is likely to affect the transplanted cells whereas in $LOGM_2G$ a real correction of the metabolic abnormality could replace damaged motor neurons by normal ones.

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Chapter 10

Viral infections of lower motor neurons

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10.1. Introduction

Many viruses that infect the human nervous system will occasionally cause lower motor neuron disease. However, for some viruses, infection of lower motor neurons is an especially common and/or important clinical problem. Poliovirus is the archetypal example, but as the global polio eradication campaign nears completion, the importance of other viruses is increasing. These include not only other enteroviruses, such as enterovirus 71 (EV71), but also flaviviruses such as West Nile and Japanese encephalitis virus (JEV). In addition rabies and other lyssaviruses, though better known for causing furious rabies, are also an important viral cause of acute flaccid paralysis. Recent experience has shown an alarming ability for these viruses to spread and cause new outbreaks. After a brief review of the clinical approach to the patient with acute flaccid paralysis, this chapter will consider what is known about the pathogenesis and clinical features of the major viral causes of acute flaccid paralysis.

10.2. Clinical approach to the patient with acute flaccid paralysis

Flaccid weakness with reduced or absent reflexes is the hallmark of anterior horn cell damage. The common causes of acute flaccid paralysis can be divided into diseases in which viral infection and inflammation directly attack cell bodies of the lower motor neurons in the anterior horn of the spinal cord (viral myelitis) and diseases in which an immunologically mediated parainfectious or post-infectious process causes damage to the lower motor neurons in the peripheral nerves or nerve roots (Guillain-Barré syndrome) (Fig. 10.1) (Solomon and Willison, 2003). In general viral myelitis and the different forms of Guillain-Barré syndrome can be distinguished according to the clinical features, with subsequent confirmation by neurophysiological and other tests (Table 10.1).

Viral invasion of the anterior horn cells tends to occur as part of an acute viral illness (with fever, headache and meningism) and causes asymmetrical weakness which is often painful, with few sensory symptoms or signs and a moderate pleocytosis in the cerebrospinal fluid (reflecting the inflammatory process in the spinal cord). In contrast, Guillain-Barré syndrome occurs typically weeks after an acute infection (or vaccination), causes symmetrical weakness and is associated with elevation in cerebrospinal fluid protein without pleocytosis. Thus it is important to determine in the history whether weakness onset is during or immediately after a febrile illness, as opposed to weeks later, whether there is pain in the back or legs, sensory involvement and whether the weakness is symmetrical or asymmetrical.

Although Guillain-Barré syndrome and viral myelitis are the most common causes of acute flaccid paralysis, other diseases to consider include diseases of the neuromuscular junction (e.g. myasthenia gravis, botulism), of the muscles themselves (myopathy, acute polymyositis, periodic paralysis) and toxic causes (diphtheria). There may be etiological clues in the history or examination. It is important to determine whether this is an isolated case or occurring in the context of other patients with central nervous system (CNS) disease or mucocutaneous syndromes such as

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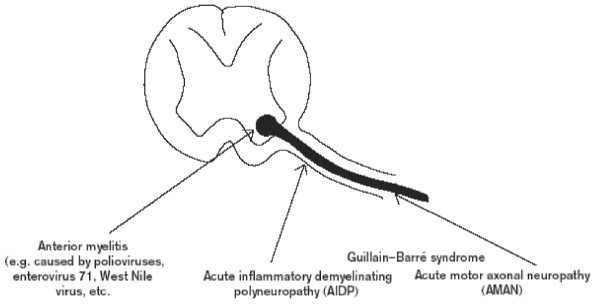

Fig. 10.1. Schematic representation of the anatomical sites of damage in viral myelitis and Guillain-Barré syndrome. In viral myelitis the lower motor neuron cell bodies are damaged in the anterior horns of the spinal cord; in Guillain-Barré syndrome there is damage to the myelin sheaths (acute inflammatory demyelinating polyneuropathy) or the motor axons (acute motor axonal neuropathy or acute motor and sensory axonal neuropathy).

hand, foot and mouth disease (HFMD) (as in EV71 infection). Sick birds in the neighborhood may point to West Nile virus as the etiology (Julian et al., 2002). It is important to determine if there may be a history of exposure to toxins or to poorly preserved food, as in botulism. A preceding history of sore throat with neck swelling and lymphadenopathy may indicate that this is diphtheritic neuropathy; a prior dog or bat bite may be reported for paralytic rabies (Fooks et al., 2003b; Solomon et al., 2005). The history of an intramuscular injection is important, not only because it may make poliomyelitis worse (see below), but also because a misplaced buttock injection may be the cause of paralysis through trauma to the sciatic nerve. Depending on the geographical location, the examination should include careful scrutiny for a tick, which may be hidden in a crevice or hairy area of the body, and for the puncture marks of a snake bite.

10.3. Poliovirus, enterovirus 71 and other enteroviruses

10.3.1. Introduction

The earliest record of a withered shortened leg with the characteristic appearance of poliomyelitis is an Egyptian stele of the 18th dynasty (1580–1350 BC). The name derives from the Greek *polio* meaning gray and *myelos* meaning marrow or spinal cord. Originally this was a pathological description of the findings in patients with "Heine-Medin" disease or "infantile paralysis." Subsequently the term poliomyelitis was applied to all patients with the clinical syndrome of fever, meningeal irritation and acute flaccid paralysis. However, once poliovirus (or strictly the three poliovirus types I, II and III) was identified as the most common cause of this syndrome, the term poliomyelitis was used to mean the *disease* caused by polioviruses (World Health Assembly, 1988; World Health Organization, 1993). However with the recent increasing recognition that West Nile virus (WNV) causes an acute flaccid paralytic syndrome there has been controversy about what the word poliomyelitis means – with some reports describing patients as having a "poliomyelitis-like illness due to West Nile virus" and others as "poliomyelitis due to West Nile virus" (Leis et al., 2003). Previously, when other viruses including other enteroviruses, flaviviruses and herpes viruses have been identified as causing acute flaccid paralysis, they have been referred to as causing "poliomyelitis-like illnesses," rather than causing "poliomyelitis" (John, 2003). Whilst some have pointed out that neither clinicians, virologists nor pathologists have exclusive rights to the term poliomyelitis (Johnson and Cornblath, 2003; Sejvar et al., 2003b), others have suggested that a lack of clarity over this issue could seriously hamper the World Health Organization's attempts for global poliomyelitis eradication (Solomon and Ravi, 2003), as well as hampering further studies of the disease in North America. For this reason it has been proposed that the term 'poliomyelitis-like illness' be used to describe the clinical presentation and 'West Nile virus myelitis' to describe the pathology (Solomon and Ravi, 2003); and that is the system that is used in this chapter.

In 1908, Landsteiner and Popper demonstrated the disease's transmissible nature by injecting human spinal cord homogenates into monkeys (Landsteiner and Popper, 1909). In 1949, Enders, Weller and Robbins cultured the virus, for which they received the Nobel Prize. The ability to culture poliovirus led to the development of the inactivated (Salk) vaccine and the live attenuated (Sabin) vaccine.

In addition to polioviruses, other members of the *enterovirus* genus (Family *Picornaviridae*), including coxsackieviruses, echoviruses and newer enteroviruses, can also cause acute flaccid paralysis (Modlin, 1995a). These non-polio enteroviruses are associated with a much wider spectrum of clinical manifestations than polioviruses including exanthemas (rashes), enanthemas (eruptions in the mouth), conjunctivitis, respiratory infections, myocarditis, pericarditis, pleurodynia (attacks of intercostal muscle inflammation causing chest pain), aseptic meningitis, encephalitis and acute flaccid paralysis (Table 10.2).

Aseptic meningitis is the most common neurological manifestation of non-polio enterovirus infections. In Western settings, group B coxsackieviruses, especially B2 and B5, and echoviruses account for more than 90% of viral meningitis cases (Modlin, 1995a). Encephalitis is a rarer complication of enterovirus infection, but accounts for 11–22% of proven viral encephalitides in

Table 10.1

Comparison of clinical and diagnostic features for viral myelitis and Guillain-Barré syndrome (modified from Solomon and Willison, 2003)

	Rabies viral myelitis	Acute viral myelitis	Guillain-Barré syndrome	
	Caused by rabies virus and related lyssaviruses	e.g. caused by enteroviruses (polioviruses, enterovirus 71) and flaviviruses (West Nile virus, Japanese encephalitis virus, etc.)	Acute inflammatory demyelinating polyneuropathy (AIDP)	Acute motor axonal neuropathy (AMAN)
Clinical features	Rapid ascending asymmetrical flaccid weakness, often with muscle tenderness. Follows exposure to rapid animal; may be feverish on admission. Can be associated with severe limb pain and skin itching. Almost always fatal	Acute viral illness, then rapid onset asymmetrical flaccid weakness, often with muscle tenderness. May be respiratory and bulbar weakness, Sensation not often affected	Gradually onset ascending symmetrical weakness with sensory involvement, often involving facial nerve, several weeks after viral infection (especially cytomegalovirus) or vaccination	Gradually ascending symmetrical weakness with no sensory involvement, often involving facial nerve, several weeks after Campylobacter jejuni, or other infection
Peripheral blood count	May be leukocytosis	May be leukocytosis or leukopenia	Usually normal	Usually normal
CSF findings	May be moderately elevated white cell count (lymphocytes or sometimes neutrophils at first), slightly elevatedprotein	Moderately elevated white cell count (lymphocytes or sometimes neutrophils at first), slightly elevated protein	Normal initially, then elevated protein with normal cell count	Normal initially, then elevated protein with normal cell count
Neurophysiological abnormalities	Low CMAP; normal SNAP; positive sharp waves and fibrillations on EMG; occasionally prolonged latencies andreduced velocities	Low CMAP; normal SNAP; positive sharp waves and fibrillations on EMG	Delayed distal latencies and reduced velocities, in sensory and motor nerves	Low CMAP; positive sharp waves and fibrillations on EMG
Additional tests	Ante-mortem: immunofluorescence of skin biopsy; PCR of saliva; virus culture; post-mortem brain biopsy, immunohistochemistry and virus culture	Positive for the appropriate virus (antibodies against West Nile virus or Japanese encephalitis virus in serum/CSF; stool culture of poliovirus;CSF PCR of enterovirus 71)	May have positive serology for cytomegalovirus; IgM antibodies against GM2	May have positive C. jejuni serology or stool culture; IgG antibodies against GM1, GM1b, GD1a, GaINAc-GD1a components of nerve

CMAP = compound muscle action potential; SNAP = sensory nerve action potential.

Table 10.2

Clinical spectrum of CNS disease caused by non-polio enteroviruses (modified from Modlin, 1995a)

Clinical pattern	Most common enteroviral cause	Other associated enteroviruses
Aseptic meningitis	Coxsackie B2, B5 Echo 4, 6, 9, 11, 13, 16, 30, 33	Coxsackie B1, B3, B4, B6 A1–11, A16–18, A22, A24
Encephalitis	Coxsackie A9, B2, B5, Echo 6, 9	Coxsackie A2, A5, A6, A7; B1-3, B6; Echo 2–4, 6, 7, 9, 11, 14, 17–19, 25
Acute flaccid paralysis	Coxsackie A7 A9, B1–5, Echo 6, 9; EV 70, 71	Coxsackie A4, A5, A10; Echo 1-4, 7, 11, 14, 16-18, 30
Guillain-Barré syndrome		Coxsackie A2, A5, A9; Echo 6, 22

* EV = enterovirus.

some series (Meyer et al., 1960). A range of enteroviruses have been associated with sporadic cases and outbreaks of acute flaccid paralysis. Of these, EV71 has proven to be a major public health problem in the Asia-Pacific region in recent years, with potential to spread beyond. The virus causes large outbreaks of hand, foot and mouth disease (HFMD), with associated aseptic meningitis, flaccid paralysis, encephalitis and systemic complications. It has been suggested that it may become the major infectious cause of acute flaccid paralysis following the eradication of poliovirus (Sabin, 1981; da Silva et al., 1996). Even though poliomyelitis has declined dramatically, a late consequence of the disease is postpoliomyelitis syndrome – a progressive weakness and atrophy seen many years after the initial infection.

10.3.2. Virology of enteroviruses

As the family name implies, *Picornaviridae* are among the smallest RNA viruses (*pico,* small). Polioviruses are grouped into three serotypes, distinguished on the basis of neutralization tests, type 1 being the most important cause of paralytic disease. The first coxsackievirus was isolated from the feces of a child with paralysis in 1948 (Dalldorf and Sickles, 1948). Many other viruses that were cytopathic, damaging cells in tissue culture, were subsequently isolated from the feces and were named enteric cytopathic human orphan (ECHO) viruses because of their unknown relation to human disease (Enders et al., 1949). Based on differences in host range and pathogenic potential, enteroviruses have conventionally been divided into polioviruses, group A coxsakieviruses, group B coxsakieviruses and echoviruses. However, because of limitations in this system, since 1970 newly characterized enterovirus serotypes have been assigned type numbers (e.g. enterovirus 68–71). A more recent classification scheme has been adopted that divides all non-polio enteroviruses into four groups, designated A to D based on genetic similarity in the VP1 gene (see below).

Enterovirus virions consist of a non-enveloped capsid surrounding a core of single-stranded, positive sense RNA of approximately 7.5 kb in size. This has a single open reading frame, flanked by untranslated regions at the 5′ and 3′ end. The 5′ un-translated region consists of several stem loops of nucleic acid that include an internal ribosomal entry site, crucial for initiation of RNA translation into protein. The open reading frame codes for four structural proteins (VP1–4), which make up the viral capsid, and seven non-structural proteins which include proteases involved in viral replication. The capsid is icosahedral in symmetry and is composed of 60 identical units (protomers), each consisting of the four structural proteins. A deep cleft on the virion surface at the junction of VP1, VP2 and VP3 is thought to function as the site of virion attachment to the cellular receptor (Hogle et al., 1985).

10.3.3. Pathogenesis of enterovirus CNS disease

10.3.3.1. Cellular entry and early viral spread

Humans are the only natural hosts and reservoir for polioviruses and all group A enteroviruses, including EV71. A major advance in our understanding of poliovirus pathogenesis followed the identification of the poliovirus receptor (Mendelsohn et al., 1989). Known as CD155, the receptor attaches to the canyons on the virion surface. It is a member of the immunoglobulin superfamily, with three extracellular domains, a membrane spanning domain and a cytoplasmic domain, and is involved in the formation of cell-cell adhesion junctions (Sato et al., 2004). The cytoplasmic

domain of CD155 interacts with Tctetx-1, a light chain subunit of the retrograde motor complex dynein (Mueller et al., 2002), which may thus facilitate retrograde transport of the virus to the cell body in the anterior horn of the spinal cord.

Enteroviruses infect humans via direct or indirect contact with virus shed from the gastrointestinal or upper respiratory tract. Virus implants and replicates in the upper respiratory or gastrointestinal tract, probably in submucosal lymph tissue, before spreading to regional lymph nodes (e.g. cervical and mesenteric) and causing a low level "minor" viremia, which is usually not detectable. During this viremia, virus spreads to the reticuloendothelial system, particularly the spleen, liver, bone marrow and deep lymph nodes. If viral infection is controlled at this point by the host defense mechanisms, the infection will remain subclinical. However, in a minority of patients continued replication occurs, resulting in a "major viremia" corresponding with the febrile illness seen in many enterovirus infections. At this point virus may disseminate to target organs, including the central nervous system (especially for polioviruses and EV71) and the skin and buccal mucosa (important for EV71 and other non-polio enteroviruses).

10.3.3.2. Entry into the central nervous system

How poliovirus and other enteroviruses enter the CNS during viremia is not completely certain; there has been much debate about the relative importance of direct permeation across the blood–brain barrier, versus retrograde transport up axons. The development of transgenic mice that express the human poliovirus receptor has helped considerably in understanding possible mechanisms. Such mice are susceptible to intravenous, intramuscular and intracranial inoculation of virus; unfortunately because they do not express CD155 in the gut, they are not susceptible to oral ingestions (which would be closest to natural transmission in humans). Experimental data indicate that intravenously inoculated virus permeates through the blood–brain barrier at a high rate (Yang et al., 1997). The fact that this occurs even if preceded by anti-CD155 monoclonal antibodies suggests it is not dependent on the poliovirus receptor. The increased blood flow in the anterior segment of the cord is postulated as one reason why this region is preferentially infected (Nathanson and Bodian, 1962). However, when the virus is injected via the intramuscular route, it travels up nerve axons to reach the anterior horn cells of the spinal cord (Ren and Racaniello, 1992); this is exclusively in the ipsilateral spinal cord and can be prevented by sciatic nerve section (Gromeier et al., 1997; Gromeier and Wimmer, 1998). In humans trauma to a limb during the viremic phase appears to predispose to poliomyelitis in that limb, which supports the concept that, at least in some circumstances, viral entry via the retrograde route is important.

10.3.3.3. Pathology

Once inside the CNS the virus appears to spread preferentially along certain nerve fiber pathways. Although the anterior horn cells are most severely affected, in severe cases the intermediate, intermediolateral and posterior gray columns are affected (Bodian, 1949). The involvement is characteristically patchy and asymmetrical. Lesions are also found in the sensory spinal ganglions, the thalamus and hypothalamus, reticular formation, vestibular nuclei, cerebellar vermis, deep cerebellar nuclei and motor cortex. There are foci of neuronophagia, comprised of neutrophils initially and subsequently lymphocytes and macrophages (Boos and Esiri, 2003). In addition, there is accompanying congestion, endothelial hyperplasia and, in severe cases, petechial hemorrhage. Subsequently there are numerous plasma cells and cuffs of perivascular inflammation. Interestingly, for both human disease and animal models, the distribution of virus and lesions is virtually the same in all cases, irrespective of the extent or severity of clinical features. The clinical features are thus thought to reflect the extent of neuronal damage and inflammation, rather than their localization.

10.3.3.4. Host cellular response

The effects of viral replication on the host cell are widespread and include inhibition of cellular mRNA translation and cellular protein synthesis and inhibition of host cell RNA synthesis. These changes lead to characteristic morphological changes known as cytopathic effects, which include condensation of chromatin, nuclear blebbing, proliferation of membrane vesicles, changes in membrane permeability, leakage of intracellular components and shriveling of the entire cell. During productive infections in cultured cells apoptosis is blocked by virus encoded inhibitors, particularly the non-structural proteins 2B/2BC, 3A and the ATPase 2C). However, if virus reproduction is hindered, cell death occurs through induction of apoptosis, which is thought to be mediated by the viral proteases 2A and 3C (Belov et al., 2003). In mice, viral infection and CNS injury in mice is associated with apoptosis (Blondel et al., 2005).

10.3.3.5. Host immune response

The humoral response to infection with wild-type or live-attenuated poliovirus includes IgM, which appears in the serum 1 to 3 days after challenge, IgG (mostly

IgG_1 and IgG_3), which appear after 7 to 10 days, and IgA, which is produced in nasal and alimentary secretions 2–4 weeks after exposure (Modlin, 1995b). Neutralizing IgG persists for life after natural infection with enteroviruses, but immunity is serotype specific. The observation that people with B-cell immunodeficiency develop persistent infections indicates an important role for antibodies in recovery from viral infection; macrophages also have a critical role in viral clearance (Modlin, 1995a), whereas T lymphocytes appear not to be important in viral control and may even contribute to the pathogenesis – see below.

10.3.3.6. Inflammation and immunopathology

There is growing evidence that some inflammatory and T-cell mediated immune responses may be deleterious in enterovirus infections. Thus, in murine models of enterovirus myocarditis, expression of pro-inflammatory cytokines and an acute inflammatory infiltrate are thought to contribute to necrosis of infected myocites. Recent evidence suggests there may also be immunopathology in severe human disease caused by EV71. In one study, children with the EV71 meningoencephalitis were more likely to possess a particular cytotoxic T lymphocyte antigen haplotype (CTLA4), but less likely to express CD40 ligand, which may promote B-cell function, but may also be cytotoxic by induction of interleukin (IL)-16 (Yang et al., 2001). In another two studies, proinflammatory cytokine IL-6 was associated with severe disease (Lin et al., 2002b; Lin and Wu, 2003). One further study showed abnormal cytokines production (IL-10; IL-13 and INF-γ) and lymphocyte depletion in severe disease (Wang et al., 2003).

10.3.3.7. Viral strain virulence determinants

There is evidence from a range of sources that properties of the enteroviruses themselves may also convey virulence determinants. This comes from comparison of the live attenuated (Sabin) vaccine poliovirus strains with the neuroinvasive wild type viruses from which they originate. For example, there are 57 nucleotide differences and 21 amino acid changes between the vaccine strain of poliovirus type 1 and its progenitor strain, whereas for poliovirus type 3 there are just 10 nucleotide and three amino acid changes. The most important changes attenuating against virulence appear to be in the VP1 capsid protein (Mueller et al., 2005). Small nucleotide changes in the 5′ non-translated region of the viral genome may also affect virulence. In this region the internal ribosomal entry site binds host translational machinery to initiate viral replication. Single point mutations are found in domain V of this site for all three Sabin vaccine strains and these mutations reduce replication in neuronal cells in vitro and also produce an attenuation phenotype on transgenic mice expressing the poliovirus receptor (CD155) (Mueller et al., 2005). Vaccine strains of poliovirus can occasionally revert to virulence and cause paralytic poliomyelitis. Genetic analysis of revertant strains has shown eversion to virulence is associated with a point mutation in the 5′ non-translated region (Evans *et al*., 1985).

For EV71 there are not yet any live vaccine strains available, nor is there a good animal model. However, there may be clues from the clinical epidemiology of the virus. Phylogenetic studies have divided EV71 strains into genogroups A, B and C, which have been further sub-divided (Brown et al., 1999; McMinn et al., 2001a; Cardosa et al., 2003). Molecular epidemiological studies have documented remarkable changes in the circulating EV71 genogroups in the Asia-Pacific region since 1997 (Wang et al., 2002; Cardosa et al., 2003; Shimizu et al., 2004). The fact that the reported incidence of neurological and severe disease has varied between outbreaks caused by different genogroups has led to speculation that genotypic differences may be responsible (Dolin, 1999; McMinn et al., 2001a; Wang et al., 2002). However, making comparisons between outbreaks has been hampered by the retrospective nature of most studies, differences in inclusion criteria, lack of specificity over what constitutes severe disease and differences in viral diagnostic capabilities. Large prospective studies which include more than one genotype of virus are needed to answer this question. Nevertheless, a comparison of virus isolates during an epidemic in Perth in 1999, in which genogroup C2 viruses were isolated from children with severe neurological disease gave intriguing results. It was suggested that a change from an alanine to a valine at amino acid 171 in the VP1 protein may have resulted in a critical change in the proteins' conformation in this region on the rim of the canyon which constitutes the viral attachment site (McMinn et al., 2001a). Clearly, further work is needed in this important area.

10.3.3.8. Pathogenesis of postpoliomyelitis syndrome

The pathogenesis of postpoliomyelitis syndrome is not known. In some patients the presence of viral RNA and antiviral IgM in the CSF suggests chronic infection and elevated pro-inflammatory cytokines suggest ongoing inflammation (Dalakas and Illa, 1991; Leon-Monzon and Dalakas, 1995). Electromyographic findings show large motor units with ongoing chronic denervation and jitter on single fiber studies. This has led to the suggestion that the deterioration is a result of the physiological attrition of the large unstable motor units that develop, usually many years after the initial infection.

10.3.4. Clinical and epidemiological features of poliomyelitis

10.3.4.1. Epidemiology

Before the late 19th century, polio was predominantly a sporadic disease, which mostly affected children under 5 years old. Subsequently, large epidemics were recognized in Scandinavia, Western Europe and the US. This increased incidence was associated with a shift in the affected age groups: in the 1950s the peak incidence in the US was in 5 to 9 year olds with more than one third of cases occurring in those over 15. Epidemiological evidence supports the concept that before the 1900s the virus was ubiquitous resulting in mostly inapparent infection during early childhood (Bodian, 1949). With improved hygiene, infection was delayed until older ages, when the pool of susceptible children was large enough to support epidemics. In younger children, passively transferred maternal antibody are postulated to play a role in producing inapparent as opposed to paralytic infection (Bodian, 1949). Following the development of the inactivated and live attenuated polio vaccines and their widespread use the epidemiology has changed considerably (see below).

10.3.4.2. Clinical features

Six clinical syndromes are described: inapparent infection (i.e. no symptoms); "abortive" poliomyelitis (i.e. a mild febrile illness only); "non-paralytic" poliomyelitis (a viral meningitis syndrome); "paralytic poliomyelitis," which may be spinal or bulbar; and "encephalitis," which is rare. Estimates of the ratio of apparent to inapparent infections vary from 1 in 60 to 1 in 1000 (Melnick and Ledinko, 1951; Nathanson and Martin, 1979).

Paralytic poliomyelitis occurs in 0.1% of all poliovirus infections. In children there is typically a biphasic course (Horstmann, 1949). Initially there is a non-specific febrile illness lasting 1–3 days that coincides with a viremia and is known as the "minor illness." Following this the patients may be asymptomatic for 2–5 days, before the "major illness" starts. This begins with fever, headache, malaise, vomiting and neck stiffness which are associated with a cerebrospinal fluid (CSF) pleocytosis. In older patients there is often spontaneous muscle pain, which may be relieved by walking (Weinstein et al., 1952). However, exercise during the major illness increases the incidence and severity of paralytic disease and so bed rest is recommended (Russell, 1949). Sensory changes such as localized cutaneous hyperesthesia and paresthesias may occur at this stage. After 1–2 days there is frank paralysis and weakness, which may range from a single portion of one muscle to quadriplegia. The paralysis is flaccid and, although deep tendon reflexes may be brisk transiently, they soon become absent. The weakness is characterized by its asymmetrical distribution, which typically involves the legs more than the arms and proximal muscles more than distal ones. Any combination of limbs may be paralyzed, but the most frequent pattern is one leg, followed by one arm, or both legs and both arms. Paralysis tends to localize in a limb that has been the site of an intramuscular injection or injury within 2–4 weeks before the onset of infection – so called 'provocation poliomyelitis' (Greenberg et al., 1952; Wyatt, 1985; Sutter et al., 1992).

The weakness usually reaches a maximum over 2–3 days. Progression of the paralysis invariably halts when the patient becomes afebrile (Horstmann, 1949). Paralysis of the bladder, which is usually associated with leg paralysis, occurs in about one quarter of adults, but is uncommon in children (Weinstein et al., 1952). Sensory loss is very rare and its occurrence suggests an alternative diagnosis. In bulbar poliomyelitis there is paralysis of the muscles innervated by the lower cranial nerves, particularly the ninth and tenth, resulting in dysphagia, nasal speech and occasionally dyspnoea (Modlin, 1995b). Its frequency has been reported as 5–35% and it is more common in adults (Weinstein et al., 1952). Rarely the medullary respiratory and vasomotor centers may be affected, leading to irregular respiratory patterns, respiratory failure, cardiac dysrrhythmias and circulatory collapse (Baker et al., 1950).

10.3.4.3. Postpoliomyelitis syndrome

Some patients who partially or fully recover from poliomyelitis develop a new syndrome of progressive weakness atrophy and fatigue many years after the initial illness. Diagnostic criteria were established to distinguish the syndrome of post-poliomyelitis progressive muscular atrophy from the non-specific symptoms of joint instability, nerve, root or plexus compression and increasing scoliosis which may be late secondary consequences of the initial weakness (Dalakas et al., 1984; Post-Polio). Typically in postpoliomyelitis syndrome the muscles affected during the original illness develop progressive atrophy, fatigue and weakness and there may be cramps and fasciculations. In addition previously unaffected muscles may become affected. Twenty to 30% of previously paralyzed polio patients may develop the syndrome, whose incidence peaks 20–25 years after the initial illness. There appears to be continuing debate as to the existence of post-poliomyelitis syndrome. In any case there soon will be few if any remaining cases originally estimated in North America as 300,000.

10.3.5. Clinical and epidemiological features of enterovirus 71

10.3.5.1. Epidemiology

EV71, one of the most important recently identified enteroviruses, is a cause of muco-cutaneous and CNS disease, including acute flaccid paralysis. First isolated from young children with encephalitis and meningitis in California in 1969 (Schmidt et al., 1974), the virus has since caused epidemics in which there is hand, foot and mouth disease (HFMD), neurological disease or both (Blomberg et al., 1974; Kennett et al., 1974; Shindarov et al., 1979). The reason why some epidemics involve mostly mucocutaneous disease, others mostly neurological disease and others a mixture is not known, but differences in the dermatotropsim and neurotropsim of different viral genogroups has been postulated (McMinn, 2002).

10.3.5.2. Clinical features

Children with HFMD typically present after 3 or 4 days of fever, with a vesicular eruption on the buccal mucosa, tongue, gums and palate and a papulovesicular rash on the hands, feet and buttocks (McMinn, 2002). Alternatively children may develop herpangina – an abrupt onset of fever and sore throat, with raised papular lesions in the pharynx, soft palate and uvula (Samuda et al., 1987). Neurological presentations of EV71 include aseptic meningitis, brainstem (or rhombo-) encephalitis, cerebellar encephalitis and acute flaccid paralysis. Although in most cases flaccid paralysis is thought to be secondary to virus-mediated destruction of the anterior horn cells (Shen et al., 1999, 2000; Chen et al., 2001), electrophysiological studies suggest that in a minority, paralysis may be due to the acute inflammatory demyelinating polyneuropathy form of Guillain-Barré syndrome (McMinn et al., 2001b). These cases usually make a better recovery.

Brainstem encephalitis is the most severe neurological manifestation of EV71 infection; it usually represents an extension of spinal cord disease and may present with myoclonus, tremor, ataxia, nystagmus and cranial nerve palsies (Huang et al., 1999). Imaging and autopsy studies show the midbrain, pons, medulla oblongata and reticular formation are frequently involved. In some cases there is associated severe pulmonary edema, which presents as cyanosis, tachycardia, tachypnoea and the production of pink frothy sputum (Chang et al., 1999; Wang et al., 1999; Yan et al., 2000). There has been some controversy as to the cause of this syndrome. Whilst some have argued that it is neurogenic pulmonary edema (Lum et al., 1998; Ho et al., 1999; Huang et al., 1999; Wang et al., 1999), similar to that described for poliomyelitis (Baker et al., 1950), others think that it is cardiogenic (Cardosa et al., 1999). Neurogenic pulmonary edema is postulated to be the consequence of an excess of sympathetic activation ("sympathetic storm") causing an increased peripheral vascular resistance, which diverts blood to the pulmonary vasculature and resulting in the edema (Chang et al., 1999; Lin et al., 2002a). However, echocardiographic studies have confirmed that in some patients acute cardiac dysfunction does indeed occur (Chan et al., 2000; Huang et al., 2002b), though histological evidence suggests that this is not due to a viral myocarditis (Hsueh et al., 2000; Yan et al., 2000) and its cause is uncertain. In other cases, normal pulmonary capillary wedge pressures would seem to rule out any hydrostatic cause of pulmonary edema, suggesting that increased pulmonary vascular permeability is the cause – perhaps as a result of cytokine activation (Wu et al., 2002).

10.3.6. Investigation of poliovirus and enterovirus 71 infections

The CSF typically has a "viral" picture with elevated lymphocytes, a slightly elevated protein and normal glucose ratio. Polioviruses can usually be isolated from throat secretions during the first week of illness and from feces subsequently. Virus is rarely isolated from the CSF in patients with paralysis; this is in contrast to patients with meningitis caused by enteroviruses, where the virus is often isolated or can be detected by the polymerase chain reaction (Davies et al., 2005). Where the clinical presentation includes HFMD (for example in EV71 infection), virus isolation from cutaneous vesicles is also often positive (Chan et al., 2003; Shah et al., 2003). Antibody detection by neutralization tests or IgM capture enzyme linked immunosorbant assays (ELISAs) may also be useful (Tsao et al., 2002), though cross-reactivity with other enteroviruses can be an issue.

10.3.6.1. Imaging

Although there are few imaging studies of poliomyelitis, magnetic resonance imaging in EV71 infection shows high signal changes in T2 weighted images in the anterior horn of the spinal cord (Fig. 10.2) (Huang et al., 1999; Shen et al., 2000; Chen et al., 2001). In patients with encephalitis there are also lesions in the the brainstem, thalamus, putamen and dentate nuclei of the cerebellum (Shen et al., 1999; Chen et al., 2001). Contrast enhancement can be found within the ventral roots and anterior horn cells on T1-weighted images. Diffusion weighted imaging may be especially useful in early detection of lesions (Maschke et al., 2004).

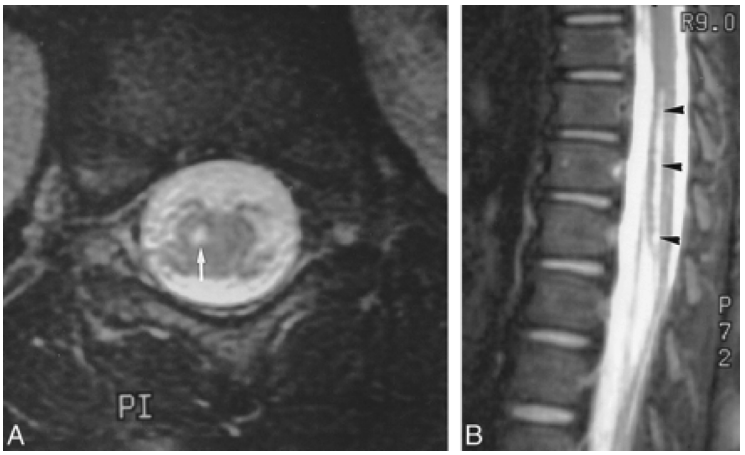

Fig. 10.2. Magnetic resonance imaging of the spine in enterovirus 71 myelitis. Axial fast spin-echo T2-weighted image at lumbosacral cord 2 months after acute right lower limb paralysis shows a hyperintense lesion in the right anterior horn region (arrow) (**A**); Sagittal section (**B**). Reproduced from Chen et al. (2001) with permission. © American Society of Neuroradiology.

10.3.7. Treatment of enterovirus infections

Pleconaril is an antiviral agent effective against some enteroviruses. It acts by stabilizing the enterovirus viral capsid protein, thus preventing viral uncoating after receptor binding. It has proved useful in some patients with meningitis caused by enteroviruses, particularly immunodeficient patients with chronic infections (Rotbart and Webster, 2001; Starlin et al., 2001). Its role in immunocompetent patients is less clear (Webster, 2005). It has limited effect against EV71 in vitro and did not appear helpful when used in enterovirus-associated brainstem encephalitis (McMinn, 2002).

Although convalescent serum was at one time thought to be valuable therapy in poliomyelitis, subsequent trials showed there was no effect (Horstmann, 1950). However there is renewed interest in immunoglobulin in EV71 infection – not because of neutralizing antibodies it may contain, but because of an immunomodulatory effect it may have against the postulated immunopathology. There is disagreement about the efficacy of intravenous immunoglobulin (Chang et al., 2004a,b) and randomized controlled trials are needed. The management of CNS disease caused by enteroviruses is therefore symptomatic. Bedrest is advised for patients with paralysis, because of the risk that movement exacerbates the disease. Physiotherapy should be initiated once the progression of paralysis has ceased. Patients with respiratory failure receive positive pressure ventilation, which has replaced the 'iron lung' tank respirators which were used in the past for poliomyelitis.

10.3.8. Prevention of poliomyelitis and enterovirus 71 disease

In 1988 the World Health Assembly resolved to eradicate polio by the year 2000 using mass vaccination and disease surveillance (Hinman et al., 1987; World Health Assembly, 1988). Although the target of global eradication has not been achieved, the campaign has undoubtedly been a success. The number of countries where polio is endemic declined from 125 in 1988 to seven in 2002, and the estimated incidence of polio has decreased by more than 99% (Anonymous, 2003). In 2001 there were only 483 confirmed cases. However, there have been several setbacks in recent years; for example in 2004 there were 1255 confirmed cases from 16 countries. Reasons include difficulty immunizing in areas of ongoing conflict, poor compliance with immunization because of mistrust in some communities, natural disasters disrupting infrastructure and issues over financing the program. Despite this, there is still optimism that ultimately the wild-type virus will be eradicated. There is not yet a vaccine for EV71. Control measures therefore include personal hygiene to reduce the risk of spread. In many Asian countries nurseries and kindergartens are closed during large outbreaks.

10.4. West Nile virus, Japanese encephalitis virus and other flaviviruses

10.4.1. Introduction

Flaviviruses (genus, *Flavivirus*, family *Flaviviridae*) are named after the prototype yellow fever virus (in Latin flavus = yellow), which was first isolated in 1927. Broadly speaking flaviviruses cause three clinical syndromes – fever arthralgia rash, hemorrhagic fevers and central nervous system infections. Because they are transmitted naturally between vertebrate hosts by insect or ticks, the ecological term arbovirus (short for arthropod-borne virus) is used. Many flaviviruses are zoonotic viruses, meaning their natural hosts are small animals or birds, rather than humans. Outbreaks of encephalitis were recognized in Japan from the 1870s and Japanese encephalitis virus (JEV) was

isolated in 1935. Similar outbreaks of encephalitis were recognized in America in the 1930s and in 1931 St Louis encephalitis virus (SLEV) was isolated from a fatal case in St Louis, MI. This virus, which was soon shown to be related to but distinct from JEV, was the most important flavivirus encephalitis in the US until the appearance of West Nile virus (WNV) in 1999. WNV was originally isolated from a woman with a febrile illness in Uganda in 1935, and until recent years was better known as a cause of a fever and rash syndrome that only occasionally caused CNS disease. In Australia, Murray Valley encephalitis virus (MVEV) was isolated in 1951, but is thought to be responsible for earlier outbreaks of viral encephalitis called 'Australian X' disease (Breinl, 1918; French, 1952). These three viruses, SLEV, WNV and MVEV, which are genetically closely related to JEV are grouped with it in the Japanese encephalitis (JE) serogroup of flaviviruses. The viruses are all transmitted naturally between birds by Culex mosquitoes. Tick borne encephalitis virus (TBEV) is a flavivirus transmitted naturally between small rodents by hard *Ixodes* ticks. Descriptions of a disease compatible with tick-borne encephalitis (TBE) appeared from the 1930s and the virus was first isolated by Russian scientists in the Far East in 1937 (Smorodintsev, 1958). However, across this vast geographical area, the disease was given a range of different names (Central European encephalitis, Russian spring-summer encephalitis, Far Eastern encephalitis, Biphasic milk fever, Taiga encephalitis, Kumlinge disease, Fruhsommer-Meningoenzephalitis) before it was realized that they are essentially the same disease. Three closely related subtypes of TBEV exist, whose names reflect the geographical areas that they principally affect – European, Siberian and Far Eastern. The TBE group serocomplex (recently renamed the Mammalian group of tick-borne flaviviruses) also includes Powassan virus (a rare cause of encephalitis in Canada) and louping ill virus (a very rare cause of CNS disease in the British Isles and Scandinavia).

10.4.2. Virology

Flaviviruses consist of a single strand of positive sense RNA, wrapped in a nucleocapsid and surrounded by a glycoprotein containing envelope. The RNA comprises a short 5′ untranslated region (UTR), a longer 3′ UTR and between them a single open reading frame (Chambers et al., 1990). This codes for a single polyprotein which is co- and post-translational cleaved by viral and host proteases into three structural proteins (core – C, pre-membrane – PrM and envelope – E) and seven non-structural (NS) proteins. The E protein is the major target for the humoral immune response and is thought to include the binding site for the cellular receptor, which is, as yet, unidentified (Roehrig et al., 1989; Rey et al., 1995).

10.4.3. Pathogenesis of flavivirus encephalomyelitis

10.4.3.1. Cell entry and early spread

The cellular receptor for flaviviruses is not known. For a number of flaviviruses there is evidence that heparin sulfates and other glycosaminoglycans may be important, perhaps acting as initial attachment factors that concentrate virus particles at the cell surface for subsequent interaction with other receptor molecules (Chen et al., 1997; Su et al., 2001). A range of molecules with different molecular masses have been postulated as putative receptors for flaviviruses, including heat shock proteins 90 (Reyes-Del Valle et al., 2005) and a 105-kDa protease-sensitive glycoprotein recently identified as a member of the integrin superfamily (Chu and Ng, 2004). Given the wide range of hosts that flaviviruses infect, it is possible that there is no single cellular receptor, but perhaps a multistep process involving multiple receptors, which may differ in different tissues (Reyes-Del Valle et al., 2005).

10.4.3.2. Early spread

Humans become infected with flaviviruses following the bite of an infected arthropod. Replication is thought to occur initially in the skin, before virus is transported to local lymph node – possibly by Langerhans dendritic cells (Johnston et al., 2000; Wu et al., 2000). The virus enters the CNS after a viremia, and in most models there is a clear relationship between the level of viremia and risk of CNS involvement (Huang, 1982).

10.4.3.3. Crossing into CNS

Although the olfactory route of CNS entry has been demonstrated in animal models of SLEV, WNV and JEV (Nir et al., 1965; Monath et al., 1983; Myint et al., 1999), the evidence suggests that in humans entry occurs across the vascular endothelium. For example, for JEV and WNV, the diffuse distribution of virus demonstrated by immunohistochemical staining supports a hematogenous, rather than an olfactory route of entry (Johnson et al., 1985; Desai et al., 1995; Shieh et al., 2000). There are some data for JEV suggesting virus is passively transported across the vascular endothelium (Liou and Hsu, 1998), whereas for WNV there appears to be replication within endothelial cells (Dropulic and Masters, 1990).

A range of factors may increase the likelihood of virus crossing the blood–brain barrier to enter the CNS. The fact that elderly and hypertensive people are at greater risk of developing CNS disease, for both JEV and WNV (Mostashari et al., 2001), support the

hypothesis that sub-clinical microvascular disease may increase the likelihood. In a similar way, co-infection with cysticercosis is thought to be a risk factor for CNS disease following JEV infection because of an effect on blood–brain barrier function (Liu et al., 1957; Shankar et al., 1983). Finally, the inflammatory response to viral infection may itself increase the blood–brain barrier permeability to virus, as discussed below.

10.4.3.4. Pathology of flavivirus encephalitis

Typically in flavivirus encephalitis, the leptomeninges are normal or slightly hazy, and histological examination shows an inflammatory infiltrate. The brain parenchyma is usually congested with focal petechiae or hemorrhage in the gray matter. Inflammatory lesions are distributed through the thalamus, basal ganglia, midbrain, cerebellum, brainstem and gray matter of the cerebral cortex (Johnson et al., 1985; Guarner et al., 2004). Necrotic "punched out" foci are characteristic of JE and are seen less often in other flaviviruses (Miyake, 1964; Guarner et al., 2004). Microscopically there is perivascular inflammation, necrosis and loss of neurons with neuronophagia (ingestion of dying neurons) and gliosis (formation of nodules by glial cells in place of degenerating lesions) (Johnson et al., 1985; Sampson et al., 2000; Shieh et al., 2000; Guarner et al., 2004). In WNV infection lesions are found in the white as well as gray matter.

In some patients with JE the gray matter of the spinal cord is confluent and discolored, resembling that of poliomyelitis (Haymaker and Sabin, 1947) and accounting for the flaccid paralysis. For West Nile, several autopsy reports have shown inflammatory changes in the cord (Kelley et al., 2003; Leis et al., 2003). Spinal cord inflammation was seen in as many as 17 of 23 people that died following WNV neuroinvasive disease in one recent series (Guarner et al., 2004).

10.4.3.5 Effect on host cells

For flaviviruses, as for enteroviruses above, virus induced cell death is thought to occur as a consequence of apoptosis and/or necrosis. Apoptosis is thought to be an active cellular process of self-destruction to limit viral replication and hence spread.

Flaviviruses activate biochemically distinct apoptotic pathways, involving NF-kappa β FasL and P53 (Liao et al., 1997, 1998; Parquet et al., 2001; Yang et al., 2002). The envelope glycoprotein is thought to be a major trigger of apoptotic pathways (Despres et al., 1998; Catteau et al., 2004).

10.4.3.6. Host response

For dengue viruses, which are flaviviruses best known for causing hemorrhagic fever, there is evidence that pre-existing cross-reactive antibodies from related flaviviruses may increase the risk of severe disease (called antibody dependent enhancement) (Halstead, 1981). However, for the JE serogroup flaviviruses, the clinical epidemiological evidence suggests that antibodies are protective. For example, in Southeast Asia, patients with JE who have been previously infected with a different flavivirus (presumed to be dengue) are at less risk of severe disease than those with a primary infection (Libraty et al., 2002; Winter et al., 2004). However caution is used in post-exposure treatment of TBEV infection with intravenous immunoglobulin (see below), because of fears of antibody dependent enhancement (Arras et al., 1996; Waldvogel et al., 1996).

Cellular immunity also appears to be important in protection against CNS flavivirus disease. In animal models, the cellular immune response may contribute to the prevention of disease during acute infection by restricting virus replication before the CNS is invaded (Chambers and Diamond, 2003). Athymic nude mice have increased susceptibility to experimental infection with JEV (Yu et al., 1985) and transfer of spleen cells from mice immunized with live attenuated virus conveys immunity to infection (Jia et al., 1992). Recent studies have shown the role of CD8 T cells in controlling WNV infection in mice (Shrestha and Diamond, 2004). In humans infected with SLEV, impairment of T-cell function by human immunodeficiency virus appears to increase the risk of developing encephalitis (Okhuysen et al., 1993). Immunosuppressed patients are at increased risk of severe disease following WNV infection (Petersen et al., 2002a).

10.4.4. Inflammation and immunopathology

Perivascular cuffing with infiltration of inflammatory cells (T-cells and macrophages) is a feature of both human JE and WNE, though in neither disease do the cells appear to transport viral antigen across the blood–brain barrier (Johnson et al., 1985; Shieh et al., 2000). In JE, the T-cells include both CD8+ (thought to be cytotoxic/suppressor cells) and CD8− (presumed to be CD4+ helper/inducer cells) (Johnson et al., 1985). In fatal WNV encephalitis, immunohistochemical analysis of the lymphocyte populations showed numerous CD8+ T-cells and fewer CD4+ T-cells. CD20+ B-cells were scattered and most prominent around blood vessels (Sampson et al., 2000). In vitro WNV infection causes upregulation of the cellular adhesion molecules E-selectin, ICAM and VCAM, which may be important in initiating adhesion and migration of neutrophils and macrophages (Shen et al., 1997).

Cytokines may also play a role in disrupting the blood–brain barrier. In humans, the proinflammatory

cytokines, tumor necrosis factor-alpha and interleukin (IL)-6 and the chemokine CXCL-8 (IL-)8 which is involved in polymorphonuclear cell recruitment and RANTES (regulated on activation, normally T-cell expressed and secreted) are all upregulated in the CSF and/or serum of humans with JE and are higher in fatal than non-fatal cases (Ravi et al., 1997; Winter et al., 2004; Samuel and Diamond, 2005). IFN alpha is also elevated in JE (Burke and Morill, 1987; Winter et al., 2004). In mice, a macrophage-derived neutrophil chemotactic factor has been shown to increase the blood–brain barrier permeability for JEV (Mathur et al., 1992) and recent studies with knockout mice have shown that, for WNV, viral penetration of the central nervous system appears to follow stimulation of toll-like receptors resulting in increased levels of TNF-α, which increases permeability of the blood–brain barrier (Liou and Hsu, 1998). IFN-alpha/beta controls WNV infection by restricting tropism and viral burden and by preventing death of infected neurons (Samuel and Diamond, 2005) and a recent study in a mouse model of WNV infection has shown the receptor for RANTES, CCR5, promotes leukocyte trafficking and survival (Glass et al., 2005).

10.4.5. Viral strain difference

The observation that JEV isolates from northern Asia, where the disease is epidemic, are of a different genotype than isolates from southern Asia, where the disease is endemic, led to the suggestion that strain-specific differences in neurovirulence may determine the clinical presentation (Chen et al., 1990, 1992). However, more recent studies have shown the distribution of virus genotypes is probably best explained by the virus originating in the Indonesia–Malaysia region and evolving here into the different genotypes, the most recent of which have subsequently spread across Asia (Solomon et al., 2003c).

However, more subtle strain differences may have a role in determining tissue tropism. Wild-type isolates of JEV with differing phenotypes in terms of neurovirulence and neuroinvasiveness in mice do occur (Huang and Wong, 1963; Hasegawa et al., 1990; Ni and Barrett, 1996) and the same is true for WNV and TBEV. For example, the strain of WNV isolated in New York in 1999 is more virulent in American crows (*Corvus brachyrynchos*) than strains from Kenya and Australia; both the New York strain and the Kenyan strain experimentally killed house sparrows whereas the Australian strain did not (Komar et al., 2003). For TBEV, disease caused by the Far Eastern subtype of TBE virus is thought to be more severe than that caused by the European subtype, with case fatality rates of 20–60% compared with 1–3%.

10.4.6. Clinical and epidemiological features of neurotropic flaviviruses

The epidemiology of flavivirus encephalitis is governed by a complex interplay of climatic, entomological, human behavioral, viral and host factors that are not completely understood (Solomon et al., 2003d). For the JE serogroup viruses (JEV, WNV, SLEV and MVEV), viruses are transmitted naturally in enzootic cycles involving birds and Culex mosquitoes; pigs act as additional amplifying hosts for JEV itself. For TBEV and related viruses, the natural cycle involves small rodents and ticks. Humans become infected inadvertently when they encroach upon the flavivirus cycle, but they are considered "dead end" hosts because they do not normally have sufficiently high or prolonged viremias to transmit the virus further. In addition to arthropod-borne transmission, less common routes of transmission that have been documented include infected transplanted organs and blood products for WNV (Iwamoto et al., 2003), transplacental transmission, for WN and JE (Chaturvedi et al., 1980) and transmission by ingestion of unpasteurized goats' milk for TBEV.

JE is mostly a disease of children, whereas in America WNE and SLE are more likely to affect adults. This apparent paradox is probably largely explained by the increased intensity of transmission of JE in Asia – which means that most children in rural Asia meet the virus in childhood and are immune by adulthood. In contrast when non-immune populations are exposed to flaviviruses, it is typically adults that are more likely to present with neurological disease. This applies to most SLEV outbreaks in America and WNV outbreaks in America and southern Europe (Monath, 1980), but also occurs when immunologically naïve adults meet JEV for the first time. This occurs when the virus spreads to new areas (Solomon et al., 2000) or when immunologically naïve adults travel to endemic areas, for example holiday makers (Wittesjö et al., 1995) or service personnel. The incidence of TBE varies across northern Europe and Northern Asia, but typically affects adults because of their occupational or recreational exposure to ticks in forests.

10.4.6.1. Clinical features of neurotropic flaviviruses

Patients typically develop neurological disease after an incubation period of 5–15 days and a short non-specific febrile prodrome. For most neurotropic flaviviruses, encephalitis is a more common presentation than aseptic meningitis or acute flaccid paralysis (Table 10.3). Seizures are common in children with flavivirus encephalitis (Kumar et al., 1990; Burrow et al., 1998; Solomon et al., 2002) and may include subtle motor seizures and status epilepticus (Wasay et al., 2000;

Table 10.3

Epidemiological features of flaviviruses that cause acute flaccid paralysis (modified from Solomon, 2004)

	Japanese encephalitis virus	West Nile virus	St Louis encephalitis virus	Murray Valley encephalitis virus	Tick-borne encephalitis virus
Geographical area	South Asia, Southeast Asia, China, Pacific Rim, North Australia	Africa, the Middle East, South Asia, Malaysia, Australia, Southern Europe, North America	North, Central and Southern America	Australia, New Guinea	Eastern Europe, Northern Asia (former USSR)
Main vectors	*Culex tritaeniorhynchus*, *C. vishnui*, *C. gelidus*, *C. pipiens*	*Culex pipiens*, *C. restuans*, *C. quinquefasciatus*	*Culex pipiens*, *C. tarsalis*, *C. quinquefasciatus*	*Culex annulirostris*, *C. quinquefasciatus*, *Aedes normanensis*	*Ixodes ricinus*, *I. persulcatus*
Main vertebrate hosts	Migrating birds, especially Asiatic cattle egret (*Bubulcus ibis coromandus*); domestic fowl, pigs	Family *Corvidae* (crows, blue jays) and other *Passeriformes* (finches, blackbirds, warblers)	Pigeons, blue jays, sparrows	Birds, esp night heron (*Nycticorax caledonicus*), ?feral pigs	Small rodents, esp. *Apodemus* and *Clethrionomys* mice
At risk groups	Children in endemic areas and non-immune adults	Elderly, chronically ill and immunocompromised	Elderly	Children and non-immune adults	Occupational/recreational exposure in forests
Ratio symptomatic to asymptomatic infections	1 in 25 (non-immune adults) to 1 in 250–1000 (children)	1 in 5 (fever); 1 in 140–320 (CNS disease)	1 in 250	1 in 700 to 1 in 1200	1 in 20 to 1 in 200
Presenting with acute flaccid paralysis	10–15	10–20	NK	NK	7–20
Presenting with encephalitis (%)*	75	58–62	58–85	50	40–50
Presenting with meningitis (%)	5–10	15–40	5–40	50	40–50
Case fatality rates (%)	20–30	4–14	3–30	15–30	1–-5
Sequelae at hospital discharge (%)	50–60	50	30–50	50	30–50

Note: Data presented here are based on a limited number of studies and may not be directly comparable because of differences in study methodologies.
* Many patients with encephalitis also have lower motor neuron flaccid paralysis, but this is not the main presenting feature.
NK = insufficient data.

Solomon et al., 2002). In addition movement disorders are common and may include a Parkinsonian syndrome, opisthotonus, generalized rigidity, choreoathetosis, orofacial dyskinesias and myoclonic jerks (Misra and Kalita, 1997b; Solomon et al., 2002, 2003b; Pepperell et al., 2003; Sejvar et al., 2003a). These disorders are thought to reflect involvement of the basal ganglia, particularly the thalamus and substantia nigra – as seen on magnetic resonance imaging and at autopsy (Zimmerman, 1946; Bennet, 1976; Brinker and Monath, 1980; Misra and Kalita, 1997b; Cerna et al., 1999; Weiss et al., 2001; Solomon and Vaughn, 2002; Bosanko et al., 2003; Kienzle and Boyes, 2003; Solomon et al., 2003b). In some patients, intention tremors and ataxia may suggest cerebellar involvement (Pepperell et al., 2003).

10.4.6.2. Flaccid paralysis

Motor weakness is common in flavivirus encephalitis. As well as upper motor neuron weakness, which is reported for 30–50% of patients, flaccid limb weakness, with reduced or absent reflexes, is also common. This is often associated with respiratory or bulbar paralysis and is reported for approximately 20–60% of patients (Misra and Kalita, 1997a; Burrow et al., 1998; Nash et al., 2001; Pepperell et al., 2003). In addition to causing flaccid weakness in comatose encephalitis patients, flaviviruses can also cause a poliomyelitis-like acute flaccid paralysis in fully conscious patients. The earliest outbreaks of MVE were thought to be an "aberrant form of poliomyelitis" (Breinl, 1918) and poliomyelitis-like illness has been described for JEV and WNV (Gadoth et al., 1979; Solomon et al., 1998a; Leis et al., 2002; Li et al., 2003; Sejvar et al., 2003b). Flaccid paralysis is typically seen in a single lower limb, but there can be involvement of all four limbs and respiratory muscle weakness too. In TBEV infection, flaccid paralysis usually affects the neck and upper limbs to cause pain, sometimes with periodic muscle contractions and numbness, then upper limb weakness with winging of the scapula, wrist drop or a "hanged head" due to neck extensor weakness (Haglund and Gunther, 2003). Muscle atrophy begins after the second or third week and persists (Kaiser, 1999).

Nerve conduction studies in myelitis caused by flaviviruses typically show reduced or absent compound muscle action potentials, with preserved sensory nerve action potentials and normal conduction velocities (Breinl, 1918; McCordock et al., 1934; Zimmerman, 1946; Newman and Southam, 1954; Solomon et al., 1998a; Kelley et al., 2003; Li et al., 2003; Sejvar et al., 2003b). Electromyography typically shows positive sharp waves and spontaneous fibrillations, consistent with denervation. Acute retention of urine, due to an atonic bladder, may be an early clue that paralysis is due to a flavivirus (Solomon et al., 1998a; Solomon and Vaughn, 2002). In some patients the combination of upper and lower motor neuron damage can lead to bizarre mixtures of clinical signs, which may change hourly during the acute stages of infection.

Although anterior myelitis is the most important cause of paralysis for JE serogroup viruses, in some patients flaccid paralysis reflects other pathologies such as Guillain-Barré syndrome and other radiculopathies (Jeha et al., 2003; Park et al., 2003; Pepperell et al., 2003). A recent study of WNV infection showed 27 (74%) of 32 patients with paralysis had poliomyelitis-like paralysis and four (13%) had Guillain–Barré syndrome (Sejvar et al., 2005). Similarly, some patients with TBEV infection develop a "polyradiculoneurotic form" of disease: neuropathy occurs 1 to 2 weeks after the initial febrile phase and is associated with a recurrence of fever, but there is usually complete recovery (Haglund and Gunther, 2003).

In Russia a 'chronic form' of TBE has been described and is believed to be caused only by the Siberian subtype of TBEV. Deterioration continues long after the acute disease, post mortem examination suggests chronic inflammation and viral RNA may be detected by nucleic acid hybridization or cultured (Gritsun et al., 2003). Because it was felt that some of these patients progressed to amyotrophic lateral sclerosis, there were extensive efforts to transmit this progressive neurological disease to non-human primates, but they were not successful (Haglund and Gunther, 2003; Johnson and Cornblath, 2003). Other patients with TBEV infection are asymptomatic following the initial tick bite, but present years later with a progressive form of disease – with virus being isolated at autopsy (Gritsun et al., 2003). Spontaneous regular contractions (myoclonic jerks) of the limbs are seen in about a quarter of patients with neurological forms of TBEV disease and may persist as epilepsia partialis continua (Kozhevnikov's epilepsy).

10.4.7. Investigations of neurotropic flavivirus infections

Usually there is a moderate CSF pleocytosis of a few hundred lymphocytes/mm^3, protein is moderately elevated and the glucose is normal. Approximately 50% of JE patients and 30% of SLE patients have elevated CSF opening pressures. Attempts at isolating virus from the blood of patients with flavivirus encephalitis are usually negative because of the transient and low viremias. Virus is occasionally isolated from the CSF of patients that do not yet have antibody, particularly those that subsequently die (Burke et al., 1985a; Huang et al.,

2002a), and from post mortem brain tissue (Burke et al., 1985a; George et al., 1987; Iwamoto et al., 2003). Viral RNA may occasionally be detected in the CSF by the reverse transcriptase polymerase chain reaction (PCR) (Igarashi et al., 1994; Petersen and Marfin, 2002). For WNV real-time PCR has proved more useful (Lanciotti et al., 2000). IgM capture ELISAs that detect antibody are often positive on a single CSF or serum sample and have therefore become the accepted standard for diagnosing flavivirus encephalitis (Solomon et al., 2000; Petersen et al., 2002b). Not all patients have antibody on admission to hospital and the test should be repeated if initially negative. False positives can occur in areas where more than one flavivirus co-circulates or for patients that have received a flavivirus vaccine, but this problem can be minimized by testing for antibody against various flaviviruses in parallel (Innis et al., 1989; Solomon et al., 1998b; Martin et al., 2002). Investigations for neutralizing antibodies are more specific than ELISAs, but can only be performed in reference laboratories. Antibody may persist in the serum for many months after infection (Burke et al., 1985b; Roehrig et al., 2003).

Electroencephalograms in encephalitis caused by flaviviruses usually show generalized or focal slowing. In addition they may reveal subtle motor statues epileptics or periodic lateralized epileptiform discharges (PLEDS) (Wasay et al., 2000; Solomon et al., 2002), which are often encountered in herpes simplex encephalitis.

10.4.7.1. Imaging

Computer tomography of patients with flavivirus encephalitis shows low attenuation lesions and magnetic resonance imaging shows a high signal on T2 weighted images in the basal ganglia, particularly the thalamus and substantia nigra (Fig. 10.3) (Zimmerman, 1946; Bennet, 1976; Brinker and Monath, 1980; Misra and Kalita, 1997b; Cerna et al., 1999; Weiss et al., 2001; Solomon and Vaughn, 2002; Bosanko et al., 2003; Kienzle and Boyes, 2003; Solomon et al., 2003b). In patients with flaccid paralysis, magnetic resonance imaging of the spinal cord has shown high signal intensity on T2-weighted scans in the anterior horns of the spinal cord (Fig. 10.4) (Li et al., 2003).

10.4.7.2. Treatment

There is no established antiviral treatment for any flavivirus infection. For many years, the most promising compound was considered to be interferon alpha, which is produced naturally in flavivirus infection and has efficacy in some animal models. It was reported to show promise in open clinical trials against JE (Harinasuta et al., 1985) and SLE (Rahal et al., 2004), and, on this basis, has been given presumptively to patients with WN encephalitis. However, a randomized double-blind placebo-controlled trial of interferon alpha in Vietnamese children with JE showed it had no effect on outcome (Solomon et al., 2003a). Ribavirin and intravenous immunoglobulin have also been given presumptively to patients with WNE (Shimoni et al., 2001; Haley et al., 2003). There is supportive data from animal experiments for the use of immunoglobulin (Agrawal and Petersen, 2003) and a clinical trial has been set up by the US National Institute of Allergy and Infectious Diseases. Intravenous immunoglobulin has also been used for post exposure prophylaxis against TBEV in those bitten by ticks in areas where the virus circulates (Chiba et al., 1999). However, if symptoms of CNS infection have already developed, immunoglobulin is not recommended because of the concern that antibody dependent enhancement of viral entry may aggravate disease (Arras et al., 1996;

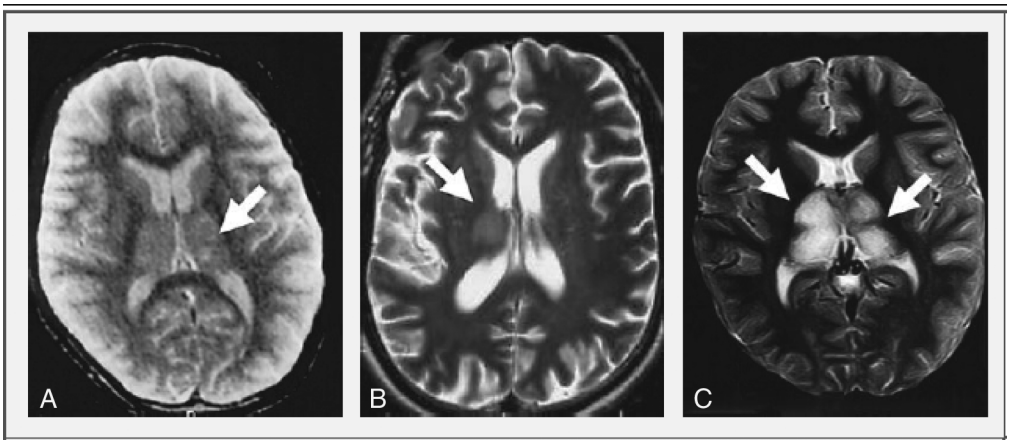

Fig. 10.3. Magnetic resonance imaging in flavivirus encephalitis. T2-weighted magnetic resonance images showing high signal intensity and swelling in the thalamus (arrows) of patients with Japanese encephalitis (**A**), West Nile encephalitis (**B**) and Murray Valley encephalitis (**C**). Adapted from Solomon (2004), Solomon et al. (2003b) and Kienzle and Boyes (2003).

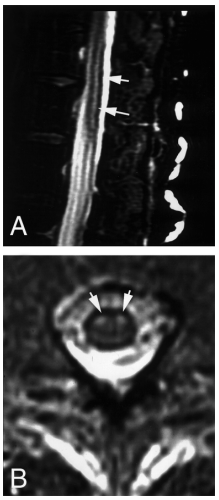

Fig. 10.4. Magnetic resonance imaging in West Nile virus myelitis. Abnormal signal intensity at the anterior horns of spinal cord (arrows) on sagittal T2-weighted MRI of the lumbar spinal cord (**A**). A transverse view of the cord at the midlumbar level. Abnormal signal intensity (arrows) is confined to the anterior horns (**B**). Reproduced from Li et al. (2003) with permission from John Wiley & Sons, Inc.

Waldvogel et al., 1996) – as is thought to occur in dengue infection (Halstead and O'Rourke, 1977; Solomon and Barrett, 2003). Once symptoms have developed, treatment of flavivirus encephalitis consists of paying attention to the complications of infection and avoiding bed sores and contractures with good nursing care and physiotherapy. Despite intensive care, severe neuropsychiatric sequelae are common in survivors of flavivirus encephalitis.

10.4.7.3. Prevention of CNS flavivirus disease

Preventative measures include personal protection to avoid being bitten by infected mosquitoes or ticks, vector control and other measures to reduce the amount of virus circulating naturally. However, vaccines offer the best protection. There are licensed formalin inactivated vaccines for TBEV (Kunz, 2002) and JEV (Hoke et al., 1988) and a live attenuated vaccine for JEV that has been used widely in China and is being used increasingly across Asia (Barrett, 2001; Bista et al., 2001; Monath, 2002). There are not yet licensed vaccines against WNV for humans, though a formalin inactivated vaccine has been used to protect horses (Siger et al., 2004).

10.5. Rabies and other lyssaviruses

10.5.1. Introduction

For thousands of years rabies has been one of the most well recognized and feared human diseases, being described by Aristotle in the 4th century BC, and probably also by the Chinese in the previous century (Warrell and Warrell, 2004). The disease is caused by rabies virus or related lyssaviruses. Worldwide, approximately 55,000 people die every year of rabies, mostly in Asian and African countries (Fu and Jackson, 2005). However, occasional cases in travelers returning to Europe (Johnson et al., 2005) and endemic cases in North America serve as a reminder that the disease also affects the West. Rabies virus is transmitted naturally in enzootic cycles between mammals principally by bites and scratches. Humans most often become infected following dog bites, though other routes have been documented (see below). There are effective pre- and post-exposure vaccines, but once symptoms develop, the disease is almost universally fatal. Approximately 80% of patients present with encephalitic or "furious rabies," which is characterized by hydrophobia and spasms; the remaining 20% present with paralytic or 'dumb' rabies and are of particular interest here.

Rabies virus belongs to genotype 1 in the genus *Lyssavirus* (Family *Rhabdoviridae*). Other genotypes of lyssavirus include Lagos bat virus (genotype 2), Mokola virus (genotype 3) and Duvenhage virus (genotype 4), European bat lyssavirus type 1 (genotype 5), European bat lyssavirus type 2 (genotype 6) and Australian bat lyssavirus (genotype 7). All the lyssaviruses have caused fatal encephalitis except Lagos bat virus, which has not caused human disease, and Mokola virus, which causes a non-fatal encephalopathy (Warrell and Warrell, 2004). Whereas rabies virus circulates naturally among dogs, foxes, racoons and bats, for other genotypes of lyssavirus, insectivorous bats are the most important natural hosts. Lyssavirus genotypes 2, 3 and 4 are found in Africa; genotype 1 (rabies virus) is found across most of the globe; but even countries that don't have this virus (e.g. the British Isles and Australia) are affected by European or Australian bat lyssaviruses.

10.5.2. Virology

Like other rhabdoviridae, the rabies virion has a characteristic bullet-shaped appearance measuring 130–240 nm by 80 nm. The genome consists of a single strand of negative sense non-segmented RNA, which codes for five proteins; G (glycoprotein), M (matrix protein), N (nucleoprotein), L (the polymerase) and NS. The viral nucleic acid is wrapped in a ribonuclear protein, which is surrounded by a matrix glycoprotein, and then the envelope membrane bilayer; this is covered with surface glycoproteins that appear as 6–7 nm spike projections and comprise the major surface antigen for the virus.

10.5.3. Pathogenesis of rabies encephalitis

Although rabies is as old as antiquity, much of our understanding of the pathogenesis of this disease has come from studies carried out in the last few decades. This has mostly been studies using experimental animals, particularly rodents, and laboratory strains of rabies virus such as the "Challenge Virus Standard" (CVS). Conditions may not be the same in the natural infection of humans.

10.5.3.1. Early events in transmission

Following the bite of an infected animal, rabies virus is transmitted centripetally along nerve axons to the brain. However, only about 50% of bites from infected animals result in human disease. Several factors determine the outcome of a bite. These probably include the amount of virus in the saliva of the infected animal, the severity and depth of the bite and the site of the bite – proximal bites being more likely to result in disease. Thus, severe deep bites to the face and neck are especially ominous. The high density of nicotinic acetylcholine receptors on muscle may explain why deep bites are so dangerous. In contrast bat bites and scratches resulting in rabies often tend to be very small and unnoticed, or ignored because they seem trivial. The risk of developing disease also depends on what first aid measures and vaccination is instituted after a bite has occurred – see below.

After virus is inoculated into subcutaneous tissue and muscle there is an incubation period during which time the virus replicates locally. The silver-haired bat virus may have undergone adaptation to allow replication at 34°C in epithelial and fibroblastic cell types present in the skin (Jackson and Fenton, 2001). Rabies virus then enters the nervous system by attaching to the nicotinic acetylcholine receptors at the neuromuscular junction. After gaining access to the peripheral neurons it travels in a retrograde fast axonal transport fashion to the spinal cord at a speed of 50–100 mm per day (Jackson, 2003). Whilst virus is transported within peripheral nerves it is thought to avoid recognition by the host immune system. When the virus reaches the central nervous system there is massive replication on membranes within neurons. Replication in the brain of animals is thought to cause the behavioral changes which result in biting. This is followed by direct transmission of virus from cell to cell across synaptic junctions. The virus then spreads centrifugally from the central nervous system in somatic and autonomic nerves and is deposited in many tissues, including the salivary glands, skeletal and cardiac muscle, kidney, pancreas, retina, cornea, adrenal glands and peripheral nerves (Warrell, 2003). Further viral replication occurs in salivary glands and virus is shed with saliva, so that during a bite it is transmitted to a new host. Although virus is also secreted in the saliva of humans with rabies, direct human to human transmission via saliva or bites has not been documented.

10.5.3.2. Effects of virus infection on neurons

One of the curious things about neurological infection with rabies virus is the relative lack of inflammatory change, compared with other viral CNS infections. Although the actual mechanism by which the rabies virus causes neuronal damage is not completely understood, there are several proposed mechanisms. Under natural conditions, rabies virus infection of the CNS only causes relatively mild neuropathological changes without much evidence of neuronal death (Iwasaki and Tobita, 2002). In human beings, the symptoms of encephalitis and even death can occur with only minor histopathological changes. Few abnormalities of organelle structure are seen on electron microscopy in neurons infected by the virus. This probably shows that during animal infection there is more neuronal dysfunction than death and, although there are a certain recognized range of MRI changes (Laothamatas et al., 2003), no consistent pattern has yet emerged.

10.5.3.3. Effects on cellular RNA and protein synthesis

Animal studies have shown that rabies virus infection suppresses host gene synthesis, which in turn leads to neuronal dysfunction, particularly in the late stages of infection (Fu and Jackson, 2005).

10.5.3.4. Effects on neurotransmission

In experimental animal studies of rabies virus there is evidence of impairment of release and binding of serotonin. This might play a role in producing the neuronal dysfunction (Fu and Jackson, 2005). Impairments of both release and uptake of GABA have been found in CVS strain infected primary rat cortical neuronal cultures (Ladogana et al., 1994). Measurements of noradrenaline, dopamine, serotonin and 5-hydroxyindoleacetic acid (5-HIAA) were found to increase gradually and peaked at 3 days post-infection; by day 7 the levels of neurotransmitter release was at or below the level prior to infection. It is possible that neurons are no longer capable of releasing neurotransmitters at the synaptic junctions and this may be the basis of clinical signs, including paralysis (Fu and Jackson, 2005).

10.5.3.5. Neurotoxicity

Increasing quantities of nitric oxide in the brains of rabies virus-infected experimental animals has been

shown to correlate with clinical progression of the disease. Nitric oxide produced by macrophages may cause neurotoxicity because it reacts with superoxide anion O_2^-. This leads to the formation of peroxynitrate, which is a reactive oxidizing agent capable of causing tissue damage (Akaike et al., 1995).

10.5.3.6. Neuronal death

Neurotropic viruses may cause death by either apoptosis or necrosis. Each of these forms of cell death is associated with typical morphological features. CVS has been observed to induce apoptotic cells in experimental animal models (Jackson and Rossiter, 1997).

10.5.3.7. Pathophysiology of paralytic rabies

Symptoms and signs in paralytic rabies are indicative of derangement of spinal cord (anterior horn cell) function or peripheral nerve damage (Mitrabhakdi et al., 2005). Evidence supporting peripheral nerve dysfunction is based on electrophysiological studies. A range of abnormalities has been found including multifocal demyelination, severe reduction in conduction velocities, marked prolongation of distal latencies and progressive loss of motor and sensory amplitudes – findings which may be indistinguishable from demyelinating and axonal variants of Guillain-Barré syndrome (Hemachudha, 2005; Mitrabhakdi et al., 2005).

Why some patients infected with rabies virus should present with paralytic disease and others with furious disease is not known (Hemachudha, 2005). Earlier suggestions that viral strain differences may be important have been shown to be unlikely by recent molecular genetic characterizations of virus isolates (Hemachudha et al., 2003). Nor are there strong data to support the hypothesis that paralytic disease is more likely in those who have been previously vaccinated (Hemachudha, 2005).

10.5.4. Pathology of paralytic rabies

Histopathology study performed on paralytic rabies suggests peripheral nerve demyelination as the prime change (Chopra et al., 1980). In a more recent histopathological study of two paralytic and one furious rabies patients, all had neuropathic symptoms. In both paralytic cases a moderate to severe degree of lymphocytic infiltration, mainly of CD3-positive T-cells, was evident in the dorsal and spinal nerve roots. The degree of inflammation appeared to be greater at the level of the bitten segment (Mitrabhakdi et al., 2005). In the patient with furious rabies, who had been bitten on the ankle, mild inflammation of the spinal nerve roots at all levels was observed.

10.5.5. Clinical and epidemiological features

10.5.5.1. Epidemiology

Rabies virus is principally transmitted to humans following animal bites, however there have been documented cases of transmission by the aerosol route in laboratory workers (Winkler et al., 1973) and in an entomologist who frequently visited bat infested caves (Dutta et al., 1992). There has also been a single reported case of human transplacental transmission (Sipahioglu and Alpaut, 1985) and apparent transmission from a mother to her breastfeeding baby (Dutta et al., 1992). Other unusual routes of transmission include via corneal transplant (Javadi et al., 1996) and recently through transplantation of other organs (Srinivasan et al., 2005).

The true incidence of rabies is not known, because of under-reporting. However, in India 30,000 deaths were reported to the WHO in 1998 (Warrell, 2003). Across Asia the reported incidence varies from 0.15 per 100,000 in Vietnam to 3 per 100,000 in India. Only 204 cases were reported for the whole of Africa in 1998, probably due to gross under-reporting. In the US, 32 deaths were reported between 1990 and 2000; 24 (75%) of these were due to bat rabies viruses; ten victims did recall contact with a bat, but only two reported a bite (Centers for Disease Control, 2000).

10.5.5.2. Clinical features of paralytic rabies

Furious rabies presenting with hydrophobia and/or aerophobia is usually unmistakable, though sometimes psychiatric disturbances early in the illness may cause confusion. Paralytic rabies is harder to diagnose and is often mistaken initially for other causes of acute flaccid paralysis, either because the history of exposure to a potentially rabid animal is not elicited or not interpreted correctly. Any exposure to any mammal in an endemic country should be considered a potential source of rabies virus. The animal may not be obviously rabid. For example it is not uncommon for owners of a domestic dog to think it is gagging because of something stuck in the throat, rather than because it is rabid. Infected bats may simply appear “friendly” or sleepy. All exposures should be taken seriously.

Paralytic rabies presents after an incubation period which is typically between 20 to 90 days, but may range from a few days to more than 1 year (Jackson, 2000). There is often localized pain and paresthesia which may cause pruritus or numbness at the site of the healed bite wound and are thought to reflect infection in the local peripheral sensory ganglia related to the site of viral entry (Jackson, 2000). The pain can occasionally be excruciating and involve the whole leg (Solomon et al., 2005). Other patients present with non-specific symptoms of headache, malaise, anorexia

and nausea, followed by ascending flaccid paralysis, with limb and then respiratory muscle involvement. Although distinguishing clinically between paralytic rabies and Guillain-Barré syndrome can be difficult, there may be clues. For example, fever and headache at presentation, asymmetry of limb weakness, bladder involvement and cells in the cerebrospinal fluid suggest virus infection of the anterior horn cells in the spinal cord rather than immunologically mediated Guillain-Barré syndrome (Solomon et al., 2005). Other features that may serve to differentiate paralytic rabies from Guillain-Barré syndrome are intact sensory function of all modalities except at the bitten region and percussion myoedema. This consists of mounding of the muscle at the percussion site, which disappears after a few seconds (Hemachudha et al., 1987). Percussion myoedema is most readily elicited on the chest, deltoid and thigh regions. The sign is also sometimes seen in extreme cachexia, hyponatremia and the syndrome of inappropriate secretion of antidiuretic hormone (Hemachudha et al., 2002).

Once the clinical features of rabies have developed, the disease is almost universally fatal (see below). Without intensive care, patients with furious rabies die within 7 days of illness, whereas those with paralytic disease tend to have slower progression and survive longer.

10.5.6. Diagnosis of rabies

Globally, most rabies is diagnosed clinically; this is not normally difficult for patients with furious rabies. Where possible, patients with suspected rabies are investigated by collecting saliva, cerebrospinal fluid, serum and a punch biopsy at the nape of the neck, which includes hair follicles containing peripheral nerve endings (Warrell, 2003). The detection of rabies virus antigen in a skin biopsy, using a fluorescent antibody test, is one of the most reliable tests (Warrell, 2003); corneal impression smears are less reliable and so are no longer recommended. Detection of nucleic acid in saliva and other samples using PCR are now used increasingly and proving to be a rapid and reliable way of making the diagnosis (Smith et al., 2003; Solomon et al., 2005). Attempts should also be made to isolate the virus from the saliva and CSF. Because initial results may be negative, investigations should be repeated daily (Fooks et al., 2003a). In some patients all ante-mortem testing will be negative and the diagnosis is only made post-mortem by examining brain material. This can be obtained at autopsy or by biopsy with a Vim-Silverman needle or other long biopsy needle, such as that used for bone marrow aspiration (Warrell, 2003; Solomon et al., 2005). In addition to virus detection, as described above, routine staining may reveal negri bodies. Virus infection can also be confirmed serologically by demonstrating the presence of neutralizing antibodies against rabies virus in patients not previously vaccinated. These antibodies are usually not present early in the illness until the second week of illness and sometimes will only become detectable just before death.

10.5.7. Management

Rapid diagnosis of rabies is important for the appropriate infection control and public health measures to be instituted, and it is a notifiable disease in the UK and elsewhere (Department of Health, 2004). Although there are no well documented cases of human to human transmission (except via organ transplantation (Srinivasan et al., 2005)) barrier nursing is used, vaccination offered to relatives and exposed staff and reassurance given to other staff members. Until recently, once clinical features developed, rabies was considered almost universally fatal. The only documented survivors (Jackson et al., 2003) had received some pre- or post-exposure vaccination, but had not received complete prompt post-exposure vaccination, making them essentially failures of the vaccination regime they were given (Hemachudha et al., 2002). However in 2004 a teenager in Wisconsin, USA, who developed rabies following a bat-bite, was successfully treated with a combination of ketamine, midazolam, ribavirin and amantadine (Willoughby et al., 2005). Quite how the treatment worked is not clear. Ketamine has both anti-viral effects against rabies virus, but is also an NMDA receptor antagonist, and so is anti-exitotoxic. Her treatment was instituted on the 5th day of disease, when she was semi-obtunded, with cranial nerve signs and ataxia.

10.5.8. Prevention of rabies

Preventative measures include eliminating infection in the animal vectors, pre- and post exposure vaccination. Domestic-dog strains of rabies account for more than 90% of the human disease worldwide. Rabies in dogs can be reduced by parenteral vaccination, fertility control and clearing rubbish to reduce the food supply that maintains the population of stray dogs (Warrell and Warrell, 2004).

10.5.8.1. Vaccination

Two types of the rabies vaccine are licensed for use in the UK and the USA: human diploid-cell vaccine (HDCV; Imovax Rabies, Aventis Pasteur, Lyon, France) and purified chick-embryo-cell vaccine (PCECV;

Rabipur, RabAvert, Chiron Behring). Both of these are sold in single-dose 1 mL vials. Elsewhere, purified vero-cell vaccine (PVRV, verorab, Aventis Pasteur) is widely available in single-dose 0.5 mL vials. Rabies vaccine adsorbed (BioPort, Lansing, MI, USA) is also licensed in the USA (Warrell and Warrell, 2004).

10.5.8.2. Pre-exposure vaccination

Pre-exposure vaccination (i.e.vaccination of travelers and others *before* they get bitten by a rabid animal) does not remove the need for post-exposure vaccination (i.e. vaccination after being bitten), but it simplifies the post-exposure regime and makes it more likely to work (Warrell and Warrell, 2004). The standard pre-exposure regimen consists of three doses of a tissue-culture vaccine intramuscularly (deltoid) on days 0, 7 and 28. Studies have shown injection of 0.1 ml intradermally is a more cost effective way of using vaccine, and this approach is being increasingly used (Warrell and Warrell, 1988; World Health Organization, 2005). A booster after 1 year increases and prolongs the antibody response (Strady et al., 1998). If an individual who has had pre-exposure vaccine subsequently is exposed to the virus, they require only two doses of vaccine post-exposure, on day 0 and day 3, and there is no need for rabies immunoglobulin. There have not been any reported cases of rabies deaths in anyone who has had pre-exposure treatment followed by a booster dose after exposure (Warrell and Warrell, 2004).

10.5.8.3. Post-exposure vaccination

If someone has been bitten by a potentially rabid animal, wound care immediately after the bite is essential. This includes cleaning with soap and water, detergent or plain water, followed by the application of ethanol, tincture or aqueous solution of iodine and removing any dead tissue (World Health Organization, 2005). Wounds should be left open, rather than sutured, and a tetanus shot should be given.

Modern post-exposure treatment is very effective. Failures of optimum treatment are uncommon. The standard anti-rabies vaccine regimen should be started as soon as possible. This involves five 1.0 ml doses given intramuscularly into the deltoid (or anterolateral thigh for children) on days 0, 3, 7, 14 and 28; again recent studies show 0.1 ml intradermally is just as effective and cheaper (World Health Organization, 2005). Passive immunization with human rabies immunoglobulin, given at the same time as the active vaccination, has been shown to lower mortality after severe exposure, e.g. a savage bite to the head or neck; the vaccine affords protection for the first 7 days after a bite, while antibody is raised against the active vaccine. It may not be important for milder rabies exposure, for example a single bite on limbs, and indeed is not available in many countries. The full dose of rabies immunoglobulin, or as much as anatomically feasible, should be administered into and around the wound site. Any remainder should be injected intramuscularly at a site distant from the vaccine administrative site (World Health Organization, 2005).

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Chapter 11

Monomelic amyotrophy of upper or lower limbs

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11.1. Introduction

Monomelic amyotrophy in which neurogenic atrophy is restricted to one limb is a heterogenous disorder, involving one upper or lower limb. Insidious onset of atrophy and weakness, presumed to be due to anterior horn cell involvement, starting in the second or third decade with male preponderance and sporadic occurrence are the characteristic features. Progression is slow and followed by stabilization within a few years, resulting in a benign outcome. Cranial nerves, pyramidal, sensory, cerebellar and extrapyramidal systems are not involved.

Hirayama et al. (1959) from Japan reported atrophy of a single upper limb and labeled it as "juvenile muscular atrophy of unilateral upper extremity." Prabhakar et al. (1981) from India reported atrophy of muscles of one lower limb and described it as "wasted leg syndrome." Since either one upper or lower limb is affected, Gourie-Devi et al. (1984a, 1986) suggested the eponym "monomelic amyotrophy" (MMA) as a more appropriate term. The authors further suggested that upper limb MMA may be called "brachial monomelic amyotrophy" to differentiate it from MMA of a lower limb, which may now be called "crural monomelic amyotrophy" (Gourie-Devi and Nalini, 2003). Focal amyotrophy has been described under a variety of descriptive names, which refer to the limb involved, the site of muscles affected and the benign and non-progressive course of the disease (Table 11.1).

11.1.1. Monomelic amyotrophy of upper limb

More than 300 cases have been reported from Japan (Hirayama et al., 1963; Hashimoto et al., 1976; Sobue et al., 1978; Hirayama, 2000a). The atrophy was distal and segmental, confined to one upper limb, but electromyographic abnormalities were noted in some patients in the non-atrophic upper limb. From India also more than 200 cases (including a personal series of 89 cases) have been reported of single upper limb atrophy, a large proportion of them with distal muscle involvement and a few with proximal muscle involvement (Singh et al., 1980; Gourie-Devi et al., 1984a,b, 1987a; Virmani and Mohan, 1985; Misra and Kalita, 1995; Pradhan and Gupta, 1997; Saha et al., 1997; Khandelwal et al., 2004; Misra et al., 2005).

Reports from many other countries including Sri Lanka (Peiris et al., 1989), Korea (Kim et al., 1994), Hong Kong (Chan et al., 1991), Taiwan (Kao et al., 1993a) and Malaysia (Tan, 1985) reaffirm the frequency of MMA in Asia. Initially there were few reports from Western countries, mostly isolated cases or a small number of patients, but with increasing awareness more publications have appeared in the literature (Pilgaard, 1968; Compernolle, 1973; Engel, 1977; Adornato et al., 1978; De Visser et al., 1988). Large series of cases, notably from France and Brazil, have been published (Serratrice et al., 1987; De Freitas and Nascimento, 2000).

Hirayama et al. (1963) referred to 10 cases reported by Marie and Foix in 1912, of isolated non-progressive atrophy of small muscles of hand, older age at onset of the disease in the fifth to eighth decades in eight cases and second decade in two cases. The autopsy findings in four of these patients are discussed later (§ 11.12).

11.1.2. Monomelic amyotrophy of lower limb

Monomelic amyotrophy of a lower limb is less frequent than MMA of an upper limb. More than 130 cases (including a personal series of 36 cases) have been

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Table 11.1

Eponyms used for single limb atrophy

A. Upper and lower limb
- Monomelic amyotrophy (Gourie-Devi et al., 1984a).
- Benign focal amyotrophy (Adornato et al., 1978; Riggs et al., 1984).
- Monomelic spinal muscular atrophy (De Visser et al., 1988).
- Spinal monomelic amyotrophy (Serratrice, 1991).
- Benign monomelic amyotrophy (De Freitas and Nascimento, 2000).

B. Upper limb
- Juvenile muscular atrophy of unilateral upper extremity (Hirayama et al., 1959).
- Juvenile non progressive muscular atrophy localized to hand and forearm (Hashimoto et al., 1976).
- Juvenile type of distal and segmental muscular atrophy of upper extremities (Sobue et al., 1978).
- Juvenile muscular atrophy localized to arms (Singh et al., 1980).
- Juvenile lower cervical spinal muscular atrophy (Kao et al., 1993a).
- Juvenile amyotrophy of distal upper extremity (Biondi et al., 1989).
- Non-familial spinal segmental muscular atrophy in juvenile and young subjects (Virmani and Mohan, 1985).
- Non-progressive juvenile spinal muscular atrophy of the distal upper limb (Hirayama's disease) (Hirayama, 1991).
- Juvenile asymmetric segmental spinal muscular atrophy (Pradhan and Gupta, 1997).
- Brachial monomelic amyotrophy (Gourie-Devi and Nalini, 2003).

C. Lower limb
- Wasted leg syndrome (Prabhakar et al., 1981).
- Benign monomelic amyotrophy of lower limb (Uncini et al., 1992).
- Benign calf amyotrophy (Felice et al., 2003).
- Crual monomelic amyotrophy (Gourie-Devi, 2004).

reported from India (Prabhakar et al., 1981; Gourie-Devi et al., 1984a,b, 1987a; Virmani and Mohan, 1985; Chopra et al., 1987; Saha et al., 1997) and more than 40 cases from Western countries (Riggs et al., 1984; Serratrice et al., 1987; Uncini et al., 1992; De Freitas and Nascimento, 2000; Felice et al., 2003). It is noteworthy that, although numerous cases of MMA of an upper limb are described from Japan, there is only one isolated report of two cases of MMA of a lower limb (Hamano et al., 1999).

11.2. Prevalence and geographic distribution

Monomelic amyotrophy constituted 8–29% of all motor neuron diseases in different series reported from India (Gourie-Devi et al., 1984a, 1987a; Saha et al., 1997). The estimated prevalence rate of MMA was 0.9, of upper limb 0.5 and lower limb 0.4 per 100,000 population (Gourie-Devi et al., 1984a; Gourie-Devi, 2004), based on the ratio of cases of monomelic amyotrophy to amyotrophic lateral sclerosis, as suggested by Kurtzke (1962), the prevalence rate of ALS having been determined to be 4 per 100,000 population (Gourie-Devi et al., 1984a, 1995). The geographic distribution of MMA of upper and lower limb in Asia and other countries is shown in Tables 11.2 and 11.3.

11.3. Classification

Monomelic amyotrophy can be classified based on the limb involved and the site of muscles affected:

Type 1. Monomelic amyotrophy of upper limb.
- Distal: Hand and forearm muscles.
- Proximal: Shoulder girdle and arm muscles.
- Global: Entire limb.

Type 2. Monomelic amyotrophy of lower limb.
- Distal: Leg and foot muscles.
- Proximal: Pelvic girdle and thigh muscles.
- Global: Entire limb.

In the majority of cases in both type 1 and type 2, the atrophy is confined to a single limb with electromyographic abnormalities in the contralateral limb in some patients. In type 1, spread to the contralateral limb with atrophy and weakness may occur in 10 to 30%, but significant asymmetry is a distinctive feature, the initially involved limb being more severely affected (Gourie-Devi et al., 1984a; Sobue et al., 1978). In contrast, in type 2, atrophy is usually restricted to a single lower limb (Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Serratrice et al., 1987) with rare instances of spread to the opposite limb (Kim et al., 1994; Felice et al., 2003).

Table 11.2

Geographic distribution of monomelic amyotrophy of upper limb

A. Countries in Asia	
India:	Singh et al., 1978; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Misra and Kalita, 1995; Pradhan and Gupta, 1997; Saha et al., 1997; Nalini et al., 2004; Khandelwal et al., 2004.
Hong Kong:	Chan et al., 1991.
Israel:	Neufeld et al., 1991.
Japan:	Hirayama et al., 1963; Hashimoto et al., 1976; Sobue et al., 1978; Mukai et al., 1985; Iwasaki et al., 1987; Kikuchi et al., 1987; Konno et al., 1997; Kohno et al., 1998.
Korea:	Kim et al., 1994.
Malaysia:	Tan, 1985.
Sri Lanka:	Peiris et al., 1989.
Taiwan:	Kao et al., 1993a.
Turkey:	Gucuyener et al., 1991.
B. Countries outside Asia	
Australia:	Kiernan et al., 1999.
Belgium:	Robberecht el al., 1997.
Brazil:	De Freitas and Nascimento, 2000.
Canada:	Oryema et al., 1990.
Denmark:	Pilgaard, 1968.
France:	Serratrice et al., 1987; Chaine et al., 1988; Biondi et al., 1989.
Germany:	Schlegal et al., 1987; Schroder et al., 1999.
Italy:	Barontini et al., 1991; Di Guglielmo et al., 1996; Polo et al., 2003.
Netherlands:	Compernolle, 1973; Thijsse and Spaans, 1983; De Visser et al., 1988.
Poland:	Drozdowski et al., 1998.
Switzerland:	Kaeser et al., 1983.
USA:	Engel, 1977; Adornato et al., 1978; Metcalf et al., 1987; Tandan et al., 1990; Liu and Specht, 1993; Donofrio, 1994; Rowin et al., 2001.

11.4. Clinical features

The age of onset in the majority (90%) varies from 15 to 35 years with a median age of 20 years in MMA of upper limb and slightly older in MMA of lower limb with a median age of 25 years (Hirayama et al., 1963; Sobue et al., 1978; Gourie-Devi et al., 1984a). In exceptional cases the age at onset can be as early as 2 years and as late as 84 years, the older age at onset being more often noted in MMA of lower limb (Sobue et al., 1978; Serratrice et al., 1987; Felice et al., 2003). However, because the condition is so insidious in onset it can be difficult to determine the age at onset. There is remarkable gender preference, with men outnumbering women with a ratio varying from 3:1 to 20:1, with more men affected in MMA of lower limb compared to MMA of upper limb (Hirayama et al., 1963; Sobue et al., 1978; Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985). The duration of illness at first consultation may vary from a few months to as long as 15 years, with a mean duration of 2.5 to 4.5 years (Hirayama et al., 1963; Prabhakar et al., 1981; Gourie-Devi et al., 1984a; De Freitas and Nascimento, 2000).

Table 11.3

Geographic distribution of monomelic amyotrophy of lower limb

A. Countries in Asia	
India:	Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Saha et al., 1997.
Japan:	Hamano et al., 1999.
Korea:	Kim et al., 1994.
B. Countries outside Asia	
Austria:	Willeit et al., 2001.
Brazil:	De Freitas and Nascimento, 2000.
France:	Nedelec et al., 1987; Serratrice et al., 1987.
Germany:	Munchau and Rosenkranz, 2000.
Italy:	Uncini et al., 1992; Di Muzio et al., 1994; Di Guglielmo et al., 1996.
Netherlands:	De Visser et al., 1988.
Spain:	Martinez et al., 1990.
USA:	Riggs et al., 1984; Felice et al., 2003.

11.4.1. Clinical features of MMA of upper limb

In monomelic amyotrophy of upper limb, the common initial symptoms are weakness and atrophy in the majority, followed by tremulousnesss of fingers. Coarse, intermittent nonrhythmic tremors of fingers present at rest, accentuated by outstretching of hands and on

voluntary action is present in 60 to 80% of patients (Hirayama et al., 1963; Gourie-Devi et al., 1984a). This feature has been observed in spinal muscular atrophy and the descriptive term minipolymyoclonus has been coined (Spiro, 1970). Minipolymyoclonus needs to be distinguished from tremors, which are generally rhythmic, and from fasciculations. Discharges by motor neurons innervating large territory of muscle are implicated in the causal mechanisms of these tremor-like movements, but probably not specific, and may be seen in hand weakness from most neuromuscular disorders.

Fasciculations are commonly observed in atrophic muscles and also in the unaffected muscles in a few patients. Hirayama (1972) described "cold paresis," an interesting phenomenon of aggravation of weakness on exposure to cold. Some of them also complain of stiffness of hands on dipping the hands in cold water, however there was no clinical or electromyographic evidence of myotonia (Gourie-Devi et al., 1984a).

In MMA of upper limb the distal muscles of hand and forearm are affected in more than 50% of patients, proximal muscles of shoulder and upper arm in 5–10% and diffuse involvement in 40% with the distal muscles more severely affected than proximal muscles. Small muscles of the hand, flexors and extensors of the wrist, chiefly C7-T1 spinal segments, are the most severely affected muscles (Figs. 11.1–11.3). Relative sparing of brachioradialis muscle among surrounding atrophic muscles (Fig. 11.2) is a characteristic feature of this disease (Hirayama et al., 1963). In the diffuse form with involvement of an entire upper limb, the additional muscles atrophied are biceps, triceps, deltoid and scapular muscles (Compernolle, 1973; Thijsse, 1983; Gourie-Devi et al., 1984a). Unilateral atrophy of scapulohumeral muscles in C5–C6 myotomes (Fig. 11.4) was described by Kaeser (1983) from Switzerland and similar cases were observed by others (Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Amir et al. 1987; De Visser et al., 1988; Kao et al., 1993a). The pattern of muscles affected in our series of 89 patients (Gourie-Devi and Nalini, unpublished observations) is shown in Figure 11.5.

11.4.2. Clinical features of MMA of lower limb

In MMA of lower limb, atrophy of the limb was noted by the patient because of pain on walking, and in nearly a third of the patients it was incidentally observed by a family member, friend or physician during consultation for unrelated illness (Prabhakar et al., 1981; Gourie-Devi et al., 1984a). Under these circumstances the precise age at onset and duration of illness may not be accurate. Muscle cramps and fasciculations have been observed in 20 to 30% of patients. Unilateral pes cavus may be a presenting feature (De Freitas and Nascimento, 2000). Unlike as in postpoliomyelitis progressive muscular atrophy there is no shortening of limb.

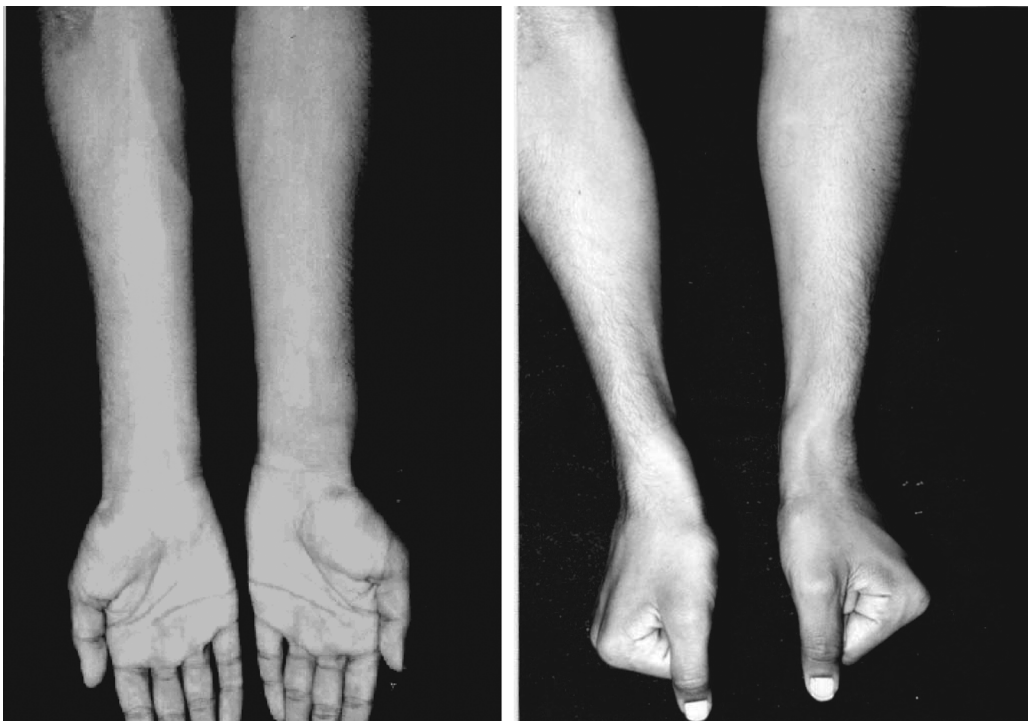

Fig. 11.1. Mild atrophy of flexors of forearm of right upper limb best seen in semiprone position.

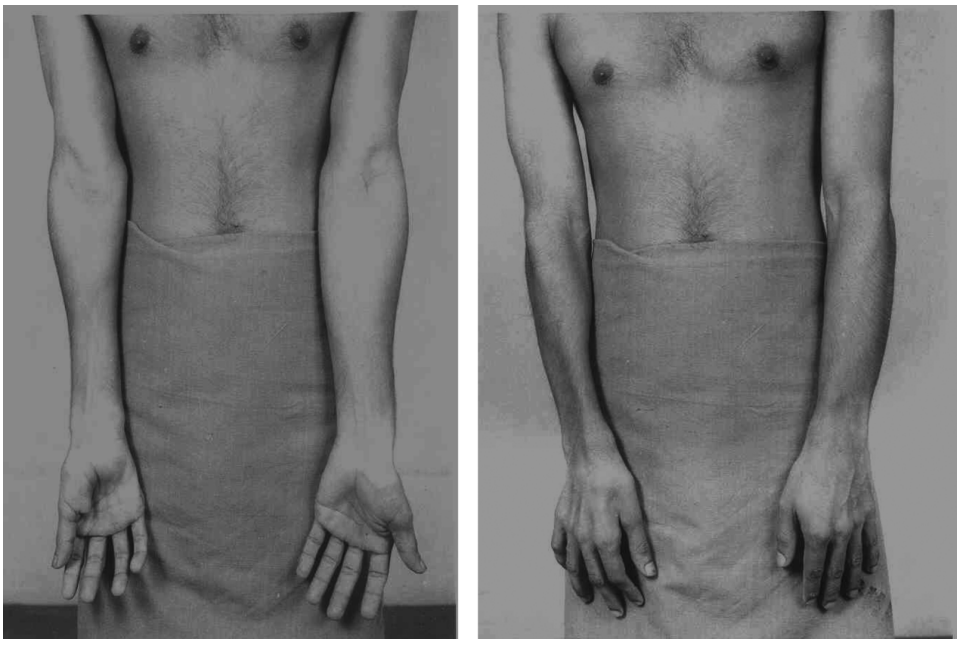

Fig. 11.2. Atrophy of flexor and extensor muscles of right forearm with sparing of brachioradialis muscle and mild wasting of hand muscles.

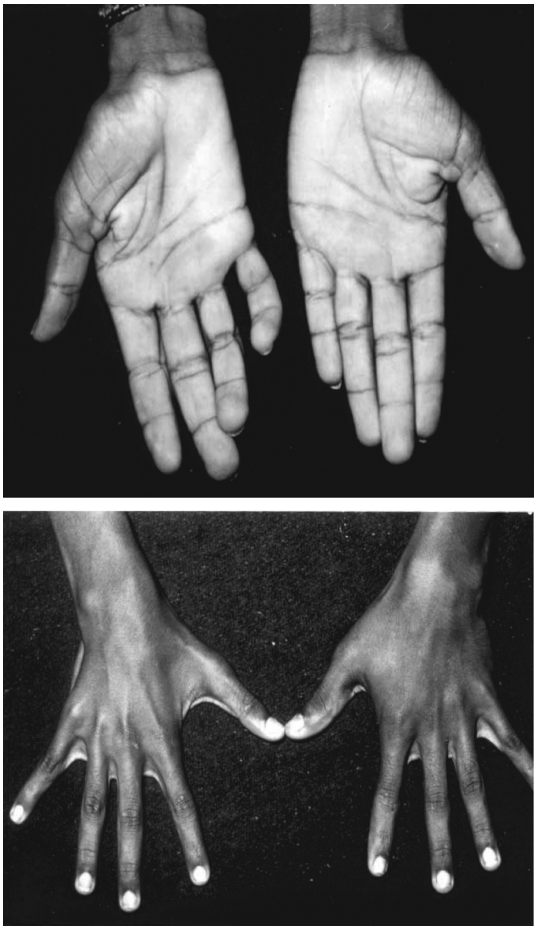

Fig. 11.3. Severe atrophy of thenar, hypothenar and interossei, particularly first dorsal interosseous muscle of right hand.

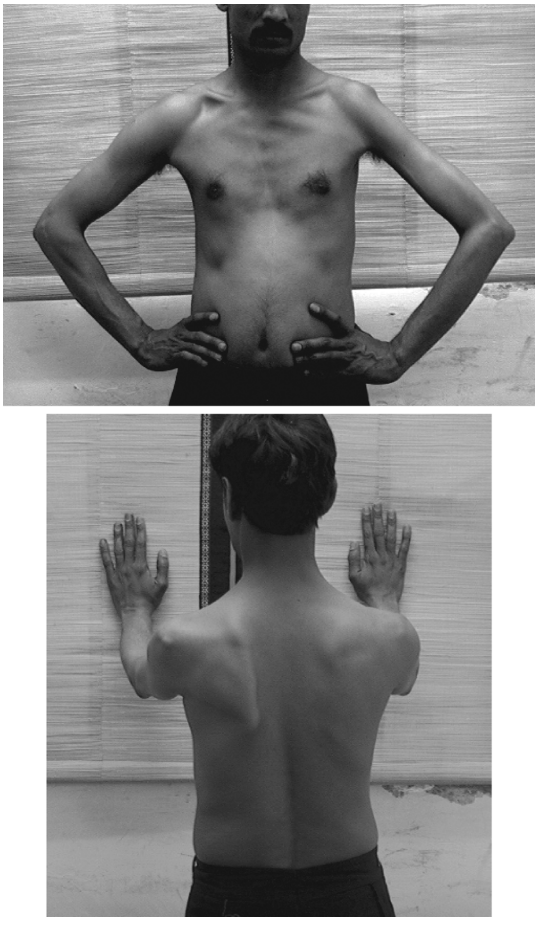

Fig. 11.4. Severe wasting of left shoulder and upper arm muscles with normal forearm muscles.

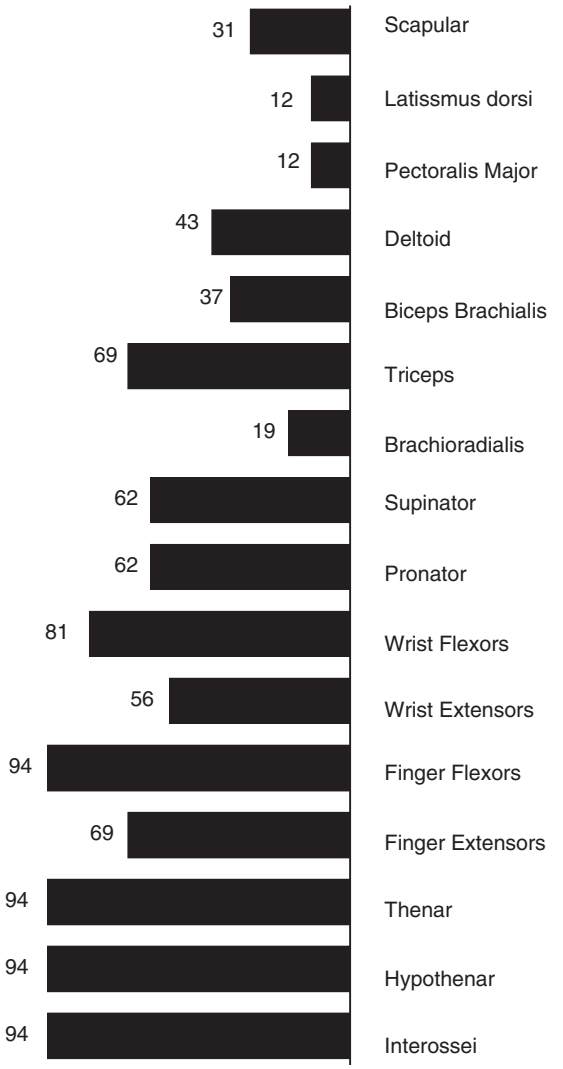

Fig. 11.5. Pattern of muscle atrophy and weakness in 89 patients of monomelic amyotrophy of upper limb (Gourie-Devi and Nalini, unpublished data).

In the distal form, which accounts for 20% of cases, with predominant calf muscle atrophy, inability to stand on tiptoe is a presenting feature (Felice et al., 2003). Anterior and posterior crural muscles are most commonly affected (Fig. 11.6), while intrinsic foot muscles are infrequently involved (Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; De Visser et al., 1988; Uncini et al., 1992; Hamano et al., 1999; De Freitas and Nascimento, 2000; Felice et al., 2003). In the proximal type, isolated atrophy of quadriceps (Fig. 11.7) may occur (Prabhakar et al., 1981; Gourie-Devi et al., 1984a) or may be involved along with hamstring muscles (Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Riggs et al., 1984; Virmani and Mohan, 1985). The commonest type is involvement of the entire limb with atrophy of proximal and distal muscles and has

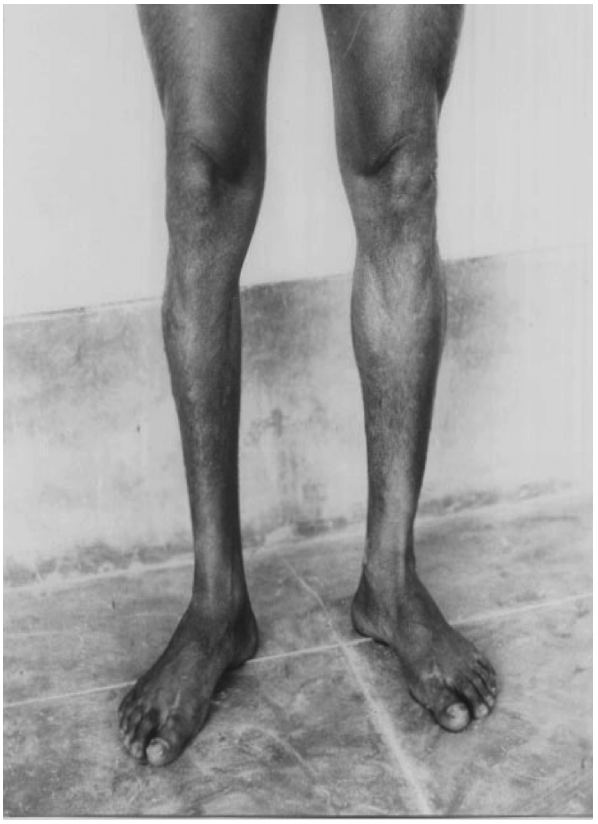

Fig. 11.6. Atrophy of calf muscles of right leg.

been observed in 70% of patients (Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Hamano et al., 1999). The pattern of muscle involvement in our series of 36 cases (Gourie-Devi and Nalini, unpublished data) is shown in Figure 11.8.

11.4.3. Other clinical features

The tendon reflexes in the affected limb in both type 1 and 2 are usually absent or sluggish. In some patients they are normal and brisk reflexes are rare, but plantar response is invariably flexor. In the unaffected homologous limb and other limbs, the reflexes were generally normal and infrequently sluggish. Although subjective symptoms of numbness have been reported, no objective sensory deficit has been documented. Excessive sweating and coldness of affected limb is a frequent feature. Cognitive function, cranial nerves, pyramidal, extrapyramidal and cerebellar systems are not involved. There is no evidence of other neurological disorders in the affected subject or their family members.

11.5. Associated factors and antecedent events

Febrile illness, vaccination, exposure to toxic substances and electric shock preceding the illness have

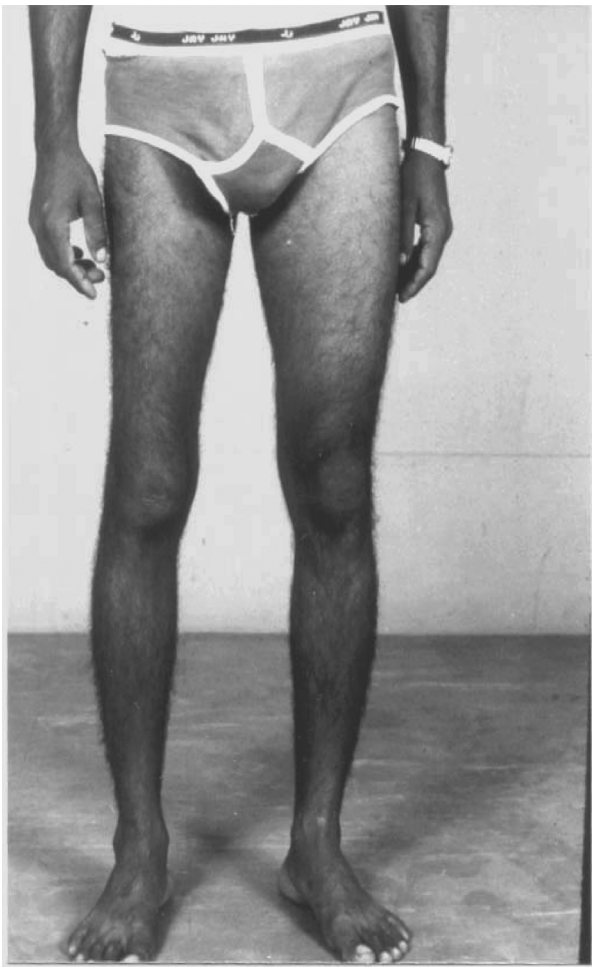

Fig. 11.7. Atrophy of thigh muscles of right lower limb with preserved calf muscles.

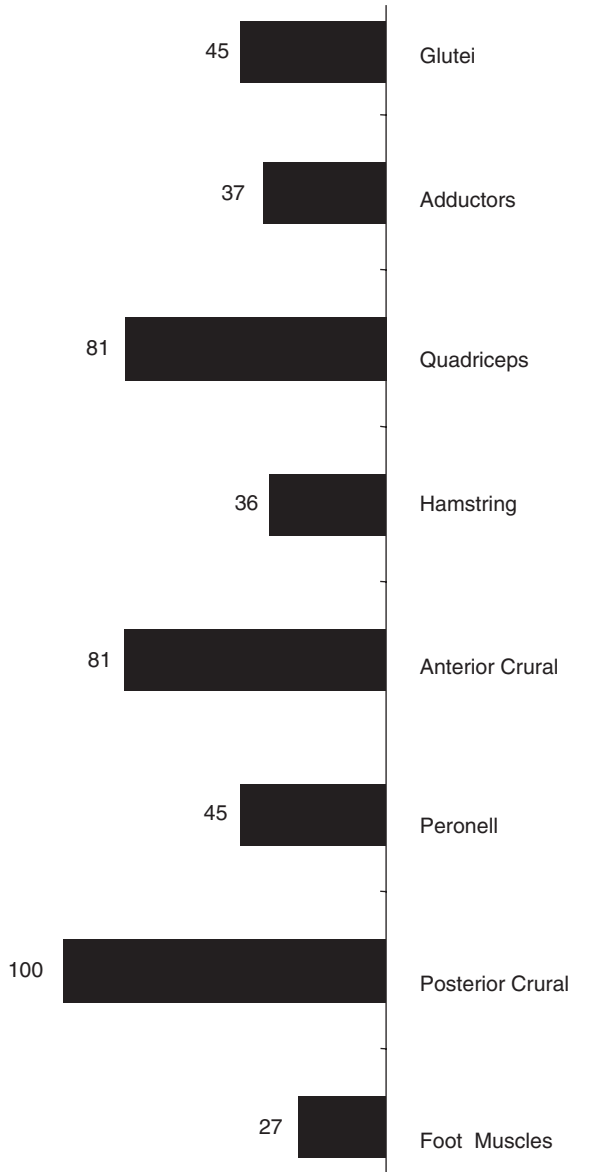

Fig. 11.8. Pattern of muscle atrophy and weakness in 36 patients with monomelic amyotrophy of lower limb (Gourie-Devi and Nalini, unpublished data).

not been observed in the majority of patients (Hirayama et al., 1963; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985, Peiris et al., 1989). In rare instances poliomyelitis in childhood has been reported (Gourie-Devi et al., 1984a; Peiris et al., 1989; Gourie-Devi, 1996). Mechanical trauma including injuries or surgery have been recorded preceding the onset of neurological symptoms by many months to years and in some of them atrophy occurred in the previously injured limb (Sobue et al., 1978; Gourie-Devi et al., 1993; Paradiso, 1997). In a case control study which examined the risk factors in 21 cases and 63 age and gender matched control subjects, strenuous physical activity was observed to be a significant associated factor (Gourie-Devi et al., 1993). Occupations involving heavy manual exertion and participation in competitive sports have also been recorded in patients with MMA (Hashimoto et al., 1976; Prabhakar et al., 1981; Biondi et al., 1989).

11.6. Familial monomelic amyotrophy

Familial occurrence of MMA is extremely rare. Gourie-Devi et al. (1984a) did not detect muscle weakness, wasting or sluggish tendon reflexes in 48 siblings and parents of 17 patients. A total of 15 families have been reported so far from countries in Asia, Europe and USA (Table 11.4). Two brothers were affected in each of six families, father and son in four, mother and son in two families, sister and brother, identical twin brothers and two half brothers in one family each. In 13 families the upper limb was involved and in two families lower limb was affected. The age at onset was in the second or

Table 11.4

Familial case of monomelic amyotrophy

Author (year)	Country	Families	Affected	Limb
Igata et al. (1966)	Japan	1	Father–son	UL
Hirayama (1972)	Japan	3	Brothers (2)	UL
Sobue et al. (1978)	Japan	1	Father–son	UL
Hirayama et al. (1987)	Japan	1	Brothers (2)	UL
Schlegel et al. (1987)	Germany	1	Father–son	UL
Serratrice et al. (1987)	France	1	Mother–son	LL
Nedelec et al. (1987)	France	1	Brothers (2)	LL
Tandan et al. (1990)	USA	1	Identical twin-brother	UL
Gucuyener et al. (1991)	Turkey	1	Sister–brother	UL
Misra and Kalita (1995)	India	1	Brothers (2)	UL
Robberecht et al. (1997)	Belgium	1	Brothers (2)	UL
Nalini et al. (2004)	India	1	Mother–son	UL
Misra et al. (2005)	India	1	Brothers (2)	UL

Figure in parenthesis indicates number of affected members.

third decade in 13 families, first decade in one family (Gucuyener et al., 1991) and fifth decade and beyond in one family (Serratrice et al., 1987). There were 25 males and three females with a M:F ratio of 8.3:1. These reports suggest autosomal recessive inheritance in some families and autosomal dominant inheritance with variable expression in others (De Visser et al., 1991; Robberecht et al., 1997; Nalini et al., 2004). Occurrence of disease predominantly in males and two half brothers may indicate X-linked recessive inheritance which needs to be further examined (Nedelec et al., 1987; Misra et al., 2005).

Only a few genetic studies have been done. In one family in two affected brothers, five exons of superoxide dismutase 1 (SOD 1) gene were normal and the SOD activity in patients' RBC was comparable to the values in control subjects (Robberecht et al., 1997). Subsequently, Mezei et al. (1999) describe a family with a D90A SOD1 mutation in which the father of the proband has clinical features typical of lower limb monomelic amyotrophy. DNA analysis revealed him to be heterozygous for D90A mutation. Survival motor neuron gene (SMN) deletion in the region of 5q13 has been demonstrated to be associated with phenotypic expression of spinal muscular atrophy (SMA) (Lefebvre et al., 1995) and for confirmatory diagnosis of SMA, SMN1 and SMN2 gene deletion study is advocated (Scheffer et al., 2001). It has also been shown that deletions in SMN gene occur in adult onset SMA (Brahe et al., 1995). Since MMA has been considered as a focal form of SMA, studies have been done to examine the deletion of SMN gene. Recent reports from Italy, USA and India show that MMA of upper and lower limb are not associated with deletions in exons 7 and 8 of the SMN gene (Di Guglielmo et al., 1996; Felice et al., 2003; Misra et al., 2005). Mutation of mitochondrial DNA, the 7472 insC in the gene coding the tRNA Ser (UCN), has been reported from Italy in a patient with monomelic amyotrophy and sensorineural hearing loss in the patient, his mother and an elder sister (Fetoni et al., 2004). Association of lower motor neuron involvement with mt DNA mutation needs further elucidation.

11.7. Secondary monomelic amyotrophy

Monomelic amyotrophy may be secondary to demonstrable causes including irradiation, atopy and human immunodeficiency virus (HIV) infection. Lower motor neuron syndrome may develop months to years after irradiation for malignant disorders encompassing the spinal cord. In most cases paraparesis has been reported but rarely cases with monomelic amyotrophy have been documented (Lamy et al., 1991; Jackson, 1992; Serratrice et al., 1993). The period between radiotherapy and development of MMA ranged from 9 to 17 years. It is possible that radiotherapy damaged a critical number of motor neurons and the compensatory efforts of surviving motor neurons in reinnervation of muscles could not be maintained over many years, leading to focal atrophy (Jackson, 1992). However, radiation necrosis more commonly affects the plexus and proximal nerves.

Asthmatic amyotrophy, a polio-like syndrome, is characterized by an asymmetrical lower motor neuron paralysis following an acute episode of asthma (Hopkins, 1974; Batley and Johnson, 1991). Importance of atopy, airways allergy in precipitating 'circulatory insufficiency' and its causal linkage to acute myelitis and to the

chronic disorder of monomelic amyotrophy has been suggested (Kira et al., 1998; Horiuichi et al., 2000; Kira and Ochi, 2001).

In HIV infection, several neurological disorders are described, but motor neuron disease has been very rarely reported (Huang et al., 1993; Moulignier et al., 2001). A significant proportion of these patients were young, the initial presentation was monomelic amyotrophy with subacute progression to other limbs and involvement of corticospinal tracts. The striking response to antiretroviral therapy convincingly establishes the etiological relationship between HIV and motor neuron disease, in these select patients (Jubelt and Berger, 2001; Moulignier et al., 2001).

11.8. Investigations

11.8.1. Laboratory tests

Routine blood and cerebrospinal fluid analysis is usually normal, but a mild rise of CSF protein has been seen in a few patients (Hirayama et al., 1963; Gourie-Devi et al., 1984a). A slight increase in serum creatine kinase level, just above the normal range, has been reported in occasional patients (Gourie-Devi et al., 1984a). Antibodies to viruses such as polio, Coxackie B, Echo, influenza A and B, adeno and herpes simplex were not detected in CSF (Sobue et al., 1978; Virmani and Mohan, 1985). Lower serum neutralizing antibody titers for poliovirus were found in patients compared to controls suggesting that patients with MMA may be immunologically unresponsive to a neutralizing epitope of poliovirus (Kao et al., 1993b). Intrathecal immunoglobulin synthesis was not detected and ganglioside antibodies, particularly anti-GM 1 antibodies, were not detected (Willeit et al., 2001).

11.8.2. Muscle biopsy

Variable findings of normal to small groups of angulated muscle fibers, group atrophy, nuclear clumping, fiber type grouping to end stage disease with diffuse fatty infiltration and prominent increase in connective tissue, all features suggestive of neurogenic atrophy in the affected limb, have been noted in various studies (Hirayama et al., 1963; Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Kao and Tsai, 1994; Kim et al., 1994). Necrotic fibers with central nuclei, basophilic fibers with large vesicular nuclei indicating secondary myopathic changes, were observed in a few patients (Prabhakar et al., 1981; Gourie-Devi et al., 1984a). Subclinical diffuse involvement of anterior horn cells was supported by evidence of mild muscle fiber type grouping in the unaffected limb (Uncini et al., 1992). Sural nerve biopsy did not show any abnormality (Gourie-Devi et al., 1984a; Kim et al., 1994).

11.8.3. Electrophysiology

11.8.3.1. Electromyography

Needle electromyography shows fibrillations or positive sharp waves, long duration, large amplitude polyphasic potentials with poor recruitment indicating both active denervation and chronic reinnervation, respectively, in the atrophic muscles of the affected limb in MMA of upper or lower limbs (Hirayama et al., 1963; Sobue et al., 1978; Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Serratrice et al., 1987; Peiris et al., 1989; Kao et al., 1993a; Kim et al., 1994; Khandelwal et al., 2004; Misra et al., 2005). Active denervation, a consistent feature in the majority of cases, irrespective of the duration of illness ranging from few months to 5 or more years, was not seen in the patients who had attained a stationary course after an initial phase of progression (Kao et al., 1993c; Misra and Kalita, 1995; Gourie-Devi and Nalini, 2003). Rarely fibrillations or positive sharp waves have been observed in a clinically stationary phase of many years, suggesting a subclinical progression (Kao et al., 1993c).

In the clinically unaffected muscles of the involved limb chronic reinnervative changes have been reported in 25 to 50% of patients with amyotrophy of upper limb (Gourie-Devi et al., 1984a; De Visser et al., 1988; Hirayama, 2000a), however no abnormalities have been reported by other authors (Virmani and Mohan, 1985; Kim et al., 1994; Misra et al., 2005). It is important to note that the relatively well preserved brachioradialis muscle usually does not show any EMG abnormalities (Hirayama et al., 1963; Gourie-Devi et al., 1984a; Misra and Kalita, 1995), with few exceptions (Sobue et al., 1978). In the contralateral unaffected upper limb, the homologous muscles show denervation and chronic reinnervation in 7–88% of patients (Hirayama et al., 1963; Hashimoto et al., 1976; Sobue et al., 1978; Singh et al., 1980; Gourie-Devi et al., 1984a; De Visser et al., 1988; Gourie-Devi and Nalini, 2003; Khandelwal et al., 2004; Misra et al., 2005) but were found to be normal by some authors (Virmani and Mohan, 1985). In the lower limbs which are clinically never affected, EMG abnormalities have not been demonstrated in the vast majority of patients (Hirayama et al., 1963; Hashimoto et al., 1976; Singh et al., 1980; Sobue et al., 1978; Gourie-Devi et al., 1984a; Willeit et al., 2001; Gourie-Devi and Nalini, 2003) with rare exceptions of mild chronic denervation (De Freitas and Nascimento, 2000).

In MMA of lower limb, denervation and chronic reinnervation have also been noted in the clinically unaffected muscles of the atrophic limb but very rarely in the contralateral lower limb (Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Riggs et al., 1984; Virmani and Mohan, 1985; Uncini et al., 1992; Munchau and Rosenkranz, 2000; Felice et al., 2003). The upper limbs in this group do not show any abnormalities.

Electromyography did not reveal any evidence of myotonic discharges, particularly in the context of appearance of stiffness of hands on exposure to cold (Gourie-Devi et al., 1984a). Aggravation of weakness of fingers induced by exposure to cold has been attributed to impairment of muscle membrane conduction since high frequency repetitive nerve stimulation showed waning of amplitude of compound muscle action potentials (Kijima et al., 2002). Only in a single case of MMA of upper limb were myokymic discharges observed (De Visser et al., 1988).

Lower cervical paraspinal muscles (C8-T1) involvement on electromyography was not observed in MMA of upper limb, although active denervation and chronic reinnervation could be demonstrated in the muscles of C7-T1 myotomes in the affected upper limbs, independent of the clinical stage of the disease or the duration of illness (Kao et al., 1993c). In contrast, paraspinal muscle involvement, an early and consistent sign demonstrable by EMG in amyotrophic lateral sclerosis (Kuncl et al., 1988), can help in differentiating ALS from monomelic amyotrophy, particularly when the initial feature is single limb involvement (Kao et al., 1993c).

Single fiber EMG done in a few patients showed increased fiber density and jitter with occasional blocking in the affected limb, indicating unstable neuromuscular transmission due to new regeneration (Thijsse and Spaans, 1983). During the stage of stabilization of the disease, there is further increase of fiber density, but jitter decreases suggesting maturation of reinnervation (Hirayama, 2000a).

11.8.3.2. Nerve conduction

Motor conduction studies are usually normal in patients with mild to moderate atrophy of muscles (Hirayama et al., 1963; Sobue et al., 1978; Singh et al., 1980; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; De Visser et al., 1988; Peiris et al., 1989). Slight slowing of motor conduction velocity may be observed consistent with loss of fast conducting axons and the compound muscle action potential amplitude is reduced (Kim et al., 1994) and occasionally motor distal latency may be prolonged (Tan, 1985). Conduction block has not been demonstrated in amyotrophy of upper or lower limb (Kim et al., 1994; Misra and Kalita, 1995; Gourie-Devi and Nalini, 2001; Willeit et al., 2001; Khandelwal et al., 2004). Sensory conduction studies are normal in all patients.

F-wave latency and H-reflex are within normal limits (Uncini et al., 1992; Kao et al., 1993c; Misra and Kalita, 1995; Willeit et al., 2001) with few exceptions of slight increase in latency and low persistence of F-wave (Kuwabara et al., 1999).

11.8.3.3. Evoked potentials

Somatosensory evoked potentials (SEP) from upper and lower limbs are normal in amplitude and latency (Kao et al., 1993c; Pradhan and Gupta, 1997; Willeit et al., 2001). Conflicting results show decrease of amplitude of Erb's point potentials and N13 spinal responses but with normal latencies and normal N20 potential (Polo et al., 2003). There was no correlation of these abnormalities with the clinical features. However, SEPS were found to be normal following tibial nerve stimulation.

11.8.3.4. Central motor conduction

Central motor conduction time (CMCT) determined by electrical stimulation of cortex or by transcranial magnetic stimulation was normal in all patients, providing evidence that in MMA upper motor neuron is not involved (Misra and Kalita, 1995; Khandelwal et al., 2004). Contrary to these findings, slight but significant prolongation of CMCT has been observed in some patients (Polo et al., 2003). Cortical threshold intensity (TI) which reflects a balance of cortical and spinal excitability was also found to be normal (Khandelwal et al., 2004). In motor neuron disease the CMCT and TI have been found to be abnormal confirming upper motor neuron involvement (Triggs et al., 1999), while in MMA there is no evidence of pyramidal tract dysfunction. The absence of upper motor neuron involvement in MMA has also been substantiated by normal H/M ratio, vibratory inhibition and reciprocal inhibition of soleus H reflex (Misra and Kalita, 1995).

11.8.3.5. Dynamic electrophysiology

Dynamic electrophysiological studies showed increased latency and decreased amplitude of motor evoked potentials after transcranial magnetic stimulation, decrease in F-wave persistence and decrease of amplitude of N13 somatosensory evoked potential during neck flexion (Shizukawa et al., 1994; Kuwabara et al., 1999; Restuccia et al., 2003).

11.8.4. Autonomic function tests and sympathetic skin response

Increased sweating of hands and cyanosis of fingers have been observed in nearly 50% of patients with MMA of upper limb (Hirayama et al., 1963; Gourie-Devi et al., 1984a). Decreased skin temperature in distal portion of upper limb, plethysmographic abnormalities indicative of vasomotor dysfunction and confirmation of hyperhidrosis by sweat tests have been documented (Hirayama, 1991).

A recent study of sympathetic skin response (SSR) in MMA showed that SSR latency in the affected upper

limb was significantly prolonged compared to controls confirming the involvement of sympathetic nervous system (Gourie-Devi and Nalini, 2001). Interestingly, increase in latency was seen in the contralateral unaffected upper limb but not in lower limbs. The abnormalities of SSR did not strictly correlate with clinical symptoms of autonomic dysfunction in the atrophic limb. Prolonged SSR latency may indicate subclinical involvement of sympathetic nervous system in unaffected upper limb (Shahani et al., 1984). These observations coupled with the pathological finding of decrease in number of nerve cells in the inferior cervical sympathetic ganglion, suggest lesion in the efferent sympathetic pathway at this level (Hirayama et al., 1987; Gourie-Devi and Nalini, 2001).

11.8.5. Imaging

11.8.5.1. Imaging of muscles

CT and MRI of muscles in monomelic amyotrophy provide valuable information about the selectivity of muscle affected (Fig. 11.9), delineate the sequence of muscle involvement and enable correlation with disease duration. Imaging can disclose affected deep muscles of thigh and leg, particularly in early stages or with mild changes, when clinical and electrophysiological examination fails to detect the involvement. In the distal form of MMA of lower limb, gastrocnemius followed by soleus are involved and in later stages muscles of anterior compartment, particularly tibialis anterior are affected (Hamano et al., 1999). In the thigh, quadriceps, semimembranosus, semitendinosus and biceps femoris are sequentially involved. (De Visser et al., 1988; Di Muzio et al., 1994; Munchau and Rosenkranz, 2000).

Involvement of periphery of muscles, selective and bilateral symmetric pattern without significant atrophy of muscles, the distinctive features of myopathy, distinguish it from neurogenic disorder (Bulcke et al., 1979; De Visser and Verbeeten, 1985; Schwartz et al., 1988; Termote et al., 1980). Early stages of ALS with evidence of involvement of a single limb may be differentiated from MMA by the demonstration of selective muscle atrophy on imaging in the latter disorder (Di Muzio et al., 1994).

11.8.5.2. Imaging of spinal cord

In MMA of upper limb earlier studies had reported straight neck due to obliteration of cervical lordosis on radiographs (Hashimoto et al., 1976) and, recently, CT myelography and MRI have demonstrated focal cord atrophy (Fig. 11.10) at lower cervical level with maximal changes at C5–C6 level, corresponding to segmental distribution of weakness (Matsumura et al., 1984; Mukai et al., 1985; Metcalf et al., 1987; Biondi et al.,

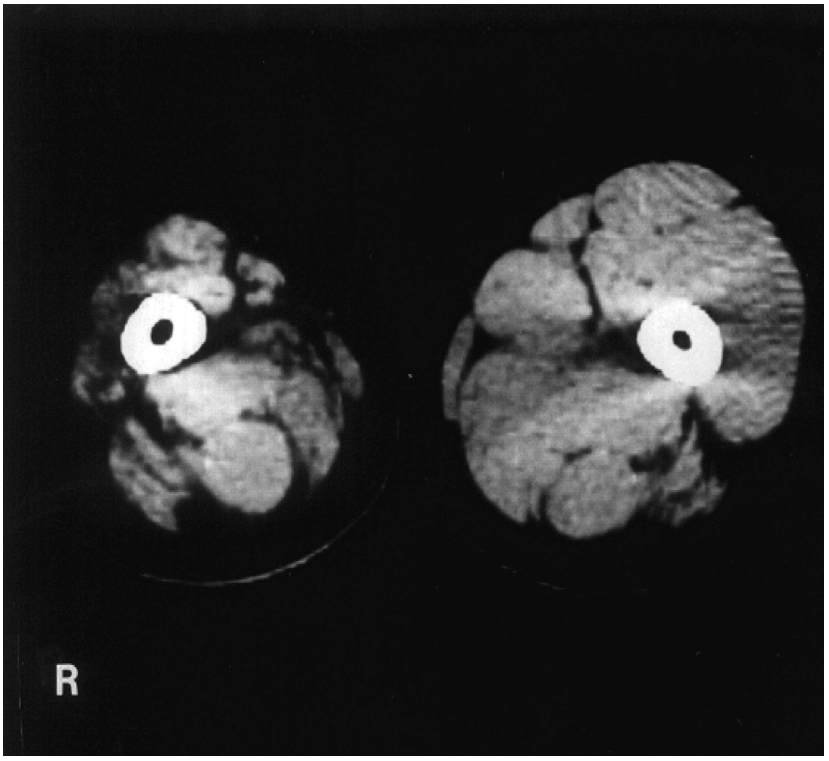

Fig. 11.9. CT of (**A**) right thigh shows severe atrophy of vastus lateralis, vastus medialis, biceps femoris with mild atrophy of all other muscles and (**B**) left thigh is normal.

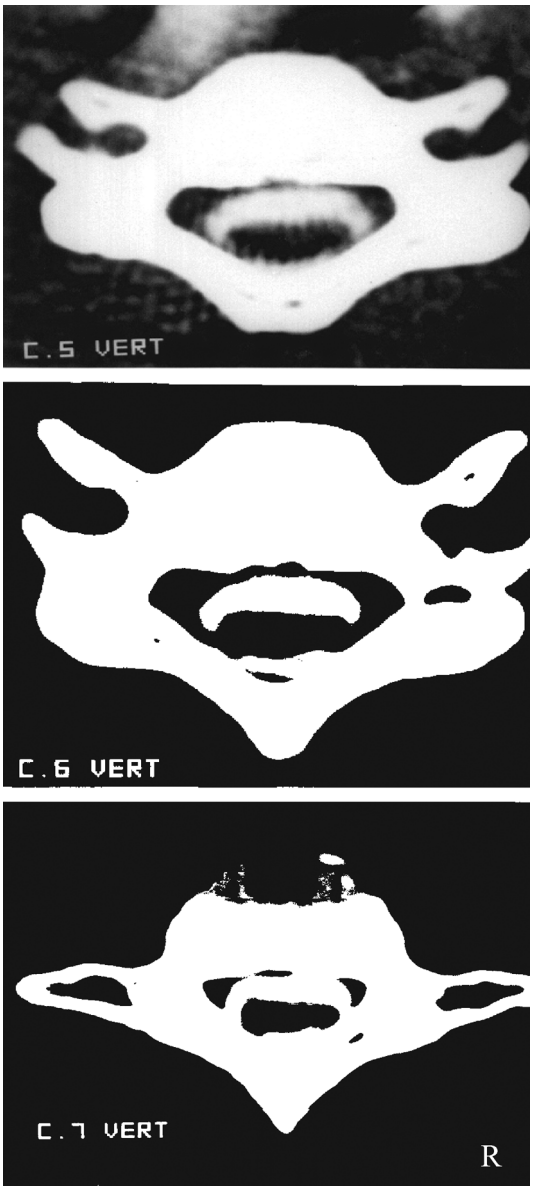

Fig. 11.10. CT myelography shows cord atrophy at C5 to C7 levels with more severe changes on right side, ipsilateral to the atrophic limb.

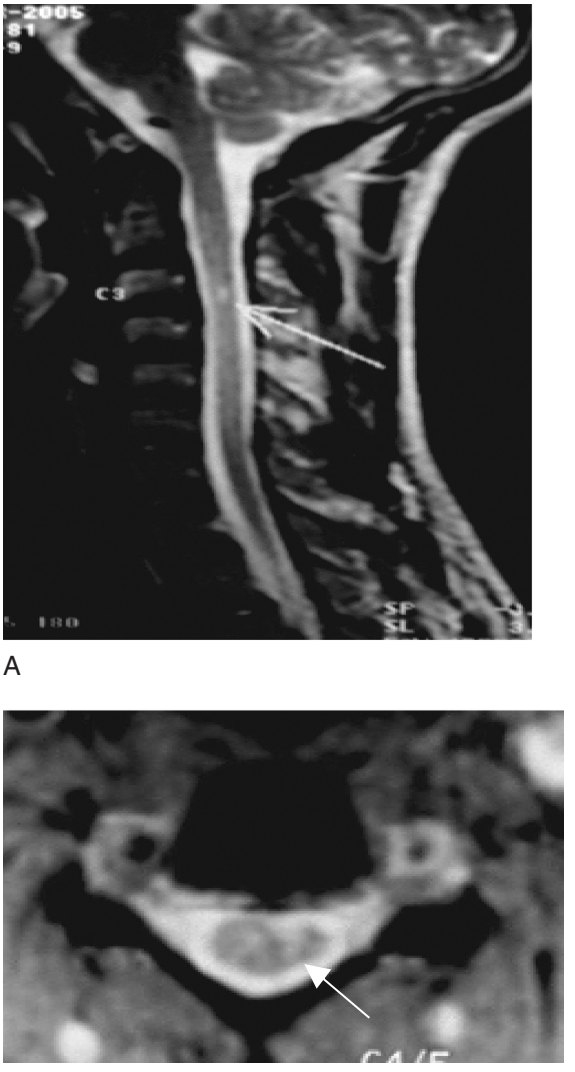

Fig. 11.11. T2-weighted image shows hyperintense signal in spinal cord, (**A**) extending from C3 to C7 and (**B**) localized to anterior and lateral horns of gray matter.

1989; Gourie-Devi et al., 1992; Kao et al., 1993a; Pradhan and Gupta, 1997; Misra et al., 2005). The atrophy was more marked on the side of the affected limb in patients with atrophy and weakness restricted to one upper limb while EMG changes were bilateral, but were more severe on the affected side. In others, focal and unilateral atrophy of the lower cervical cord limited to the anterior horn region has been reported (Biondi et al., 1989; Gourie-Devi et al., 1992). High intensity signals localized to the anterior and lateral horns of the gray matter on T2 weighted images (Fig. 11.11) have been reported (Pradhan and Gupta, 1997; Chan et al., 1998; Schroder et al., 1999; Willeit et al., 2001).

In MMA of lower limb, however, atrophy of lower thoracic or lumbar cord was not observed and there was no evidence of lumbar canal stenosis (Gourie-Devi et al., 1992; Kim et al., 1994).

Rarely syringomyelia may present with only atrophy and weakness of hand muscles without sensory changes (Mukai et al., 1984). Therefore in MMA delayed scans on CT myelography or MRI is mandatory to exclude cavity (Gourie-Devi et al., 1992).

11.8.5.3. Dynamic imaging of spinal cord

Forward displacement of cervical dorsal sac and spinal cord along with flattening of lower cervical cord has been demonstrated with dynamic conventional myelography,

CT myelography and MRI, during neck flexion (Mukai et al., 1985; Iwasaki et al., 1987; Toma and Shiozawa, 1995; Pradhan and Gupta, 1997; Hirayama and Tokumaru, 2000). The posterior dura mater also moved forward obliterating subarachnoid space leaving a large posterior epidural space with prominent epidural venous plexus. In normal subjects and in ALS the cord moved forward with slight flattening of cervical cord, but there was no displacement of the posterior dura mater (Pradhan and Gupta, 1997). It has been shown that cervical dorsal roots are short and asymmetric in patients while they are slack in normal subjects (Toma and Shiozawa,1995). It is postulated that the growth of cervical roots does not keep pace with growth spurts in adolescence. This fact may be responsible for overstretching and forward displacement of cord (Pradhan and Gupta, 1997; Toma and Shiozawa, 1995; Hirayama and Tokumaru, 2000). Interestingly, a recent report provides evidence that the cervical spinal cord was stretched even in the neutral position in patients due to a disproportion between cervical spine and shorter cervical spinal cord (Kohno et al., 1998). Contrary to these observations, dynamic imaging in neutral and maximum flexion of neck in the patients, significant compression of cervical spinal cord, forward displacement of dural space and prominent epidural veins were not observed (Schroder et al., 1999; De Freitas and Nascimento, 2000; Willeit et al., 2001). The posterior subarachnoid space and epidural space were normal. All these findings were similar to the observations in healthy control subjects.

11.9. Diagnosis

Insidious onset of atrophy and weakness restricted to a single limb in the second or third decade, male preponderance, absence of sensory and upper motor neuron signs, slow progression for 2 to 5 years followed by stabilization, are all distinctive clinical features of monomelic amyotrophy. Extrapyramidal, cerebellar and cognitive functions are preserved. Normal CPK levels, electromyographic features of neurogenic pattern, normal nerve conduction studies and absence of conduction block provide confirmation of localization of lesion to anterior horn cells. Imaging of spinal cord to exclude mass lesions, syringomyelia and vascular lesions is mandatory. An algorithm (Fig. 11.12) provides a practical approach to diagnosis of monomelic amyotrophy.

11.10. Differential diagnosis

Before considering the diagnosis of MMA of upper limb, a number of disorders which "mimic" this disease (Table 11.5) have to be excluded by appropriate and relevant investigations. The presence of sensory involvement, upper motor signs and imaging findings provide evidence for structural lesions of spinal cord. In rare instances, sensory deficit may be absent in syringomyelia with only lower motor neuron signs in one or both upper limbs, making it mandatory to do imaging of spinal cord in MMA (Mukai et al., 1984). Cauda equina lesions can also be excluded by imaging studies.

Spinal muscular atrophy, especially the distal form, characteristically is bilateral with symmetric involvement of upper or lower limbs, slowly progressive course and positive family history in many cases with autosomal recessive or dominant inheritance (McLeod and Prineas, 1971; O'Sullivan and McLeod, 1978; Harding and Thomas, 1980). In some patients, in the early stages, distal SMA may be unilateral resembling MMA (O'Sullivan and McLeod, 1978; Harding et al., 1983; Peiris et al., 1989). In juvenile spinal muscular atrophy (Kugelberg–Welander disease), the proximal limb muscles are affected and the atrophy and weakness are bilateral. In chronic neurogenic quadriceps amyotrophy, considered a forme fruste of Kugelberg–Welander disease, the atrophy of quadriceps muscles is bilateral with occasional involvement of pelvic girdle and EMG shows generalized involvement of unaffected muscles of upper and lower limbs (Furukawa et al., 1977; Tetsuo et al., 1977).

Early stage of ALS with single limb involvement can be misdiagnosed as monomelic amyotrophy. Selective involvement of muscles in MMA demonstrable on imaging may be useful in distinguishing the two disorders (Di Muzio et al., 1994). Spread to other limbs usually within 3 years, the presence of pyramidal signs and inexorable progression to develop bulbar palsy characterize ALS.

The age at onset in Madras motor neuron disease (MMND) described from India is similar to MMA, however high incidence of cranial nerve palsies (facial, bulbar and tongue muscles), sensorineural deafness, bilateral atrophy of the limbs and pyramidal signs have been described in MMND (Meenakshisundaram et al., 1970; Jagannathan and Kumaresan, 1987; Gourie-Devi and Suresh, 1988). In this context a single case report from Italy, of a young man from South India with MMA, after a stationary phase of 11 years, developed fresh neurological features, suggestive of Madras MND, is of interest (Massa et al., 1998).

The criteria for "late progression of poliomyelitis" suggested by Mulder et al. (1972) are (a) a credible history of poliomyelitis, (b) partial recovery of function, (c) a minimum 10-year period of stabilization of this recovery from acute poliomyelitis, and (d) the subsequent development of progressive muscle weakness. New weakness or atrophy can involve either the

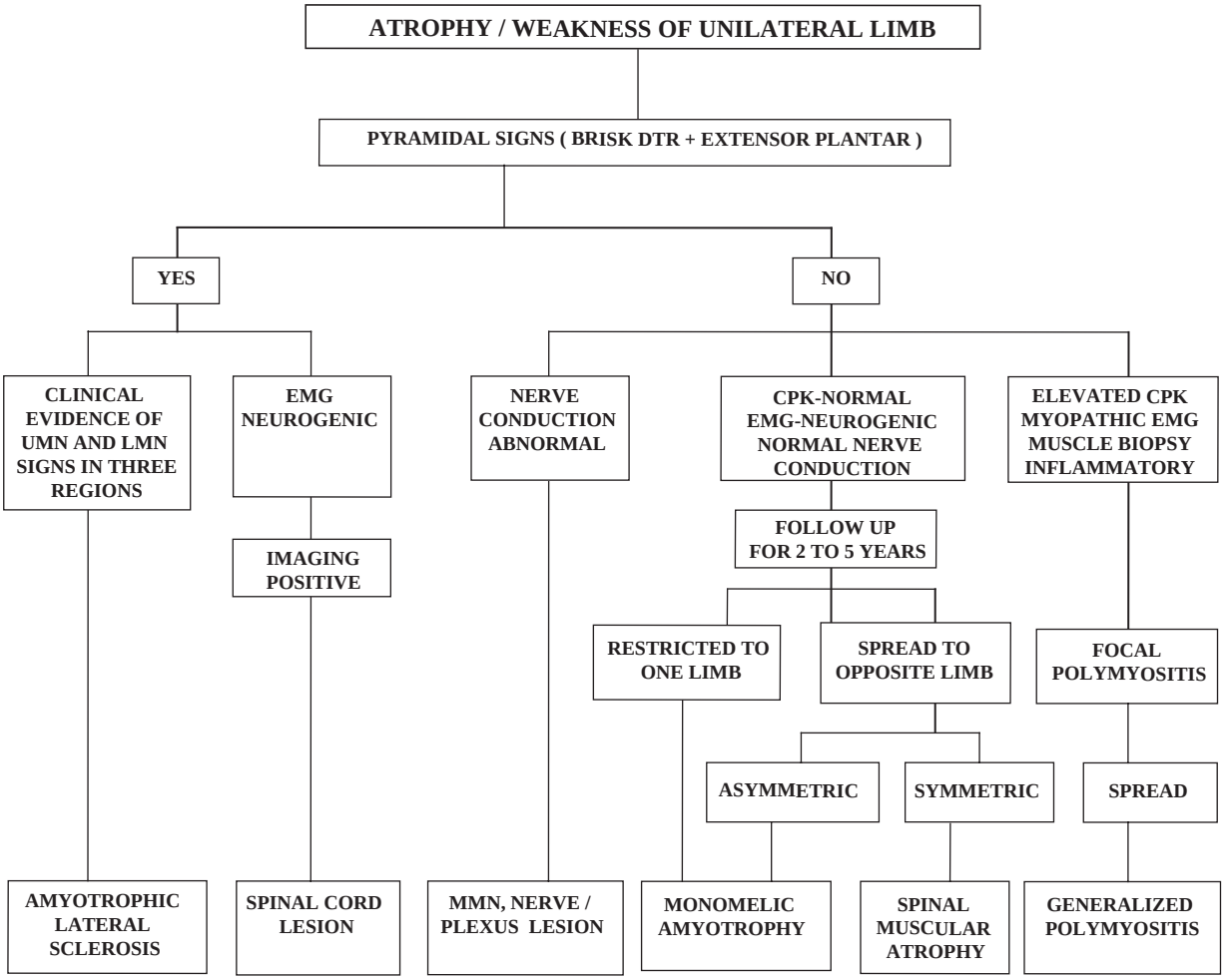

Fig. 11.12. Algorithm for approach to a patient with single limb atrophy.

previously affected or unaffected muscles (Dalakas et al., 1986; Gourie-Devi, 1996, 2001). History of poliomyelitis in childhood has not been documented in large series of patients of MMA (Hirayama et al., 1963; Sobue et al., 1978; Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Serratrice et al., 1987) or shortening of limb, a common feature in postpoliomyelitis progressive muscular atrophy, has not been reported (Gourie-Devi, 1996).

Radiculopathy, plexopathy (thoracic outlet syndrome) entrapment neuropathy, multifocal motor neuropathy, focal demyelinating neuropathy (Thomas et al., 1996) and mononeuropathy multiplex can manifest as focal atrophy of one limb. Electromyography and nerve conduction studies provide confirmation of diagnosis. Special mention needs to be made of multifocal motor neuropathy (MMN) with the characteristic features of pattern of muscle involvement in peripheral nerve distribution, association with GM1 antibodies in 50–80% of patients and conduction block of one or more nerves in proximal or distal segments (Parry and Clark, 1988; Pestronk et al., 1990; Visser et al., 2002). In recent years multifocal motor neuropathy with evidence of demyelination but without conduction block and multifocal motor axonopathy without conduction block have been recognized as distinct forms and potentially treatable disorders, which need to be distinguished from MMA (Katz et al., 1997, 2002; Pakiam and Parry, 1998).

Rare cases of focal inflammatory polymyositis, fascioscapulohumeral dystrophy and distal muscular dystrophy can be differentiated by elevated CPK, EMG and muscle histopathologic findings (Lederman et al., 1984; Serratrice, 1991; Takemitsu et al., 1993; Uncini et al., 2002). Congenital hypoplasia of one limb in which all tissues are affected and congenital unilateral absence of pectoralis muscle (Poland's syndrome) have to be differentiated from MMA (Gourie-Devi and Mehta, 1981; Serratrice, 1991).

Table 11.5

Disorders which mimic monomelic amyotrophy

Spinal cord lesions
Syringomyelia
Intramedullary tumors
Cervical disc prolapse
Arteriovenous malformation
Compressive lesions
Cauda equina lesion
Anterior horn cell disorders/motor neuron disease
Distal spinal muscular atrophy
Amyotrophic lateral sclerosis
Madras motor neuron disease
Postpolio progressive muscular atrophy
Radiculopathy
Plexopathy – Brachial, Lumbar
Neuropathy
Entrapment neuropathy
Multifocal motor neuropathy
Focal demyelinating neuropathy
Muscle disorders
Focal inflammatory myopathy
Fascioscapulo humeral dystrophy
Distal muscular dystrophy

11.11. Course and prognosis

Following an insidious onset or an accidental observation of atrophy and weakness of one limb, there is usually a slow progression over a period of 2 to 5 years followed by stabilization (Hashimoto et al., 1976; Sobue et al., 1978; Singh et al., 1980; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Serratrice et al., 1987). In a few patients the period of progression may be beyond 5 years (Kao et al., 1993a; Gourie-Devi and Nalini, 2003). The indicators of progression of the disease were worsening atrophy and weakness of the initially affected muscles, or involvement of new muscles in the affected limb or spread to the contralateral limb. These observations were essentially based on cross-sectional studies and very few long term prospective studies have been reported (Peiris et al., 1989; Barontini et al., 1991; Liu and Specht, 1993; Massa et al., 1998; Rowin et al., 2001; Gourie-Devi and Nalini, 2003). Peiris et al. (1989) and Gourie-Devi and Nalini (2003), in a large series of patients with long term follow-up of clinical status and EMG, observed that there was clinical arrest of the disease within 5 years in 75% to 80%. In 5–7%, the disease had progressed up to 8 years, followed by a stationary phase (Gourie-Devi and Nalini, 2003). Slight atrophy and tremors of the contralateral upper limb was present in 16% (seven of 44 patients) at presentation and during the follow-up period another 2% (one patient) developed the disease in the opposite limb (Gourie-Devi and Nalini, 2003). These authors also reported that in 44 patients during a mean follow-up period of 9.7 years (range 2.5 to 23 years), after stabilization of the disease there was no evidence of late progression with appearance of new symptoms in the affected upper limb, the contralateral upper limb and there was no spread to involve the lower limbs. There are, however, isolated case reports of involvement of contralateral upper limb after a quiescent period ranging from 10 to 40 years (Hirayama et al., 1987; Serratrice, 1991). Late clinical progression to involve one or both lower limbs is indeed an extremely rare occurrence and has been reported only in five patients (Thijsse and Spaans, 1983; Liu and Specht, 1993; Massa et al., 1998; Rowin et al., 2001). In many large series of patients, however, involvement of lower limbs in MMA of upper limb or involvement of upper limbs in MMA of lower limbs have not been documented (Hirayama et al., 1963; Sobue et al., 1978; Singh et al., 1980; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Peiris et al., 1989; De Freitas and Nascimento, 2000). It is also reassuring that MMA, in general, and specifically patients with brisk reflexes, did not evolve to ALS during a long follow-up mean period of 12.9 years (range 8.6 to 20) and a mean duration of illness of 14.9 years (range 6 to 22) (Gourie-Devi and Nalini, 2003). There have been no deaths due to the disease and, in the two autopsy cases, the cause of death was due to unrelated disorders (Hirayama et al., 1987; Araki et al., 1989).

11.11.1. Disability and quality of life

Adequate attention has not been focused on the problem of disability experienced by the patients and the consequent impact on quality of life. Difficulty in writing, feeding, dressing due to atrophy and weakness of intrinsic hand muscles were further aggravated by tremors and on exposure to cold.

The disability was graded as mild in 68%, moderate in 23%, severe in 4% and there was no disability in 5% (Gourie-Devi and Nalini, 2003). Few patients with significant disability were compelled to transfer activities from atrophic limb to unaffected side. In the MMA of lower limb, except for mild difficulty in walking and running, there was no significant disability (Gourie-Devi et al., 1984a).

11.12. Pathology

The earliest pathological description of spinal cord in elderly patients above 70 years of age with clinical features resembling MMA is by Marie and Foix (1912).

Softening of anterior horn of spinal cord corresponding to the side of the involved limb led to the nomenclature of tephromalacia (Tephra = ashes). The posterior horn and white matter were well preserved. The ischemic changes were attributed to syphilitic arteritis or arteriosclerosis with occlusion of spinal arteries. More recently two cases with clinical and autopsy findings have been reported from Japan (Hirayama et al., 1987; Araki et al., 1989). The changes in spinal cord were seen essentially at levels of C7–C8 with extension to C5 to T1. Atrophy of spinal cord at C7–C8 levels, thinning of C7 to T1 anterior roots, marked shrinkage of anterior horns, decrease of large and small nerve cells, chromatolysis, lipofuscin accumulation, occasional basophilic inclusions in the remaining neurons and mild astrogliosis were the salient observations. There was no evidence of vascular or inflammatory changes. Loss of myelinated fibers in the anterior roots and decrease in number of nerve cells in cervical sympathetic ganglia were the other significant findings. The posterior horn and posterior roots were normal. Based on the pathological findings, circulatory insufficiency leading to focal cervical ischemic poliomyelopathy has been suggested (Hirayama et al., 1987; Hirayama, 2000b).

11.13. Etiopathogenesis

In the etiopathogenesis, various hypotheses have been considered, but the precise mechanism underlying this disorder remains uncertain. Latent infection with viruses having a selective propensity to induce damage of anterior horn cells like poliomyelitis and other enteroviruses, which may remain dormant with reactivation appears to be an attractive hypothesis, but there is no evidence to support this contention, since antibodies to these viruses have not been found in serum and cerebrospinal fluid (Sobue et al., 1978; Virmani and Mohan, 1985; Kao et al., 1993b). Since the earlier studies were based on detection of neutralizing antibodies, there is a case for re-examining this concept using recent technique of reverse transcriptase-PCR to detect enteroviral sequences in CSF samples (Julien et al., 1999). Since the criteria defined by Mulder et al. (1972) for 'late progression of poliomyelitis' (dealt in the earlier section) are not satisfied, MMA stands out quite distinct from postpoliomyelitis progressive muscular atrophy (Prabhakar et al., 1981; Gourie-Devi et al., 1984a; Virmani and Mohan, 1985; Uncini et al., 1992; Gourie-Devi, 1996; De Freitas and Nascimento, 2000). It has been suggested that mechanical injury and heavy physical activity in occupation and sports, which are associated risk factors, may cause progressive loss of anterior horn cells due to vascular lesion of spinal cord segments corresponding to the limb used (Hirayama et al., 1963; Prabhakar et al., 1981; Gourie-Devi et al., 1993; Saha et al., 1997). Similarly predominance of right hand involvement has also been attributed to excessive use of the limb used (Hirayama, 1972; Hashimoto et al., 1976). If an injury mechanism is responsible for focal anterior horn cell lesion, the damage would not be confined only to anterior horn but should also involve sensory pathways, which are spared in MMA (Polo et al., 2003).

Imaging studies showing focal atrophy and stretching of cervical cord with forward displacement of dural sac during flexion of neck resulting in traction, compression and vascular insufficiency in the anterior spinal artery territory leading to "flexion myelopathy" has been considered in the pathogenesis (Mukai et al., 1987; Pradhan and Gupta, 1997; Hirayama and Tokumaru, 2000), further supported by autopsy findings suggestive of ischemic myelopathy (Hirayama et al., 1987; Hirayama, 2000b). A careful appraisal of pathological findings reveals that there is no convincing evidence of ischemia or vascular abnormality (Misra and Kalita, 1995; Robberect et al., 1997). Failure to consistently demonstrate forward displacement of dural sac, selective focal atrophy of one limb and the absence of sensory deficit and upper motor neuron signs, do not support the hypothesis of ischemic myelopathy (Misra and Kalita, 1995; Schroder et al., 1999; De Freitas and Nascimento, 2000; Willeit et al., 2001). Vascular insufficiency or direct compression of spinal cord does not appear to be a likely possibility in the pathogenesis also of MMA of lower limb, since the pattern of muscle atrophy does not conform to vascular territory or somatotopic representation of muscles in the ventral gray matter of lumbar spinal cord (Sharrard, 1955; Munchau and Rosenkranz, 2000) and imaging does not show spinal cord atrophy (Gourie-Devi et al., 1992; De Freitas and Nascimento, 2000; Felice et al., 2003).

Monomelic amyotrophy has been considered as a variant of spinal muscular atrophy that remains focal for many years (Pearce and Harriman, 1966; McLeod and Prineas, 1971; Riggs et al., 1984; De Visser et al., 1991). Absence of deletion of exons 7 and 8 of spinal motor neuron gene suggests that MMA is genetically a separate entity from spinal muscular atrophy (Di Guglielmo et al., 1996; Misra et al., 2005). Further, abnormality of SOD1 gene found in familial ALS was not detected in this order (Robberecht et al., 1997). In view of the male preponderance, X linked inheritance has been suggested, which needs further investigation (Misra et al., 2005). Ethnic predisposition to development of disease is suggested by predominance of cases reported from Asian countries, particularly Japan and India, possibly implicating a shared environment (Tan, 1985).

The relationship with motor neuron disease has been discussed and MMA has been considered as a focal and

benign form of motor neuron disease (Gourie-Devi et al., 1984a; Riggs et al., 1984; Rowland, 1998). Loss of motor neurons with gliosis, accumulation of lipofuscin and basophilic inclusions without overt signs of ischemia reported by Hirayama et al. (1987) while refuting the vascular hypothesis suggests an intrinsic motor neuron disease (Robberecht et al., 1997; Schroder et al., 1999).

11.14. Treatment

Coarse tremors of the hands interfering with fine activities leading to considerable disability can be partially ameliorated by propranolol. Based on the hypothesis of flexion myelopathy, cervical collar has been recommended (Hirayama, 2000a). Follow up studies of 26 patients showed that the duration of progression was significantly less compared to control patients. Duraplasty in combination with posterior spinal fusion or anterior stabilization of lower cervical vertebrae, in a few patients, has shown promising results (Konno et al., 1997; Hirayama, 2000a). Since MMA is a self-limiting disease with spontaneous arrest, the results of use of cervical collar and surgery should be validated in a larger series of patients. Since the precise pathogenesis of MMA remains unresolved and dynamic imaging had not uniformly demonstrated displacement of spinal cord in all patients, forceful arguments against surgery in a benign, self-limiting disease have been put forth by Schroder et al. (1999) and Willeit et al. (2001).

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Chapter 12

Multifocal and other motor neuropathies

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12.1. Introduction

Acquired motor neuropathies that mimic motor neuron disease are multifocal motor neuropathy (MMN) and, to a lesser extent, the pure motor form of chronic inflammatory demyelinating polyneuropathy (CIDP). As both disorders are potentially treatable neuropathies, the differentiation from motor neuron disease is important. MMN and pure motor CIDP most likely share important features in their (immuno)pathogenesis as both disorders have common clinical and electrophysiological features of a motor neuropathy without sensory abnormalities, motor conduction block on electrophysiological examination, unresponsiveness to steroids and, at times, a good response to intravenous immune globulin (IVIG) therapy. In this chapter we discuss the extensive literature on MMN reported in recent times much of which is also applicable to pure motor CIDP.

12.2. Multifocal motor neuropathy

12.2.1. Clinical diagnostic features

MMN is characterized by slowly progressive, asymmetric weakness initially without muscle atrophy of limbs that develops gradually or in a stepwise manner over several years (Parry and Clarke, 1988; Pestronk et al., 1989; Krarup et al., 1990; Biessels et al., 1997; Taylor et al., 2000; Nobile-Orazio, 2001; Van Asseldonk, et al., 2003, 2005a). Men are more frequently affected than women (approximate ratio of 2.6:1). The mean age at onset is 40 years with a range of 20–70 years, which is different from CIDP that also occurs in children and in the elderly (Chaudhry, 1998; Taylor et al., 2000; Van den Berg-Vos et al., 2002a). Almost 80% of patients present their first symptoms between 20 and 50 years. The most common initial symptoms are wrist drop, grip weakness or foot drop. Weakness develops asymmetrically and is more prominent in the arms than in the legs (Taylor et al., 2000; Van Asseldonk et al., 2003). In the majority of patients with onset in the legs, the arms also become affected at a later stage and will eventually predominate (Van den Berg-Vos et al., 2002b). Symptoms and signs of distal muscles prevail for a long time, but eventually weakness in proximal muscle groups of arms, but not of legs, may develop (Van den Berg-Vos et al., 2002a,b). Weakness is often more pronounced than the degree of atrophy suggests (Taylor et al., 2000; Nobile-Orazio, 2001). This is a characteristic component of a conduction block. Nevertheless, atrophy of affected muscles may be substantial in patients with a long duration of disease. Other motor symptoms include muscle cramps and fasciculations in about two-thirds of the patients (Roth et al., 1986; Bouche et al., 1995). Myokymia has been reported occasionally (Roth et al., 1986; Bouche et al., 1995; Le Forestier et al., 1997). Tendon reflexes are usually reduced in affected regions, but may be brisk in the arms (Parry and Clarke, 1988; Pestronk et al., 1988; Krarup et al., 1990; Le Forestier et al., 1997; Taylor et al., 2000). Single cases of cranial nerve involvement have been reported (Kaji et al., 1992; Magistris and Roth, 1992; Le Forestier et al., 1997; Pringle et al., 1997). Respiratory failure due to unilateral or bilateral phrenic nerve palsy may occur, even at the beginning of the disease, but this is very rare (Magistris and Roth, 1992; Cavaletti et al., 1998; Beydoun and Copeland, 2000). Subjective feeling of paresthesia or some numbness may be present in some patients, but objective sensory loss on neurological or neurophysiological examination is absent.

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The differential diagnosis of MMN includes motor neuron disease (Parry and Clarke, 1988; Pestronk et al., 1990; Bentes et al., 1999; Donaghy, 1999; Ellis et al., 1999; Molinuevo et al., 1999; Traynor et al., 2000; Van den Berg-Vos et al., 2003a) on the one hand and demyelinating neuropathies on the other (Hughes, 1994; Leger, 1995; Saperstein et al., 2001). The first signs and symptoms in patients with MMN may be similar to motor neuron disease, and patients may be initially diagnosed as having amyotrophic lateral sclerosis (ALS) or lower motor neuron disease (Traynor et al., 2000; Van den Berg-Vos et al., 2003a). The slowly progressive disease course and the absence of upper motor neuron signs or bulbar signs and the presence of demyelinative features, in particular significant conduction block, on electrodiagnostic examination will eventually differentiate MMN from ALS. However, the differentiation from lower motor neuron disease may be more difficult. In a previous study, we categorized, based on the distribution of weakness, lower motor neuron disease into slowly progressive spinal muscular atrophy, distal spinal muscular atrophy, segmental distal spinal muscular atrophy and segmental proximal spinal muscular atrophy (Van den Berg-Vos et al., 2003a). Clinically, it is difficult to differentiate MMN from slowly progressive spinal muscular atrophy or segmental distal spinal muscular atrophy (Van den Berg-Vos et al., 2003b; Vucic et al., 2004b). The finding of persistent motor nerve conduction block on nerve conduction studies outside nerve compression sites, a positive titer of anti-GM1 antibodies or increased signal intensity on T_2-weighted MR images of the brachial plexus may be helpful to differentiate MMN from lower motor neuron disease (Van den Berg-Vos et al., 2000a).

Within the demyelinating neuropathies it is important to differentiate MMN from CIDP, in particular the pure motor form of CIDP and the multifocal inflammatory demyelinating neuropathy (MIDN) (Barohn et al., 1989; Kornberg and Pestronk, 1995; Leger, 1995; Oh et al., 1997; Lewis, 1999; Mezaki et al., 1999; Saperstein et al., 1999, 2001; Van den Berg-Vos et al., 2000a; Katz and Saperstein, 2001). In CIDP proximal, symmetrical weakness and generalized areflexia are common, whereas weakness in MMN is asymmetrical and distal, and reflexes are only lowered or absent in affected limbs. A remitting and relapsing disease course or a progression of symptoms in weeks is common in CIDP or MIDN but not in MMN. As opposed to CIDP, the cerebrospinal fluid protein in MMN is normal or slightly elevated but rarely higher than 1 g L^{-1} (Van den Berg-Vos et al., 2000a; Nobile-Orazio, 2001; Van Asseldonk et al., 2003). As a consequence, the cerebrospinal fluid protein may help to differentiate CIDP from MMN. Sensory signs and symptoms also differentiate between MMN and CIDP. On nerve conduction studies, motor conduction block is found in both CIDP and MMN, but other features of demyelination such as slowed conduction velocities, prolonged distal motor latencies and prolonged F-waves are prominent in CIDP (Barohn et al., 1989; Van Asseldonk et al., 2003). MIDN has similarities with MMN as well as with CIDP (Oh et al., 1997; Katz and Saperstein, 2001). Patients with MIDN have an asymmetric sensory or sensorimotor demyelinating neuropathy that may remain localized in one arm or leg for several years, sometimes associated with neuropathic pain or focal nerve tenderness. The latter may result in consideration of non-immunological diseases such as tumors or neurofibromatosis and often a long diagnostic delay and late treatment. Nerve conduction studies are necessary to diagnose MIDN and may be helpful to differentiate from MMN as decreased distal SNAP amplitudes are often found in patients with MIDN. Patients with MIDN may benefit from treatment with corticosteroids, whereas patients with MMN or the pure motor form of CIDP do not, or may even deteriorate (Feldman et al., 1991; Kaji et al., 1992; Nobile-Orazio et al., 1993; Donaghy et al., 1994; Le Forestier et al., 1997; Van den Berg et al., 1997). Table 12.1 shows the most important similarities and differences between MMN, lower motor neuron disease, CIDP and MIDN.

12.2.2. Electrodiagnostic features

Conduction block on motor conduction studies is the electrophysiological hallmark of MMN (Fig. 12.1) (Parry and Clarke, 1985; Chaudhry et al., 1994; Bouche et al., 1995; Jaspert et al., 1996; Parry, 1996; Katz et al., 1997; Le Forestier et al., 1997; Taylor et al., 2000; Van Asseldonk et al., 2003). Conduction block is defined as the failure of a nerve impulse to propagate through a structurally intact axon. Conduction block arising in a sufficient number of axons can be detected as a CMAP amplitude or area decrement on proximal versus distal stimulation (P/D), i.e. the CMAP amplitude or area on proximal stimulation of a nerve segment is smaller than that on distal stimulation of that segment (Fig. 12.1). A good definition of conduction block in MMN is "paralysis or paresis of a muscle with the ability to stimulate the motor nerve distal to the block." This is a clinical–physiological definition. Besides conduction block, two other mechanisms may give rise to CMAP decrement P/D (Rhee et al., 1990; Oh et al., 1994). First, with certain differences of conduction times between axons within a nerve (known as temporal dispersion), the positive phase of fast motor unit action potentials (MUAPs) will coincide with the negative phase of slower MUAPs, yielding increased duration of the proximal

Table 12.1

Comparison of general features of MMN, LMND, CIDP and MIDN

	MMN	LMND	Pure motor CIDP	MIDN
Symptoms				
Distribution	Asymmetric	Asymmetric	Symmetric	Asymmetric
Arms > legs	Yes	Yes	No	Yes
Prominent sensory symptoms	No	No	No	Yes
Reflex pattern	Decreased in aff. regions	Decreased in aff. regions	Generalized areflexia	Decreased in aff. regions
Disease course	Slowly progressive	Slowly progressive	Progressive or relapsing	Progressive or relapsing
Laboratory features				
CSF protein > 100 mg dl^{-1}	No	No	Yes	Rare
Anti-GM1 antibodies	30–50% of patients	10% of patients	Rare	No
Abnormal MR-imaging of brachial plexus	Asymmetric*	No	Symmetric	Asymmetric*
Response to treatment				
Intravenous immunoglobulins	Yes	No	Yes	Yes
Corticosteroids	No**	No	Yes	Yes**

MMN = multifocal motor neuropathy; LMND = lower motor neuron disease; CIDP = chronic inflammatory demyelinating polyneuropathy; CSF = cerebrospinal fluid; Aff. = affected. * corresponding with neurological deficit. ** deterioration may occur.

compared to the distal CMAP, phase cancellation and CMAP decrement P/D. Furthermore, polyphasia of the MUAPs that contribute to the CMAP (due to collateral sprouting) has been assumed to yield increased phase cancellation and, consequently, increased CMAP decrement P/D. As the occurrence of temporal dispersion and polyphasic MUAPs can yield CMAP decrement P/D and mimic conduction block in peripheral polyneuropathies and lower motor neuron disease (Sumner, 1991; Lange et al., 1993) criteria are required to separate conduction block from the other mechanisms that may cause CMAP decrement P/D. A simulation study in which CMAPs were reconstructed from MUAPs that were recorded in healthy rats showed that maximal temporal dispersion could result in a CMAP area decrement P/D of up to 50% (Rhee et al., 1990). Simulation studies using human polyphasic MUAPs and realistic temporal dispersion have not been performed. Consequently, a CMAP area decrement P/D of more than 50% is at present the best proof that conduction is actually blocked in one or more axons of a nerve that fulfils these criteria. In most cases of MMN the extent of block is a very large > 80%. This proof is lacking for various other criteria for conduction block which are expert opinions that have been established on the basis of consensus (Albers et al., 1985; Feasby et al., 1985; Oh et al., 1994; Capellari et al., 1997; Van den Berg-Vos et al., 2000b; Olney et al., 2003).

Whether conduction block should be considered a mandatory finding in MMN is an important issue and depends on the criteria for conduction block and the number of investigated nerves. To explore this issue, we reviewed the studies in which patients with a lower motor neuron syndrome were treated with IVIg, whether conduction block was present or not (Table 12.2). In nerves with limited temporal dispersion (< 30%), a criterion consisting of a CMAP (area or amplitude) decrement P/D of at least 50% was fulfilled in none or in a small number of patients who responded positively to IVIg (Katz et al., 1997). This number increased when conduction block criteria allowed more temporal dispersion, required less CMAP decrement P/D or by a combination of both (Van den Berg-Vos et al., 2000b, 2003a). The American Academy of Electrodiagnostic Medicine proposed research criteria that were specified for the degree of temporal dispersion, for nerves and for segments within nerves and revealed conduction block in 60% to 70% of patients with a favorable response to IVIg (Van den Berg-Vos et al., 2000; Nobile Orazio et al., 2002). Criteria less stringent than those proposed by the American Academy of Electrodiagnostic Medicine, requiring a CMAP area decrement P/D of at least 50% in arm or leg nerves or a CMAP amplitude decrement of at least 30% in arm nerves were fulfilled in all patients with a favorable response to IVIg when a large number of arm

	DUR	AMP	AREA	DML MCV
A1 wrist	6.2	6.7	23.3	3.2
A2 elbow d	6.3	6.2	21.9	55
A3 elbow p	6.0	6.4	21.6	55
A4 axilla	4.8	1.4	4.4	23
A5 Erb	4.8	1.9	4.9	58

	DUR	AMP	SCV
A1 wrist	0.8	43.3	56
A2 elbow d	0.8	25.1	61
A3 elbow p	0.9	21.6	77
A4 axilla	1.0	18.1	64

	DUR	AMP	AREA	DML MCV
A1 wrist	5.1	8.0	21.5	4.3
A3 elbow p	10.4	5.0	17.7	54
A4 axilla	12.4	2.6	17.9	49
A5 Erb	14.5	2.5	17.7	51

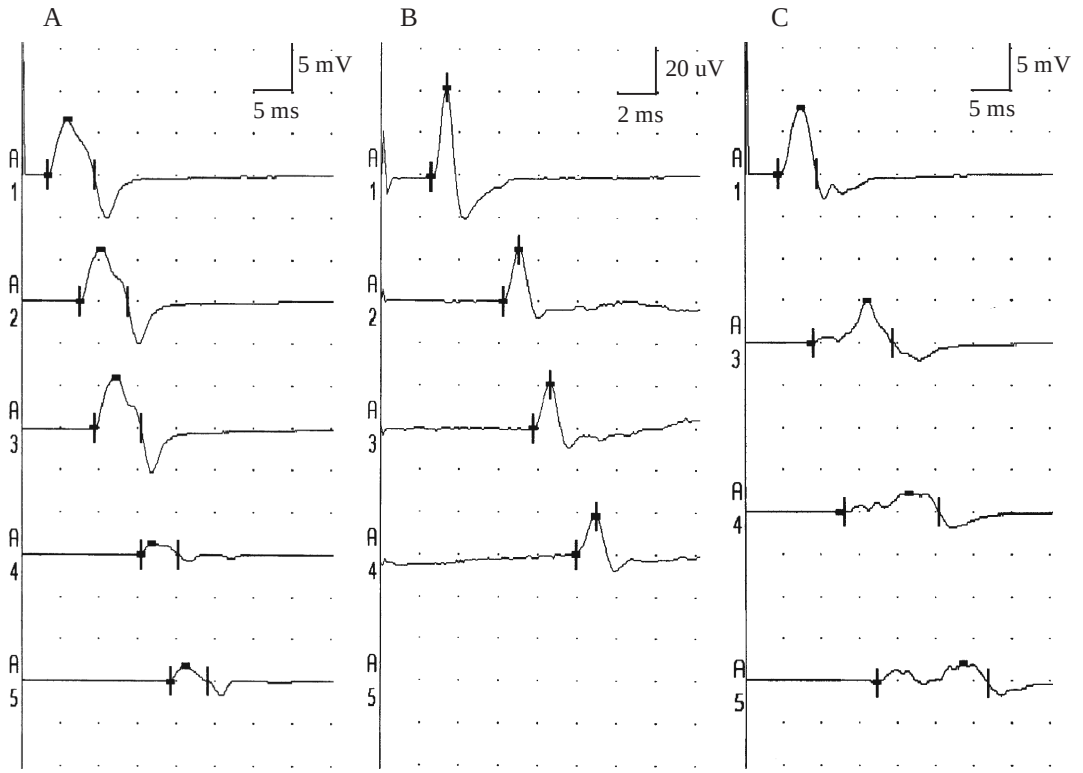

Fig. 12.1. Conduction studies in patients with multifocal motor neuropathy. (**A**) Motor conduction in the ulnar nerve, with recording from the m. abductor digiti V. Definite CB (CMAP area decrement P/D > 50%) and MCV compatible with demyelination were found in the upper arm segment. (**B**) Sensory conduction in the same nerve as under A, with recording from digit V. No abnormalities were found. (**C**) Motor conduction in the median nerve of a different patient as under A and B, with recording from the m. abductor pollicis brevis. Increased temporal dispersion (CMAP duration prolongation P/D > 30%) and probable CB (CMAP amplitude decrement P/D > 30%) were found in the lower arm segment and probable CB was found in the upper arm segment. elbow d = stimulation 5 cm distally from elbow; elbow p = stimulation 5 cm proximally from elbow; DUR = duration in ms; AMP = amplitude in mV or μV; DML = distal motor latency in ms; MCV = motor conduction velocity in ms; SCV = sensory conduction velocity in m s^{-1}. Area in mVms.

and leg nerves, including those innervating non-weakened muscles, were bilaterally investigated (Van den Berg-Vos et al., 2000a; Van Asseldonk et al., 2003). For this reason we prefer criteria for conduction block that require a CMAP area decrement P/D of at least 50% or a CMAP amplitude decrement P/D of at least 30%. These criteria were not fulfilled in all patients with a favorable response to IVIg when a limited number of arm and leg nerves was investigated on one side (Katz et al., 2002). In MMN, conduction block according to these criteria is most likely to be found in long arm nerves innervating weakened muscles. If conduction

Table 12.2

The presence of conduction block in patients with a lower motor neuron syndrome who were treated with IVIg

Reference / number of patients		Standardized protocol		CB criteria		No of patients with CB	Positive response to IVIg in patients	
		Investigated nerves	Bilateral	CMAP decrement P/D	Duration prolongation P/D		with CB	without CB
Katz et al., 1997	16	Med, Uln, Per, Tib	?	Ampl. and Area > 50%	< 30% > 30%	5/16 6/16	2/3 4/4	0/2
Pakiam and Parry, 1998	5	?	?	Ampl. > 50%	< 15%	0/5	—	3/4
Ellis et al., 1999	10	Med, Uln, Per, Tib	−	Ampl. and Area > 50%	< 30%	0/10	—	4/10
Katz et al., 2002*	9	Med, Uln, Rad, Mus, Per, Tib	−	Ampl. or area > 30%	—	0/9	—	3/6
Van den Berg-Vos, 2001**	37	Med, Uln, Rad, Mef, Mus, Per, Tib	+	Area > 50 % Ampl.> 30%	—	21/37 12/37	17/21 6/12	0/4
Nobile-Orazio et al., 2002	23	Med, Uln, Per, Tib	−	AAEM AAEM − 10%	AAEM AAEM	4/23 6/23	12/14 4/6	1/3
Van Asseldonk et al., 2003***	39‡	Med, Uln, Rad, Mef, Mus, Per, Tib	+	Area > 50 % Ampl. > 30%	—	30/39 9/39	30/30 9/9	—

Med = median nerve recorded from m. abductor pollicis brevis; Uln = ulnar nerve recorded from m. abductor digiti V; Rad = radial nerve recorded from m. extensor carpi ulnaris; Mef = median nerve recorded from m. flexor carpi radialis; Mus = musculocutaneus nerve recorded from m. biceps brachii; Per = deep peroneal nerve recorded from m. extensor digitorum brevis; Tib = tibial nerve recorded from m. abductor hallucis; ? = not specified in article. AAEM = criteria proposed by the American Academy of Electrodiagnostic Medicine; AAEM − 10% = criteria that require 10% less decrement in amplitude or area P/D for CB as compared to those proposed by the AAEM. Ampl = amplitude; * patients were selected on the basis of absence of CB; ** patients were selected on the basis of CB or other features of demyelination on nerve conduction studies; *** patients were selected on the basis of a positive response to IVIg; ‡ 33 patients were already reported in a previous study (Van den Berg-Vos).

block cannot be found in these nerves in patients with a lower motor neuron syndrome, electrophysiological examination should be extended to other nerves, including five long, intermediate size or short arm nerves innervating weakened or non-weakness muscles on both sides, until conduction block is found (Van Asseldonk et al., 2003). Since a favorable response to immune-modulating treatment was never reported for a patient with a lower motor neuron syndrome without conduction block according to these criteria on extensive nerve conduction studies, we restrict treatment to patients with a lower motor neuron syndrome and conduction block on extensive nerve conduction studies.

The detection of conduction block may be further improved by fatigability testing (Kaji et al., 2000) or root stimulation (Menkes et al., 1998; Kaji et al., 2000), both of which were shown to reveal conduction block in nerves in which conduction block was not found on conventional nerve conduction studies in a restricted number of nerves. Motor conduction is frequently slowed in MMN, as it is in CIDP and sporadically in motor neuron disease (Barohn et al., 1989; Cornblath et al., 1992; Van Asseldonk et al., 2003). Sensory nerve conduction studies are required to exclude sensory abnormalities (at the site of conduction block) in MMN and may be helpful to differentiate MMN from CIDP. Decreased distal CMAP amplitudes on nerve conduction studies, suggestive for axonal degeneration, as well as signs of de- and reinnervation on needle EMG, occur in MMN, lower motor neuron disease and CIDP (Barohn et al., 1989; Van Asseldonk et al., 2003; Van den Berg-Vos et al., 2003a; Vucic et al., 2004a). The occurrence of axonal degeneration in MMN indicates that needle EMG is unable to differentiate between MMN and lower motor neuron disease and once again stresses the importance of nerve conduction studies in patients with a lower motor neuron syndrome.

12.2.3. Laboratory diagnostic features

Routine analysis of blood and urine is normal in patients with MMN, despite slightly to moderately increased serum creatine kinase activity consistent with a slowly progressive axonal degeneration in up to two-thirds of patients (Chaudhry et al., 1993; Van den Berg-Vos et al., 2000b). Protein and immunoelectrophoresis occasionally reveal mono- or polyclonal increase of immunoglobulins, mostly of IgM isotype (Freddo et al., 1986; Latov et al., 1988; Pestronk et al., 1988; Le Forestier et al., 1997; Bentes et al., 1999). In the cerebrospinal fluid oligoclonal bands are not found, the IgG index is normal and the protein level is normal or slightly elevated (< 1 g l^{-1}) in most patients with MMN (Bouche et al., 1995).

Initial reports on increased anti-GM1 ganglioside antibody titers in serum of patients with MMN raised the hope for a diagnostic marker for MMN (Latov et al., 1988; Pestronk et al., 1988, 1990; Krarup et al., 1990; Pestronk 1991; Willison et al., 1994; Taylor et al., 2000). Positive findings for anti-GM1 antibodies in approximately half of the patients with MMN (range 22–85%), as well as in patients with lower motor neuron disease, ALS and CIDP and even in healthy subjects, suggested that the sensitivity and specificity of antibody testing was limited (Latov et al., 1988; Nobile-Orazio et al., 1990; Sadiq et al., 1990; Shy et al., 1990; Lamb and Patten 1991; Azulay et al., 1994; Kinsella et al., 1994; Kornberg and Pestronk 1994). However, anti-GM1 antibody titers in ALS and CIDP were shown to be in the range of anti-GM1 antibody titers of healthy subjects, while higher titers occur in serum of patients with MMN or occasionally progressive spinal muscular atrophy (PSMA). Within 173 consecutive patients referred for anti-GM1 antibody testing, high titers of IgM anti-GM1 only occurred in MMN (Taylor et al., 1996). Furthermore, a positive GM1 IgM test was associated with MMN within a selection of patients with a lower motor neuron syndrome (Van den Berg-Vos et al., 2000a). Moreover, a meta-analysis on the diagnostic value of IgM anti-GM1 in MMN showed that pre-test probabilities between 20 and 60% for having MMN on the basis of clinical characteristics changed to post-test probabilities between 50 and 85% when IgM anti-GM1 was found (Van Schaik et al., 1995). Overall, these studies indicate that an elevated anti-GM1 antibody titer in a patient with a lower motor neuron syndrome is supportive but not conclusive for MMN and should prompt extensive electrophysiological examination, whereas a negative test does not exclude the diagnosis of CIDP.

In patients with MMN, asymmetrically increased signal intensity on T2-weighted images of the brachial plexus was found, corresponding with the distribution of symptoms (Fig. 12.2) (Van Es et al., 1997; Van den Berg-Vos et al., 2000a). Asymmetrically increased signal intensity was also shown on T1 weighted images after gadolinium enhancement which co-localized with CB in the brachial plexus (Parry, 1996) and in the forearm segment of the median nerve. The findings in MMN resemble the symmetrical increased signal intensity seen in CIDP and distal demyelinating polyneuropathy associated with IgM monoclonal gammopathy, and may be due to demyelination (Van Es et al., 1997; Duggings et al., 1999; Eurelings et al, 2001). Increased signal intensity on MRI, occurring in approximately 40–50% of patients with MMN, may be helpful to differentiate MMN from lower motor neuron disease, in which MR images were normal (Van Es et al., 1997).

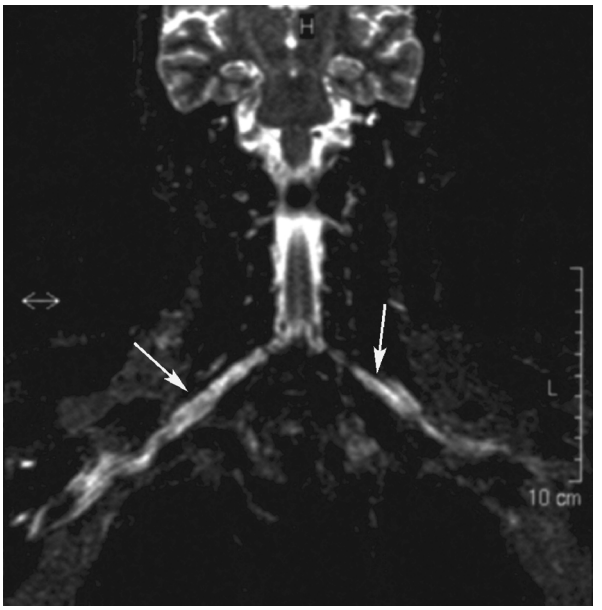

Fig. 12.2. MR imaging of the brachial plexus of a patient with MMN. Arrows indicate swellings and increased intensities on the T2-weighted images of the brachial plexus.

12.2.4. Diagnostic criteria

Most diagnostic studies in MMN focused on the diagnostic yield of criteria for CB, whereas information on additional diagnostic value of clinical and laboratory characteristics is limited. However, not only the presence of conduction block, but also the age of onset, number of affected limb regions, increased signal intensity on T2-weighted images of the brachial plexus and elevated anti-GM1 antibodies predict a positive response to IVIg treatment in patients with a lower motor neuron syndrome (Van den Berg-Vos et al., 2000a). On the basis of these findings, a criteria set consisting of combined clinical, laboratory and electrophysiological characteristics was proposed for definite, probable and possible MMN (Table 12.3) (Van den Berg-Vos et al., 2000a). In a group of patients with a lower motor neuron syndrome and conduction block or conduction slowing compatible with demyelination on nerve conduction studies the likelihood of responding to IVIg treatment was 81% for definite MMN, 71% for probable MMN and 11% for possible MMN (Van den Berg-Vos et al., 2000a). These criteria were proposed for clinical practice since they improved identification of patients who may respond favorably to IVIg and should be subject of future validation studies.

12.2.5. Treatment

The hypothesis that MMN is an immune-mediated neuropathy has led to the trial of several immunological treatments. In contrast to CIDP, prednisolone and plasma exchange were ineffective in most patients and were even associated with clinical worsening in some patients with MMN (Dyck et al., 1982, 1986; Parry and Clarke, 1988; Pestronk et al., 1988; Krarup et al., 1990; Kaji et al., 1992; Chaudhry et al., 1993; Nobile-Orazio et al., 1993; Donaghy et al., 1994; Jaspert et al., 1996; Le Forestier et al., 1997; Van den Berg et al., 1997; Carpo et al., 1998; Claus et al., 2000). Of the immunosuppressants, only high dose cyclophosphamide seems to be effective (Pestronk et al., 1988; Krarup et al., 1990; Feldman et al., 1991; Chaudhry et al., 1993). Unfortunately, the considerable side-effects of cyclophosphamide, especially the increased risk of neoplasia, limits its utility in patients with MMN, who are of relatively young age.

Several non-controlled studies have shown a beneficial effect of intravenous immunoglobulin (IVIG) treatment (Charles et al., 1992; Kaji et al., 1992; Kermode et al., 1992; Chaudhry et al., 1993; Nobile-Orazio et al., 1993; Yuki et al., 1993; Comi et al., 1994; Bouche et al., 1995; Van den Berg et al., 1995b; Jaspert et al., 1996; Le Forestier et al., 1997; Pakiam and Parry, 1998; Vucic et al., 2004a). The effect of IVIg in MMN was confirmed in four double-blind placebo-controlled trials (Azulay et al., 1994; Van den Berg et al., 1995a; Federico et al., 2000; Leger et al., 2001). However, as the effect of IVIG treatment occurs within a week but lasts only several weeks, IVIG maintenance is necessary to maintain the effect on muscle strength in most patients (Bouche et al., 1995; Van den Berg et al., 1995a; Azulay et al., 1997; Van den Berg-Vos et al., 2002b; Terenghi et al., 2004). Side effects were minor, the most disabling were skin changes (eczema) in hands and trunk (Brannagan et al., 1996; Wittstock et al., 2003).

Maintenance IVIG treatment is expensive, and the frequent infusions may be burdensome to patients, but at present there is no therapeutic alternative to IVIG therapy. Therefore, long-term studies on the effect of IVIG treatment are important. In a study evaluating the effect of long-term (4 to 8 years) IVIG treatment in 11 patients with MMN (Van den Berg-Vos et al., 2002b), muscle strength improved significantly within 3 weeks of the start of IVIG treatment and was still significantly better at the last follow-up examination than before treatment, even though it decreased slightly and significantly during the follow-up period (Fig. 12.3). IVIG treatment did not induce remission in any of our patients; once IVIG treatment was stopped, substantial progression of weakness occurred. In another study of 10 patients with IVIg maintenance treatment varying from 5 to 12 years, it was shown that the effectiveness of IVIG tends to decrease during prolonged treatment even when the IVIG dosage is increased (Terenghi et al., 2004).

Table 12.3

Proposed diagnostic criteria for MMN

I. Clinical criteria

1. Slowly progressive or stepwise progressive limb weakness
2. Asymmetric limb weakness
3. Number of affected limb regions < 7. Limb regions are defined as upper arm, lower arm, upper leg or lower leg on both sides (max. 8)
4. Decreased or absent tendon reflexes in affected limbs
5. Signs and symptoms are more pronounced in upper than in lower limbs
6. Age at onset of disease: 20–65 years
7. No objective sensory abnormalities except for vibration sense
8. No bulbar signs or symptoms
9. No upper motor neuron features
10. No other neuropathies (e.g. diabetic, lead, porphyric or vasculitic neuropathy, chronic inflammatory demyelinating polyneuropathy, Lyme neuroborreliosis, post radiation neuropathy, hereditary neuropathy with liability to pressure palsies, Charcot–Marie–Tooth neuropathies, meningeal carcinomatosis)
11. No myopathy (e.g. fascioscapulohumeral muscular dystrophy, inclusion body myositis)

II. Laboratory criteria

1. CSF protein < 1g L^{-1}
2. Elevated anti-GM1 antibodies
3. Increased signal intensity on T_2-weighted MR images of the brachial plexus

III. Electrodiagnostic criteria

1. Definite motor CB: CMAP area reduction on proximal versus distal stimulation of at least 50%, over a long segment (between Erb and axilla, upper arm, lower arm, lower leg) or a CMAP amplitude reduction on proximal versus distal stimulation of at least 30% over a short distance (2.5 cm) detected by inching. CMAP amplitude on stimulation of the distal part of the segment with motor CB of at least 1 mV
2. Probable motor CB: CMAP amplitude reduction on proximal versus distal stimulation of at least 30% over a long segment of an arm nerve. CMAP amplitude on stimulation of the distal part of the segment with motor CB of at least 1 mV
3. Slowing of conduction compatible with demyelination: MCV < 75% of the lower limit of normal; DML or shortest F-wave latency > 130% of the upper limit of normal or absence of F waves all after 16–20 stimuli. CMAP amplitude on distal stimulation of at least 0.5 mV.
4. Normal sensory nerve conduction in arm segments with motor CB. Normal SNAP amplitudes on distal stimulation.

Definite MMN:	I 1–11	and	II 1	and	III 1 + 4
Probable MMN:	I 1–3, 6–11	and	II 1	and	III 2 + 4
Possible MMN:	I 1, 7–11	and	II 2 or 3	or	III 3 + 4

CSF = cerebrospinal fluid; MR = magnetic resonance; CB = conduction block; CMAP = compound muscle action potential; MCV = motor conduction velocity; DML = distal motor latency; SNAP = sensory nerve action potential.

During the first few years of maintenance treatment the reduced response to IVIg could be restored by increasing the dosage, whereas later this increase was only partially effective. However, this was contradicted in another study in which a higher monthly maintenance IVIG dosage showed improvement of muscle strength and functional disability during long-term follow-up (Vucic et al., 2004).

As the majority of patients with MMN respond to IVIG treatment, a prospective study on the natural course of MMN without any treatment is not feasible. Two retrospective studies concerning the natural history of MMN showed that MMN runs a slowly progressive course (Taylor et al., 2000; Van den Berg-Vos et al., 2002b). Occasionally, a step-wise (Nobile-Orazio et al., 1993; O'Leary et al., 1997) or spontaneously remitting (Bouche et al., 1995) course has been described. In a study of 38 patients with MMN we showed that longer disease duration was associated with more weakness as well as electrophysiological abnormalities (Fig. 12.4), and that the patients who responded to initial IVIG treatment had a disease duration of up to 24 years and could have severe weakness (Van den Berg-Vos et al., 2002). Non-responsiveness to IVIG was not associated with disease variables like upper or lower limb involvement, muscle strength, disability and

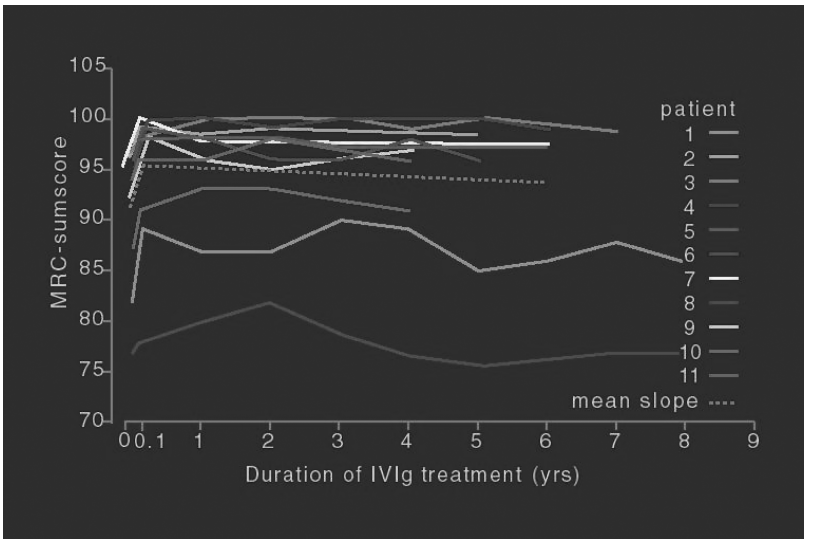

Fig. 12.3. For full color figure, see plate section. Muscle strength of 11 patients with multifocal motor neuropathy, expressed as MRC sumscores, during IVIg maintenance treatment. Sumscore of muscle strength was measured according to the Medical Research Council (MRC) in five muscle groups in both arms and in five muscle groups in both legs, yielding a maximal MRC sumscore of 100. Horizontal axis: 0 = before the onset of IVIg treatment, 0.1 = after the first full course of IVIg, 1–8 = during and after follow-up. Dotted line represents the average of all 11 patients.

electrophysiological variables. Nobile-Orazio et al. (2002b) showed that the response on initial IVIg was less pronounced for patients with longer disease duration. These results provide indirect evidence that progression of weakness in MMN is caused by an ongoing immunological process and that early treatment may prevent future progression of weakness and disability in patients with MMN. Although the overall prognosis of patients with MMN appears to be relatively good, especially in comparison with patients who suffer from lower motor neuron disease, most patients with MMN are impaired in their daily life by reduced dexterity in manual activities (Taylor et al., 2000). Other patients are disabled by fatigue (Kaji et al., 2000) a symptom which in our opinion has so far been underestimated in MMN and needs further investigation. Only two patients were reported to have a fatal outcome after several years of disease (Magistris and Roth, 1992) while, in others, death was related to concomitant diseases (Bentes et al., 1999; Beydoun and Copeland, 2000).

12.2.6. Pathophysiology

We reviewed the pathological, immunological and electrophysiological studies that improved understanding of the following observations in MMN: the typical pattern of weakness (asymmetric, predominantly distal, more in arm than in leg muscles), the presence of weakness in atrophic and in non-atrophic muscles, the absence of sensory involvement, the partial reversibility of weakness after IVIG treatment, the decreased effect of IVIG as the disease progresses, the slowly progressive course despite long-term IVIG treatment and the presence of IgM anti-GM1 antibodies in some but not in all patients with MMN.

The reluctance to take biopsy samples from motor nerves has limited the number of pathological studies in MMN. In an ulnar nerve biopsy at the site of a previously documented conduction block, Auer et al. (1989) reported onion bulb formation, a feature characteristic of multiple episodes of demyelination and remyelination and axons that were thinly myelinated in relation to axon diameter. Furthermore, Kaji and colleagues showed large diameter axons almost devoid of myelin in the median pectoral nerve with CMAP decrement P/D on intra-operative recording. On the contrary, Taylor et al. (2004) showed that multifocal loss and degeneration of axons, as well as prominent clusters of regenerating axons, predominated over myelin pathology in motor fascicular nerve biopsies with evidence of conduction block on intra-operative recording in seven patients with MMN. Besides intermediate sized fibers with thin myelin, which may have represented previous remyelination, paranodal demyelination, internodal demyelination and onion bulb formation were not found. Although these findings suggest that axonal pathology is more prominent than demyelinating pathology in MMN, they fail to explain the finding of conduction block and the rapid response to IVIG in MMN. The finding that, unlike CIDP, inflammatory cellular infiltrates are sporadic in MMN implies that MMN and CIDP are unlikely to share underlying disease mechanisms (Prineas and McLeod, 1976; Auer et al., 1989; Kaji et al., 1993; Taylor et al., 2004). The findings in biopsied sural nerves from patients with MMN were either normal or showed mild axonal degeneration, mild demyelination or both, which is consistent with the infrequent sensory impairment in patients with MMN (Corse et al., 1996; Nobile-Orazio, 2001).

The positive response to immune modulating treatment, the finding of anti-GM1 antibodies in 20–80% of

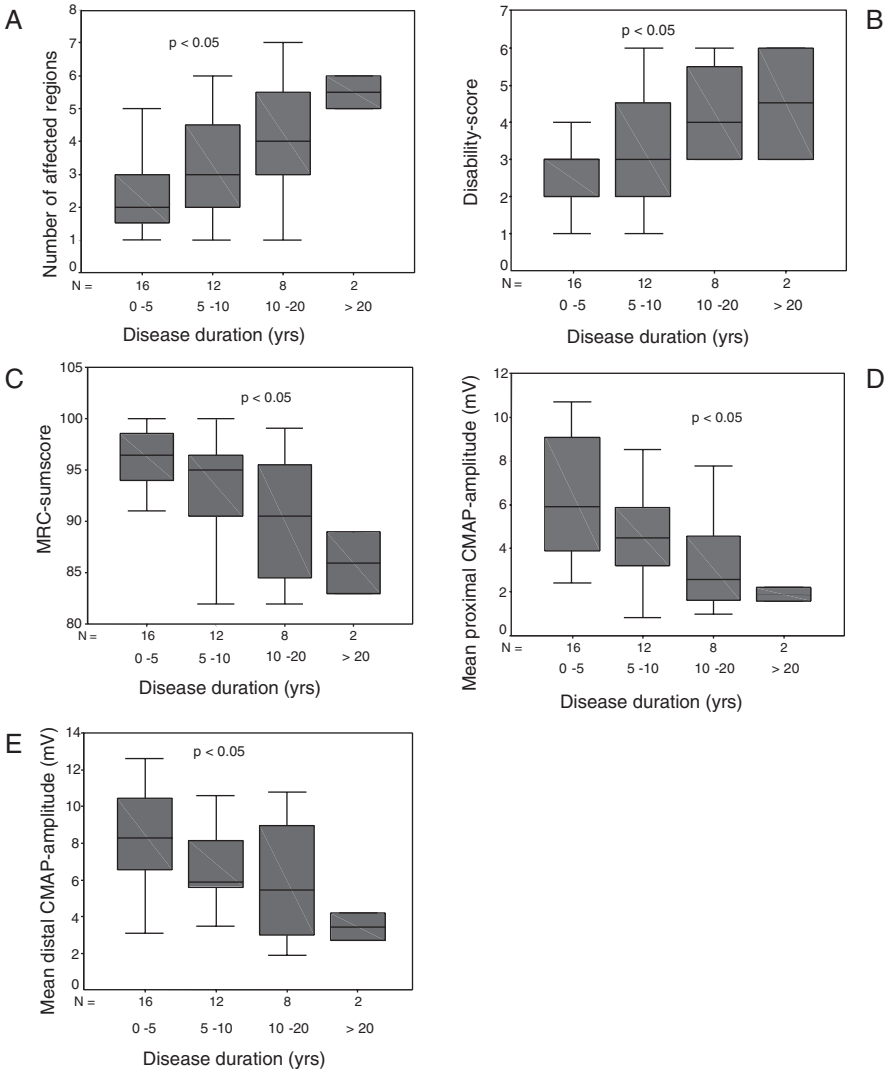

Fig. 12.4. Boxplots with median value (horizontal bar), 25th–75th interquartile range (box), maximum and minimum values of variables per category of disease duration: A. affected regions; B. disability; C. MRC sumscore; D. Mean proximal CMAP-amplitude; E. Mean distal CMAP-amplitude.

MMN patients (Freddo et al., 1986; Latov et al., 1988; Parry and Clarke 1988; Pestronk et al., 1988; Le Forestier et al., 1997; Bentes et al., 1999; Van den Berg-Vos et al., 2000a) and the expression of GM1 on axon and myelin membranes (Latov et al., 1988; Schluep and Steck, 1988; Thomas et al., 1989; Corbo et al., 1992; O'Hanlon et al., 1996, 1998) suggests that anti-GM1 antibodies may be pathogenic in MMN. In this context, difference between the fatty acid and long chain base composition of peripheral nerve ganglioside GM1 of sensory and motor nerves, resulting in different affinities of anti GM1 antibodies, may contribute to selective involvement of motor fibers (Thomas et al., 1990; Corbo et al., 1992; Ogawa-Goto et al., 1992). Evidence for antibody mediated demyelination or blocking of the voltage gated sodium channels at the node of Ranvier was shown in some in vivo and in vitro animal experiments (Santoro et al., 1992; Arasaki et al., 1993; Uncini et al., 1993; Takigawa et al., 1995) but not in others (Harvey et al., 1995; Hirota et al., 1997; Benatar et al., 1999; Paparounas et al., 1999). Furthermore, anti-GM1 binding sites and voltage gated sodium channels were not co-localized (Quattrini et al., 2001). Moreover, human IgM anti-GM1 auto-antibodies were shown to modulate intracellular calcium homeostasis in neuroblastoma cells, most likely due to activation of L-type voltage gated calcium channels that are also present on motor neurons (Quattrini et al., 2001). Overall, these experiments have not confirmed nor excluded the pathogenicity of IgM anti-GM1 antibodies in MMN. The finding that distal motor nerve conduction in mice was blocked by sera from patients with, and by sera from patients without high anti-GM1 antibody titers suggests that factors other than anti-GM1 antibodies may be pathogenic in MMN (Roberts et al., 1995).

Conduction block, the electrophysiological hallmark of MMN, was considered to underlie weakness in MMN.

Improvement of conduction block on nerve conduction studies in patients with MMN following initial and long-term IVIG was found in most, but not in all studies (Kaji et al., 1992; Nobile-Orazio et al., 1993; Chaudhry et al., 1994; Comi et al., 1994; Bouche et al., 1995; Van den Berg et al., 1995a,b; Capellari et al., 1996; Leger et al., 2001; Vucic et al., 2004a). The inability of nerve conduction studies to assess proximal nerve segments may explain the lack of improvement in conduction block in some patients. These findings suggest that conduction block may cause weakness that could, at least partially, be reversed by IVIG. In 39 patients with MMN, long nerves had more segments with conduction block due to a random distribution of conduction block in arm nerves (Van Asseldonk et al., 2003). Furthermore, distal CMAPs were more often decreased in long nerves. Taken together, these findings indicate length-dependent axonal degeneration due to the high number of conduction blocks in these nerves (Van Asseldonk et al., 2003).

Electrophysiological studies in patients with MMN who were treated with IVIG raised important questions regarding the determinants of weakness in MMN. Although muscle strength in patients with MMN improves after IVIG treatment, it rarely fully recovers to normal strength (Chaudhry et al., 1993; Nobile-Orazio et al., 1993; Azulay et al., 1994, 1997; Leger et al., 1994, 2001; Kornberg and Pestronk, 1995; Van den Berg et al., 1995a; Capellari et al., 1996; Federico et al., 2000; Van den Berg-Vos et al., 2002a; Vucic et al., 2004b). Irreversible weakness may be due to irreversible conduction block or, alternatively, due to axonal degeneration. Several observations imply that axonal degeneration contributes to weakness in patients with MMN. Atrophic muscles, decreased distal CMAP amplitudes on nerve conduction studies and evidence of denervation and reinnervation on needle electromyography are all found in patients with MMN, even in those with a short disease duration (Taylor et al., 2000; Nobile-Orazio, 2001; Van den Berg-Vos et al., 2002b; Van Asseldonk et al., 2003; Vucic et al., 2004a). In a study of patients with MMN who had never received IVIG treatment, a longer disease duration was not only associated with more segments with conduction block, but also with more nerves with low distal CMAP amplitudes, the latter being consistent with progressive axonal degeneration (Fig. 12.4) (Van den Berg-Vos et al., 2002a).

Weakness due to progressive axonal degeneration was suggested to underlie the less pronounced response on initial (or long-term) IVIG for patients with a longer disease duration (Nobile-Orazio et al., 2002). Three long-term follow-up studies of patients on IVIG maintenance treatment studies showed a mild decrease in muscle strength as well as evidence for ongoing axonal degeneration as measured by distal CMAP amplitude (Van den Berg et al., 1998; Van den Berg-Vos et al., 2002a; Terenghi et al., 2004). In contrast, weakness was not progressive and evidence for ongoing axonal degeneration as measured by distal CMAP amplitude was absent, in a recent study that used a higher monthly maintenance dosage of IVIG (Vucic et al., 2004a).

In univariate analysis weakness was associated with nerve length, years treated and years untreated, as well as with the presence of conduction block, demyelinative slowing and decreased distal CMAP amplitudes on motor nerve conduction studies (Van den Berg-Vos et al., 2002a; Van Asseldonk et al., 2003). To determine the independent contribution of these determinants to chronic progressive weakness, a multivariate analysis in 20 patients with MMN on long-term IVIg treatment was performed (Van Asseldonk et al., 2005a). In this study, axon loss, scored according to strict criteria for denervation and reinnervation on needle EMG, occurred frequently in MMN: 61% of all muscles in the 20 patients showed needle EMG abnormalities that were also quite pronounced in most muscles. In contrast, only 2% of limb muscles found spontaneous muscle fiber activity (denervation) in older normal subjects. EMG abnormalities were also frequent in patients with a short disease duration indicating that axon loss is an early feature of MMN. Importantly, in the multivariate analysis, axon loss and not conduction block had the strongest relation to weakness, whereas conduction block alone had the strongest relation to axon loss (Van Asseldonk et al., 2005a). These findings suggest that IVIG treatment may have its effect on reversible conduction block whereas an axon which is affected by a process resulting in irreversible conduction block may eventually degenerate despite continuous IVIG treatment. Mechanisms leading to axonal degeneration may play the most important role in the outcome of the neurological deficit in patients with MMN.

Generally, conduction block occurs when the action current at one node does not induce a sufficiently large depolarization at the next node to generate an action potential, either because there is less current available or because there is more current needed (Kaji, 2003). The finding that conduction block and demyelinative slowing were independently related to each other in arm nerves of patients with MMN and the finding of demyelination at the site of conduction block on most biopsy studies indicates that conduction block is likely to result from a primary demyelinating process (Van Asseldonk et al., 2003). In animal experiments paranodal demyelination was shown to impair saltatory conduction; the current available for depolarization was low because the outward capacitive sodium current,

which leads to depolarization of a node to be activated, was dissipated over the node and the adjacent damaged paranodal region (Sumner et al., 1982). The period to depolarize the node to threshold for an action potential will be longer when moderate amounts of current are lost, yielding conduction block (Kaji, 2003). The loss of current may be aggravated when demyelination exposes paranodal or internodal potassium channels. Motor axons in arm nerves have a more prominent slow potassium conductance than motor axons in leg nerves (Kuwabara et al., 2000). These differences in conductances could contribute to the greater tendency of motor axons in arm nerves to develop conduction block (Kuwabara et al., 2000; Burke et al., 2001; Van Asseldonk et al., 2003).

The mechanism that underlies axon loss in MMN is poorly understood. The finding that axon loss and conduction block were independently related with each other may be explained by a common disease mechanism that leads to conduction block in some axons and degeneration of other axons. A common disease mechanism is supported by pathological studies showing that the antibodies to the ganglioside GM1 bind to epitopes of the nodal axolemma and paranodal myelin, possibly leading to conduction block and axon loss, and to epitopes of spinal cord motor neurons possibly leading to axon loss (Santoro et al., 1992; Roberts et al., 1995; Sheikh et al., 1999; Van den Berg-Vos et al., 2000b; Kaji, 2003) Conduction block was found to be randomly distributed in arm nerves and, consequently, long nerves have more segments with conduction block: in addition distal CMAPs were more often decreased in long nerves, indicating length-dependent axonal degeneration due to the high number of conduction blocks in these nerves (Van Asseldonk et al., 2003). In a small proportion of nerves, CMAPs evoked distally to a segment with conduction block, were shown to decrease in time during follow-up (Van den Berg-Vos et al., 2002a; Vucic et al., 2004a). The association between axon loss and conduction block may also suggest that an axon will eventually degenerate if a process resulting in conduction block affects it. This mechanism is supported by excitability measurements that revealed axonal hyperpolarization adjacent to sites with conduction block. Hyperpolarization was thought to be secondary to intra-axonal sodium-accumulation at the site with conduction block, caused by reduced activity of the sodium/potassium pump (Bostock et al., 1995; Kiernan et al., 2002). An animal study of inflammatory demyelination supported this mechanism, showing that blockade of sodium channels or sodium/calcium exchanger prevented axonal degeneration (Kapoor et al., 2003).

12.3. Pure motor form of CIDP

Pure motor CIDP is a rare variant of CIDP. Patients have no sensory loss at neurological or electrophysiological examination (Donaghy et al., 1994; Sabatelli et al., 2001; Busby and Donagh, 2003). Sural nerve biopsy specimens were also reported to be without abnormalities (Sabatelli et al., 2001). In contrast to MMN, the neuropathy may have a subacute onset, the distribution of weakness involves also proximal muscle groups of the legs and is more or less symmetrical, and the neuropathy may relapse over time. Patients with pure motor CIDP appeared to be younger than those with classical CIDP (Sabatelli et al., 2001; Busby and Donagh, 2003), which is also our experience with these patients. A striking similarity is the response to IVIG and not to steroids (Donaghy et al., 1994; Sabatelli et al., 2001; Busby and Donagh, 2003). Systematic studies on the (long-term) clinical or electrophysiological response to IVIG treatment are not available.

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Chapter 13

Amyotrophic lateral sclerosis

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13.1. Introduction and historical background

Amyotrophic lateral sclerosis (ALS) is a progressive disorder characterized by degeneration of motor neurons of the primary motor cortex, brainstem and spinal cord. As we shall see, however, this selective degeneration of the motor system is relative, and other areas and types of neurons are often involved. For the clinician, the consequences of degeneration of corticospinal, brainstem and spinal motor neurons dominate issues of diagnosis and management, and it is degeneration of spinal motor neurons that, in most cases, contributes most to disability and leads to death through progressive respiratory muscle weakness. In this article, the term ALS will be used to cover the syndromes of progressive bulbar palsy and progressive pseudobulbar palsy (PBP), classic (Charcot) ALS and progressive muscular atrophy (PMA). These syndromes are included within Russell Brain's concept of motor neuron disease, a term subsequently much used by the British school of neurology but less used elsewhere in the world. In the USA, the term Lou Gehrig's disease is often used colloquially. Lou Gehrig was a revered baseball player who died in 1941 with ALS (Kasarskis and Winslow, 1989). His plight first brought ALS to the notice of the wider American public. Lou Gehrig remains an iconic figure in the history of sport, and a model of suffering borne with courage, humor and dignity. Nevertheless, the term Lou Gehrig's disease should not be considered as a scientific synonym for ALS. If eponyms were to be applied, Charcot's disease or even Aran-Duchenne-Charcot disease would be preferable to Lou Gehrig's disease. Overall, it is probably best to use the terms ALS or MND and to avoid eponyms in this context. In this author's opinion, the term Lou Gehrig's disease should be restricted to colloquial use.

13.2. Epidemiology

The incidence of ALS varies between 1 and 3 per 100,000 person years in most studies, with point prevalence rates of 4 to 6 per 100,000 person years (Jokelainen, 1976; Kurtzke, 1991; Chancellor and Warlow, 1992; Scottish Motor Neuron Disease Research Group, 1992; Traynor et al., 1999; Worms, 2001). Most of the studies derive from more developed countries and relatively little is known of the incidence and prevalence of ALS in developing countries or in specific racial or ethnic groups (Leone et al., 1987; Kurtzke, 1991). Even within Europe and North America, there is little information on incidence and prevalence rates in minority, racial and ethnic groups. Some studies have suggested that the overall age-related incidence of ALS has increased over several decades (Lilienfeld et al., 1989; Kurtzke, 1991; Chio et al., 1999; Mitchell, 2000; Seljeseth et al., 2000; Maasilta et al., 2001), but this could be due to improved ascertainment. Prevalence can be expected to rise somewhat with the increasing age of the population and perhaps with the introduction of better supportive treatments. The explanation for the strikingly increased incidence and prevalence of ALS in geographic foci such as the Island of Guam in the Western Pacific, parts of the Kii Peninsula of Japan and in Western Papua New Guinea (Irian Jaya, Indonesia) remains enigmatic. Although the prevalence remains high in Guam compared to typical European and North American populations, there has been a decrease over the last half century (Waring, 1994; Wiederholt, 1999; Yase et al., 2001; Plato et al., 2003; Waring et al., 2004; Kuzuhara and Kokubo, 2005; see below).

The incidence of ALS increases with age, being very low before the age of 40 and peaking at around 75 years of age, although the distribution is bimodal for gender, with elderly women having a somewhat higher

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incidence compared to elderly men (Kurtzke, 1991; Scottish Motor Neuron Disease Research Group, 1992; Worms, 2001). Men are more frequently affected than women with a male/female ratio in sporadic ALS of around 1.5:1. This ratio approaches 1:1 in familial ALS (Emery and Holloway, 1982). Women are relatively over-presented in older age groups although the standardized age-related incidence is greater in older men (Forbes et al., 2004a). Bulbar onset is also more common in older patients, and particularly in older women (Haverkamp et al., 1995; Forbes et al., 2004a). The mean age of onset in sporadic ALS varies between 60 and 65 years in most studies, with a median age of onset of 64 years and a range varying between the third decade and the ninth decade (Jokelainen, 1976; Kurtzke, 1991). The average age of onset of familial ALS is about a decade earlier (Emery and Holloway, 1982). Very rarely, typical ALS presents in the second or third decade. Aside from the undoubted ALS clusters in the Western Pacific, Japan and Papua New Guinea, apparent clusters of ALS, including conjugal cases, have been described. It is questionable whether such associations are statistically or biologically meaningful (Chad et al., 1982; Kurtzke, 1991; Cornblath et al., 1993; Mitchell et al., 1998; Rachele et al., 1998; Corcia et al., 2003; Sabel et al., 2003). Some surveys have detected a relationship between ALS and latitude, but this trend is weak and inconsistent (Kurtzke, 1991). In most large clinic-based or population-based studies, about 5% of all cases are classified as familial, usually with a family history suggestive of autosomal dominant inheritance (Emery and Holloway, 1982; Holloway and Mitchell, 1986; Scottish Motor Neuron Disease Research Group, 1992; Lacomblez et al., 1996; Majoor-Krakauer et al., 2003). No association has been found between sequence variants in, or haplotype across, the SOD1 locus in sporadic ALS (Broom et al., 2004).

Many different factors have at one time or another been implicated as risk factors for ALS, but the only risk factors that are consistent across all studies are gender, a positive family history and increasing age (Kurtzke, 1991; Chancellor et al., 1993a; Cruz et al., 1999; Majoor-Krakauer et al., 2003). Other factors that have, in one or another study, been associated with increased risk include trauma, physical activity, diet, vitamin E intake, participation in athletic pursuits, residence in rural rather than urban areas, alcohol consumption, cigarette smoking and working in certain industries, for example the leather industry or electrical occupations (Hawkes et al., 1989; Kurtzke, 1991; Chancellor et al., 1993a; Strickland et al., 1996; Johansen and Olsen, 1998; Longstreth et al., 1998; Savitz et al., 1998; Nelson et al., 2000a,b; Scarmeas et al., 2002; Weisskopf et al., 2004; Ascherio et al., 2005; Belli and Vanacore, 2005). Using an evidence-based medicine approach, Armon (2003) concluded that smoking is probably associated with ALS, but that the evidence in favor of trauma, increased physical activity, place of residence and alcohol consumption being risk factors for ALS was not persuasive. Similarly, there is no firm evidence to support the notion that viral infections such as poliomyelitis might act as precipitants or risk factors for ALS. Nevertheless, interest continues to focus on the possibility that certain occupations or activities may confer increased risk of developing ALS. Attention has focused on military service, specifically deployment of US military personnel to the Gulf region during the first Gulf War (August 1990 through July 1991). In a study of about 2.5 million eligible military personnel, 107 confirmed cases of ALS were identified with an overall occurrence of 0.3/100,000 per year, yielding a significantly elevated risk of ALS amongst all deployed personnel. The relative risk was about 2, with an attributable risk associated with deployment of 18% (Horner et al., 2003). Another study of Gulf War veterans, using slightly different methodology, also concluded that Gulf War service conferred an increased risk of developing ALS (Haley, 2003). Finally, a prospective study of the risk of ALS in military personnel also revealed an increased risk of death due to ALS, with a relative risk of 1.53. The increased risk was independent of the branch of the service (Weisskopf et al., 2005). There are methodological difficulties (Rose, 2003), but if a two-fold excess risk is correct the chances of developing ALS as a result of military service in that particular context are still very small. The apparent increased risk of developing ALS for military personnel and in Italian professional footballers (Chio et al., 2005), if true, might reflect a selection bias for entry into occupations particularly associated with physical activity and physical fitness rather than exposure to toxins, drugs or other environmental insults. Such individuals might have a reproductive advantage that the very slight increased risk of developing ALS would not offset (Al-Chalabi and Leigh, 2005). Nevertheless, it has to be remembered that the numbers of cases upon which these calculations are based have been very small, particularly in the case of Italian footballers (Chio et al., 2005).

In summary, we can conclude that the incidence and prevalence of ALS are similar in Western, industrialized countries where careful population-based studies have been carried out. We are still ignorant about incidence and prevalence figures in most developing countries. No geographically localized or widespread environmental risk factors can be considered as of proven causative significance. It is, however, clear that genetic factors are important in the pathogenesis of ALS, and we would expect there to be significant

differences in incidence in communities with different ethnic and racial backgrounds, so detailed epidemiological studies on such communities are much needed. The Guam ALS syndrome may represent an example along these lines. Some studies have indicated an increase in the incidence of ALS, but it remains unclear whether this could be due to demographic factors (Worms, 2001) or might be attributable to unknown environmental risk factors. A slightly increased prevalence can be expected with improvements in care and treatment and an aging population.

13.3. Natural history and prognostic factors

Analyses of large samples of ALS patients from clinics, population studies and large prospective clinical trials have yielded fairly consistent information on important prognostic factors for survival in ALS (Chancellor et al., 1993b; Haverkamp et al., 1995; Lacomblez et al., 1996; Chio et al., 2002; del Aguila et al., 2003). The average (mean or median) survival from onset of symptoms is 2.5–3.5 years. In most studies, site of onset has been identified as an important prognostic factor for survival (Chancellor et al., 1993b), bulbar onset having a median survival of about 2.5 years compared to about 3.5 years for limb onset disease (Chancellor et al., 1993b; Lacomblez et al., 1996; Sorenson et al., 2002; Turner et al., 2002; del Aguila et al., 2003; Forbes et al., 2004a,b; Millul et al., 2005). Earlier age of onset is a potent predictor of longer survival in almost all studies. PMA has a better prognosis than typical ALS or PBP (Forsgren et al., 1983; Mortara et al., 1984; Norris, 1991; Traynor et al., 2000a; Turner et al., 2002). Gender is not an independent prognostic factor. Many other factors have been identified as independent predictors of survival and/or rate of progression including delay in diagnosis (the longer the delay, the better the prognosis), body weight (lower body weight is an adverse factor), impaired respiratory function (e.g. decreased forced or slow vital capacity-FVC, SVC or sniff nasal pressure, SNP), muscle weakness, lower scores for functional rating scales, higher scores on visual analog scales for stiffness, use of riluzole and the El Escorial category (Fig. 13.1; Chancellor et al., 1993b; Haverkamp et al., 1995; Lacomblez et al., 1996; Traynor et al., 2000a; Chio et al., 2002; Turner et al., 2002; del Aguila et al., 2003; Forbes et al., 2004a; Paillisse et al., 2005). Paradoxically, one study found that survival declined over a 10 year period (Forbes et al., 2004b), whereas others have noted a trend toward better survival over recent years in the context of multidisciplinary specialist care (Turner et al., 2002; Traynor et al., 2003).

Prognostic scores can be constructed and, although these are important in designing clinical trials and understanding the basis of phenotypic variation, they have not, and arguably should not, be applied in routine clinical practice (Haverkamp et al., 1995; Turner et al., 2002; Paillisse et al., 2005). A cluster of adverse or beneficial prognostic factors may be useful in the context of group analyses, but is of limited help in advising the individual patient. This is illustrated by a study of long survivors (10 years or longer). In a sample of

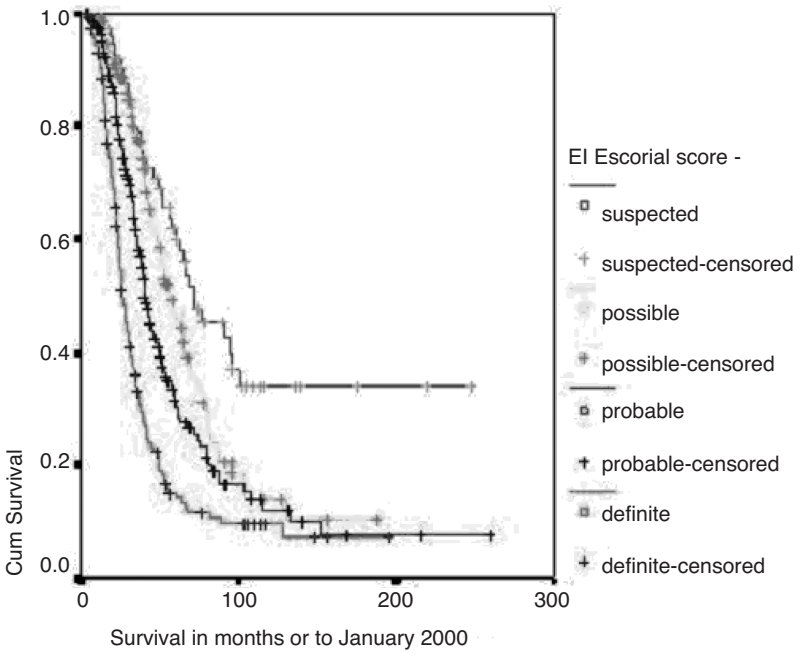


Fig. 13.1. For full color figure, see plate section. Kaplan-Meier survival curves from King's MND Centre Database showing relationship between (original) El Escorial classification and survival. Median survival for suspected ALS category was 72.0 months, compared to definite ALS 26.4 months (Log rank p <0.0001).

769 patients from the King's database, 30 (4%) survived for longer than 10 years (Turner et al., 2003). There were no significant differences for most of the usual prognostic factors between the long survival group and the rest of the cohort, with the exception that age of onset was earlier in the long survivor group.

One of the difficulties in drawing firm conclusions except for the factors with the largest effects (e.g. age of onset) is that the assumptions underlying the multivariate analysis (Cox proportional hazards model) may not be valid and that all possible prognostic factors in a specific sample cannot be known and may differ, subtly but importantly, from those in other samples. For example, several database (i.e. retrospective) studies have indicated that riluzole treatment is an independent prognostic factor for survival and that the use of riluzole may extend survival for between 4 and 20 months (Turner et al., 2002; Traynor et al., 2003). This may be true, but such analyses cannot completely exclude statistical artefacts whereby other, unknown, factors contribute to the observed difference in survival between selected groups (e.g. riluzole versus no riluzole). Nevertheless, the information acquired has been vitally important in designing clinical trials and in providing more accurate and personalized advice on disease progression for patients.

13.4. Clinical features and diagnosis of ALS

13.4.1. Diagnostic criteria for ALS

The 19th century pioneers of neurology, having defined the syndromes that could be gathered within an overarching rubric of ALS, laid the groundwork for current approaches to clinical diagnosis. The advances over the next century came with rapid developments in histopathological techniques, the introduction of clinical neurophysiology and finally the application of neuroimaging techniques beginning with X-ray computerized tomography, culminating with magnetic resonance imaging (MRI). Despite these advances ALS remains, in essence, a clinic or bedside diagnosis made by an experienced neurologist using the tools of neurological examination that were available at the end of the 19th century. There is still no generally accepted method of assessing in a standardized and reproducible fashion what might be termed the 'upper motor neuron load' (Manschot et al., 1998). Attempts have been made to quantify the 'upper motor neuron load' using a system whereby activity of the tendon reflexes is scored (Turner et al., 2004). Spasticity can be assessed using rating scales such as the Ashworth scale, but for diagnostic purposes all UMN signs are often confounded by the presence of the LMN component. New brain imaging techniques do not add significantly to the clinical detection of corticospinal tract damage, although they hold promise for the future (Leigh et al., 2002). Transcranial magnetic stimulation (TMS) of the motor cortex with measurement of central conduction in the corticospinal tracts is useful when UMN signs are equivocal or lacking (Mills and Nithi, 1998; Osei-Lah and Mills, 2004; Attarian et al., 2005). It remains to be seen whether new magnetic resonance imaging (MRI) techniques will serve to identify early damage to the motor cortex and corticospinal tracts (Ellis et al., 1999; Kaufmann et al., 2004).

The co-existence of upper and lower motor neuron signs in a distribution that cannot be explained by any pathology other than ALS requires experience and skill. The introduction of electrophysiology has not removed the need for careful evaluation of the history and thorough clinical examination. Nerve conduction studies (NCS) and electromyography (EMG) studies are as dependent upon the operator as the neurological examination. Both the neurologist and the clinical neurophysiologist must read the EMG in the light of the history and neurological examination. There are opportunities for error on both sides, however, and communication between the neurologist and electrophysiologist is of vital importance in atypical cases. Likewise, MRI greatly facilitates the exclusion of localized disorders that might mimic ALS, but coincidental disease can produce a combination of upper and lower motor neuron signs. For example, combined cervical radiculomyelopathy with lumbosacral radiculopathy is not uncommon. In an attempt to codify the diagnostic process, an international group of neuroscientists, neurologists, neuropathologists and neurophysiologists met in 1990 at the El Escorial Palace near Madrid, Spain (Belsh, 1999) to develop consensus research diagnostic criteria, now known as the El Escorial Criteria (Brooks, 1994). The 1994 criteria attempted to schematize the diagnostic process by defining categories of diagnostic certainty ranging from proven (dependent upon histopathological demonstration of selective degeneration of corticospinal tract, brainstem and spinal motor neurons), definite, probable, possible and suspected.

The original (unrevised) criteria were shown to have high specificity for a final pathological diagnosis of ALS (Chaudhuri et al., 1995), but to lack sensitivity for patients who have few or no upper motor neuron signs (Beghi et al., 2002). In practice, this excludes from entry to clinical trials not only atypical cases (as intended) but cases of ALS early in the evolution of the disease (Traynor et al., 2000a,b) arguably comprising those cases most suitable for testing neuroprotective strategies. At a subsequent meeting at Airlie House, Virginia (Brooks et al., 2000; http://www.wfnals.org), the criteria were revised (Table 13.1), creating a category of

probable ALS (laboratory supported) and possible ALS and abandoning the category of suspected ALS.

Unfortunately, this revision was not based on prospective validation of the criteria but on consensus. Agreement between independent observers for both the original and revised criteria is good, but slightly less good for the revised criteria, which has more categories (Forbes et al., 2001). As the criteria stand, in addition to the combination of upper and lower motor neuron signs, there must be evidence of progression (over a minimum of six months) and other conditions that might mimic ALS must be excluded by appropriate investigations. The El Escorial and subsequent Airlie House criteria were not designed for routine clinical practice. Patients are confused by being told that they have probable or possible ALS – they want to know whether they have the condition or not. Nonetheless, used as a means of identifying 'red flags' indicating potential diagnostic pitfalls, the El Escorial criteria may help to minimize diagnostic mistakes (Traynor et al., 2000b). As we shall see, there are some situations where it is difficult to be confident about the diagnosis, and many patients undergo repeated examinations over several years before the diagnosis is confirmed and an appropriate management plan initiated. Such delays are distressing for all concerned, and there is an intensive search for diagnostic markers in blood, muscle or cerebrospinal fluid.

Table 13.1

Summary of revised El Escorial research diagnostic criteria for ALS (Brooks et al., 2000)

The diagnosis of ALS requires:

[A:1] Evidence of LMN degeneration by clinical, electrophysiological or neuropathological examination;

[A:2] Evidence of UMN degeneration by clinical examination, and

[A:3] Progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination,

Together with the absence of:

[B:1] Electrophysiological and pathological evidence of other disease that might explain the signs of LMN and/or UMN degeneration, and

[B:2] Neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

Within these principles, one can diagnose:

Definite ALS

- UMN signs and LMN signs in three regions

Probable ALS

- UMN signs and LMN signs in two regions with at least some UMN signs rostral to LMN signs

Probable ALS – Laboratory supported

- UMN signs in one or more regions *and* LMN signs defined by EMG in at least two regions

Possible ALS

- UMN signs and LMN signs in one region (together),
- UMN signs in two or more regions, or
- UMN and LMN signs in two regions with *no* UMN signs rostral to LMN signs

UMN signs: clonus, Babinski sign, absent abdominal skin reflexes, hypertonia, loss of dexterity.

LMN signs: atrophy, weakness. If only fasciculation: search with EMG for active denervation.

Regions reflect neuronal pools: bulbar, cervical, thoracic and lumbosacral.

13.4.1.1. Symptoms and signs in ALS (Table 13.2)

Most patients with ALS present with symptoms related to local muscle weakness. Some notice wasting first (Fig. 13.2(A)) but usually weakness precedes obvious wasting. A few patients notice muscle cramps as the first symptom. Cramps are most common in muscles that become weak. Cramps tend to be more marked at night, to be more severe early in the evolution of the disease and to ease as the disease progresses. Cramps and fasciculations may precede weakness but are seldom the presenting symptoms. Fasciculations may not be perceived by the patient. Instead, the twitching may be noticed by a spouse or coincidentally by medical staff. True fasciculations are usually single twitches, reflecting contraction of single motor units. They are almost always accompanied by evidence of denervation on electromyographic (EMG) examination. Fasciculations may, of course, be frequent and multiple but will appear in different parts of the same muscle in a random fashion. They may be localized to one or two muscles, to a single limb, or they may be widespread, involving cranial nerve and limb muscles. Although the presence of early, florid and generalized fasciculations is usually an ominous sign indicating rapidly progressive disease, this is not always the case and occasionally one sees a patient with undoubted ALS, marked fasciculations and slow progression. For patients with bulbar onset ALS (Fig. 13.2(B)), the first symptom is almost always dysarthria, seldom dysphagia. Dysphagia may follow dysarthria within weeks or several months, occasionally after several years. Patients with bulbar onset ALS most frequently have the pseudobulbar syndrome comprising both upper and lower motor neuron involvement of the cranial nerve musculature, often with some lower facial weakness, a degree of nasality of speech, but predominantly with spastic dysarthria. They may first notice difficulty singing and they are commonly accused by family or friends of having drunk too much alcohol.

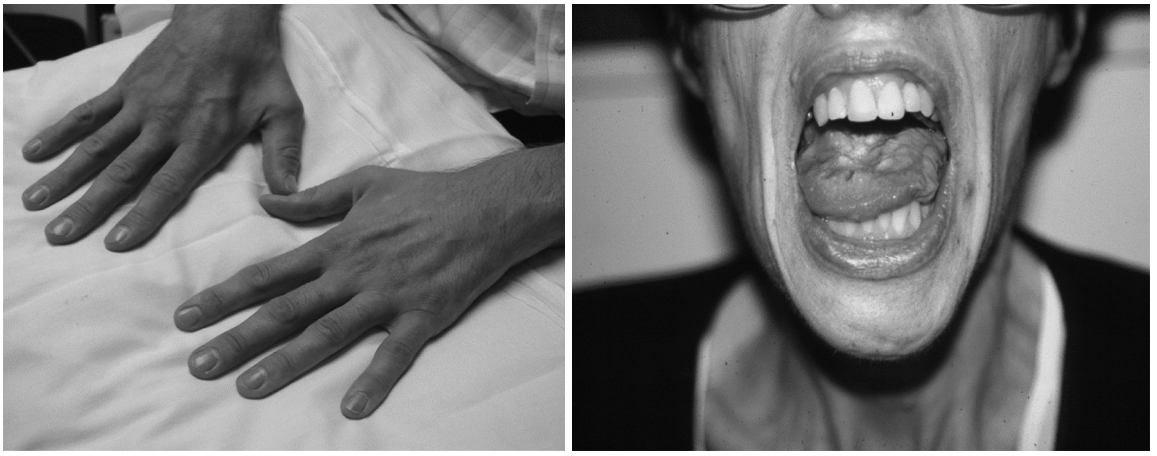
Table 13.2

Some clinical syndromes of ALS and related disorders (modified from Kato et al., 2003)

Syndrome	Main clinical features	Prognosis	References
Classical ('Charcot') ALS	Usually limb (spinal) onset of weakness; bulbar involvement usual later; combined UMN and LMN signs; M:F ratio 3:2	Median survival 3–4 years	Charcot and Joffroy, 1869; Tyler and Shefner, 1991
Progressive bulbar palsy (PBP)	Onset with dysarthria followed by progressive speech and swallowing difficulties; limb involvement usually follows within months. M:F ratio 1:1 (PBP relatively more common in older women)	Median survival 2–3 years	Duchenne, 1860
Progressive muscular atrophy (PMA)	Almost always limb onset; >50% develop UMN signs; ~85% develop bulbar symptoms; heterogeneous condition but majority are ALS; M:F ratio 3–4:1	Median survival ~5 years; more long survivors (>10 years)	Aran, 1850; Van den Berg-Vos et al., 2003a,b
'Flail arm syndrome'	A syndrome of predominantly LMN weakness of both arms; UMN signs develop in 50–70%; often slow progression; pathology is that of ALS	M:F ratio 9:1; syndrome may be more common in people of African and Asian origin	Hu et al., 1998; Tomik et al., 2000
'Flail leg syndrome'; 'pseudo-polyneuritic' form of ALS	A syndrome of progressive leg weakness, predominantly LMN.	Slow progression	
Monomelic motor neuron disease(s)	Rare ALS variant with slowly progressive focal (upper or lower limb) UMN and LMN syndrome. Rare LMN form most common in Asia (monomelic juvenile onset amyotrophy; Hirayama's syndrome)	Juvenile onset form is progressive over months or several years and then stabilizes; does not generalize; pathology unknown	Gourie-Devi et al., 1984, 1987; Gourie-Devi and Nalini, 2003a; Hirayama et al., 1987
Primary lateral sclerosis (PLS)	Clinically progressive pure upper motor neuron syndrome	20+ years	Pringle et al., 1992; Le Forestier et al., 2001
ALS with dementia (ALS-D; ALS with frontotemporal dementia-FTD)	ALS presenting as FTD or with FTD following motor signs. FH in ~50% of cases, linkage to 9q21-22; sometimes Parkinsonism; rare motor neuron degeneration in 17q and sporadic tauopathies	poor prognosis	Kew and Leigh 1992; Strong et al., 2003

Indeed, mild dysarthria is exacerbated by alcohol, so the problem often comes to light in this way. Limb weakness may develop almost simultaneously with dysarthria, or may be delayed by months or even several years and can involve first either the arms or the legs. It is usually asymmetrical but occasionally may be symmetrical. Other features that are common with bulbar onset ALS include emotional lability which may precede dysarthria or other symptoms and an increased tendency to yawn, sometimes with violent and even painful yawning. A few patients may yawn so as to dislocate their jaw. Sialorrhea is seldom a problem until patients have significant dysphagia.

Sphincter disturbance is absent at presentation in ALS, unless it is due to another coincidental disease. In the later stages of the disease urgency of micturition and even incontinence may occur in a minority of patients, reflecting the degeneration of Onuf's nucleus in the sacral spinal cord, despite its sparing relative to adjacent somatic motor neurons. Likewise, diplopia and weakness



A B

Fig. 13.2. (**A**) Wasting of small muscles of hands in ALS. (**B**) Wasting of facial, mandibular and tongue muscles in bulbar onset ALS.

of ocular muscles do not occur in ALS, reflecting selective preservation of the brainstem oculomotor nuclei. However, supranuclear abnormalities of ocular movements may appear late in the disease, occasionally to the extent of producing supranuclear palsy (Okuda et al., 1992; Kobayashi et al., 1999). Indeed, multi-system involvement generally becomes more evident late in the course of the disease and is particularly marked in patients who have been maintained for several years with assisted ventilation (Hayashi and Kato, 1989; Mizutani et al., 1992). Although studies of autonomic function have revealed subtle abnormalities (Dettmers et al., 1993; Provinciali et al., 1994; Oey et al., 2002), symptomatic autonomic disturbance is extremely rare and is usually limited to changes in the color and temperature of the paralyzed limbs, particularly the feet in patients with severe leg weakness. Sensory symptoms are not uncommon in ALS but are usually ephemeral and are not associated with abnormal sensory signs. Nevertheless, axonal loss in sensory nerves occurs in patients with undoubted ALS (Bradley et al., 1983). Although the presence of sensory axonal neuropathy in a patient with apparent ALS should trigger a thorough diagnostic review, it may represent another aspect of multi-system involvement in the disease. Cognitive abnormalities are rare at presentation and in most series less than 5% of patients present with the ALS-dementia syndrome. The use of more sensitive criteria increases that proportion to 50% (Lomen-Hoerth et al., 2002, 2003; see below).

Physical signs in bulbar onset ALS include the appearance of emotional lability, slurred speech which may have a nasal quality or be slow and slurred (spastic dysarthria) or be a combination of nasality and slurring reflecting the combined upper and lower motor neuron weakness involving the palate and glossopharyngeal muscles. Hoarseness is virtually never a feature of the dysarthria in ALS and inspiratory stridor is exceptionally rare, although it can occur. The jaw jerk is typically brisk and it may be easy to elicit a pout response. There may be weakness of the facial muscles, particularly of orbicularis oris, reflecting predominant upper motor neuron weakness. A lower motor neuron pattern of facial weakness is rather uncommon early in the course of ALS. Respiratory muscle involvement is variable in the course of bulbar onset ALS, but it is not easy to obtain an accurate record of the vital capacity and the accurate assessment of respiratory muscle weakness remains a challenge in this form of ALS (Lyall et al., 2001). The difficulty in performing the vital capacity test is partly due to difficulties with lip seal, but often in addition seems to reflect difficulty carrying out the procedure due to the upper motor neuron involvement – leading one to suspect a problem akin to apraxia. The gag reflex is usually well preserved (and may be very brisk) but the palate is often weak and the tongue shows bilateral fasciculations and often a degree of wasting laterally, even in the early stages of the disease. It can, however, be very difficult to be sure about fasciculation or fibrillation of the tongue, and it is important to observe the tongue at rest in the floor of the mouth when looking for evidence of fasciculations. Tongue movements are often slow. Lingual sounds can be tested by asking the patient to repeat "la la la" and labial sounds by asking the patient to repeat "pa pa pa." Weakness of neck flexion and extension is common in the later stages of the disease but patients may present with the "dropped head" syndrome. The latter, of course, is not restricted to bulbar onset disease. In the limbs the signs are those of any form of ALS, with a

variable combination of upper and lower motor neuron signs, upper limbs, lower limbs or both.

In limb onset ALS the symptoms start, by definition, in the arms or legs, and in the majority of cases will eventually involve the cranial nerve muscles although this is not inevitable and 10–15% of patients will remain able to speak until the terminal phases of the disease, although usually with some degree of dysarthria and dysphagia. In the arms, weakness most often manifests in the hands with loss of dexterity and difficulty with fine movements, such as using a car or door key, doing up buttons or opening bottles or cans. Less often patients present with proximal weakness, having difficulty lifting objects onto shelves, putting on clothes or carrying bags. About 5% of patients with ALS – most often limb onset ALS – present with severe respiratory muscle weakness and rapidly develop type 2 respiratory failure (de Carvalho et al., 1996; Chen et al., 1996). The first symptoms in such patients include orthopnea, disturbed sleep, morning headache, excessive daytime sleepiness, anorexia, depression, lack of concentration and other relatively non-specific symptoms that reflect nocturnal hypoventilation (Polkey et al., 1999; Leigh et al., 2003).

The syndrome of progressive muscular atrophy (PMA) comprises 10–15% of all cases with a final diagnosis of ALS and poses the greatest diagnostic challenge (Norris, 1991). Most patients with the PMA syndrome will eventually prove to have ALS and, although PMA is associated with a rather better prognosis than typical ALS (Norris, 1991), some patients presenting with PMA have rapid progression and early involvement of respiratory muscles. It has been suggested that patients with lower motor neuron syndromes (excluding multifocal motor neuronopathy (MFMN), Kennedy's disease and SMA with SMN gene mutations) with a history of more than 4 years from onset of weakness at the time of referral to a specialist center have a much better prognosis than those with a shorter history (van den Berg-Vos et al., 2003a,b). This distinction is arbitrary, of course, but probably reflects the poor prognosis of most LMN ALS patients (i.e. those with PMA) and the heterogeneous causes and clinical features of patients with slowly progressive motor neuron degeneration, labeled as having lower motor neuron disease (LMND) or late variants of spinal muscular atrophy (van den Berg-Vos et al., 2003a). In this study, most of the patients labeled as having sporadic, adult-onset LMND (spinal muscular atrophy) still had disease limited to one limb after a median follow-up of 12 years.

Two ALS variants that are particularly difficult to diagnose early in the course of the disease are the flail arm syndrome, also known as the Vulpian-Bernhart syndrome, the man-in-a-barrel syndrome, or progressive amyotrophic brachial diplegia (Hu et al., 1998; Gamez et al., 1999, 2000; Katz et al., 1999; Sasaki and Iwata, 1999; Fig. 13.3).

This distinctive variant often presents with localized weakness and wasting of one arm that only very gradually, over several years, evolves into the full syndrome of severe bilateral upper limb wasting and weakness. Patients with this syndrome quite often have strong legs, although upper motor neuron signs are relatively common in the legs and ultimately develop in about 70% of patients. Men are more often affected than women with a ratio of 9:1 (Hu et al., 1998), a striking departure from the ratio of 1.5:1 observed in more representative samples of ALS patients. The pathology is that of ALS (Sasaki and Iwata, 1999). Similarly, the flail leg syndrome poses a difficult diagnostic dilemma since it usually presents with a unilateral lower motor neuron foot drop and (as for the flail arm syndrome) it is common for weakness to progress proximally without accompanying upper motor neuron signs and to spread into the other leg in a similar fashion. All too often, it is difficult to demonstrate EMG evidence of acute or chronic partial denervation in the upper limbs and the patients remain in a diagnostic limbo for several years. The prognosis of these syndromes may be rather better than typical ALS (Hu et al., 1998) but it is variable and some of these patients are severely disabled before a firm diagnosis is made. One study indicated that the flail arm syndrome might be more common and, with a worse outlook, in patients of African descent (Tomik et al., 2000).

The syndrome of primary lateral sclerosis (PLS) also poses diagnostic difficulties, particularly in the first 3 years, since it is not uncommon for ALS to present in this way, with lower motor neuron signs emerging at a relatively late stage. The diagnostic criteria for PLS (Pringle et al., 1992) require a period of 3 years in which there are only upper motor neuron signs on examination before a diagnosis of PLS can be confirmed, but EMG studies can be misleading as evidence of chronic partial denervation may be evident in the absence of clinical signs of LMN involvement (Le Forestier et al., 2001). Furthermore, patients presenting with the PLS syndrome can develop lower motor neuron signs more than 5 years after the onset of symptoms, although this is rare (Bruyn et al., 1995; Strong and Gordon, 2005). Mills' syndrome is a term used to describe a rare form of idiopathic progressive hemiparesis. It may represent a variant of PLS (Mills, 1900; Gastaut and Bartolomei, 1994; Turner et al., 2005). Hereditary spastic paraparesis (HSP) may be confused with PLS (and ALS) and is discussed in relation to differential diagnosis below. PLS is considered in detail in Chapter 16.

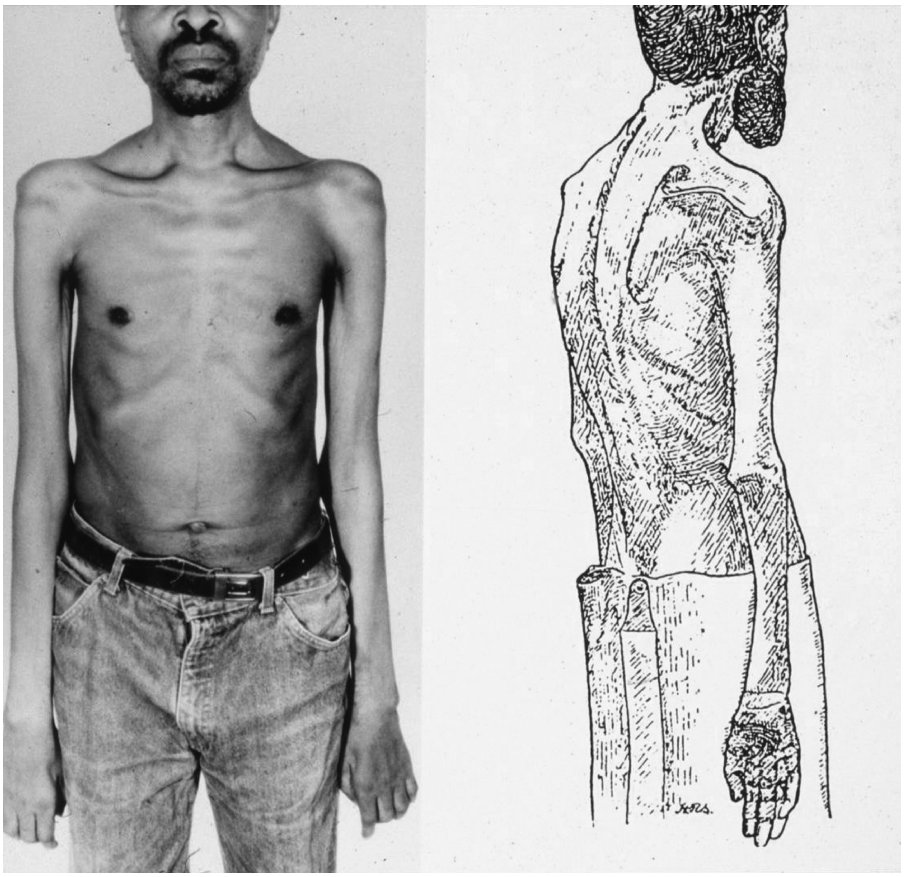


Fig. 13.3. "Flail arm syndrome," illustrating patient with typical pattern of weakness, and drawing by W. Gowers textbook (1883). Reproduced from Hu et al. (1998), with permission of authors and publisher.

13.5. Differential diagnosis of ALS (Tables 13.3 and 13.4)

In most cases, over a year elapses between onset of weakness and diagnosis (Chio et al., 2002; Sorenson et al., 2002; del Aguila et al., 2003; Millul et al., 2005). Since the average survival is about 3 years, this represents at least one-third of the duration of the disease; in some cases the diagnosis is not made until the patient only has a few months to live. It must be stressed that delay in diagnosis (and hence delay in starting treatment) arises not only through misdiagnosis. More frequently delays occur because patients present with atypical features. The flail arm and flail leg syndromes, PLS and presentation with respiratory symptoms are the main culprits here (see above). Benign fasciculation often causes consternation and diagnostic heart searching, although time will yield the truth. The syndrome comprises chronic muscle twitching with undoubted, electrophysiologically-confirmed fasciculations without progressive denervation (Blexrud et al., 1993). A survey of 121 patients (33% of them health workers) seen at the Mayo clinic with a diagnosis of benign fasciculation revealed no instance of ALS developing on long-term follow-up (Blexrud et al., 1993). The syndrome of benign fasciculation is not rare, affecting perhaps 1% of the population (Reed and Kurland, 1963; Rowland, 1985). Benign fasciculations are most common in the gastrocnemius muscle, may be associated with cramps and are exacerbated by exercise. Nevertheless, people presenting with fasciculations and cramps do very rarely develop ALS (de Carvalho and Swash, 2004).

Muscle cramps and twitching (with or without weakness) may reflect variants of autoimmune peripheral nerve hyper-excitability with myokymia (Rowland, 1982, 1985; Tahmoush et al., 1991; Hart et al., 2002; Gutmann and Gutmann, 2004; Lagueny, 2005). Myokymia typically manifests as undulating continuous muscle contractions, rather than the discrete random flickering of true fasciculation. EMG will usually distinguish myokymia from fasciculation, although fasciculation and fibrillation may accompany myokymia where there is axonal damage (Hart et al., 2002; Mills, 2003). Antibodies against voltage-gated potassium channels are present in about a third of patients with this syndrome and

Table 13.3

Motor neuron disorders (modified from Kato et al., 2003)

1. Genetically determined forms of motor neuron disorder:
Familial ALS

- Typical ALS (ALS 1, SOD1 mutations; loci at 16q, 18q, 20, Xc: Major-Krakauer et al., 2003)
- Atypical motor neuron disease (ALS 8: slow progression, predominantly LMN with Vesicle Associated Protein B gene mutations)
- ALS with frontotemporal dementia (9q21-2; 9p)

Brown-Vialetto-Van Laere syndrome (early onset bulbar and spinal ALS with sensorineural deafness)
Fazio-Londe syndrome (infantile progressive bulbar palsy)
Worster-Drought syndrome (Clark et al., 2000)
Hexosaminidase deficiency (GM2 gangliosidosis)
Hereditary spastic paraplegia (including ALS2 with *alsin* mutations; ALS4, with *senataxin* mutations; dHMNS-V or Silver syndrome with mutations of *BSCL2;* Troyer syndrome, SPG20, *spartin* mutations)
Spinal muscular atrophy (SMA)

- Proximal childhood and later onset forms of SMA (types 1–4), SMN gene related
- Adult onset proximal SMA (unrelated to SMN gene mutations)
- Congenital distal SMA (12q23-q24; van der Vleuten et al., 1998)
- Distal Hereditary Motor Neuropathy (dHMN types I–IV various phenotypes, mainly distal upper or lower limb; overlapping with Charcot-Marie-Tooth disease type 2)
- Juvenile or adult onset laryngeal and distal SMA (Harper-Young syndrome, HMN type VII; HMN type VII/SMA with vocal cord and facial weakness, *DCTN1* gene mutation)
- Severe infantile onset HMN-VI with respiratory distress (mutations in *IGHMBP2* gene; Grohmann et al., 2001)
- X-linked bulbar and spinal muscular atrophy (Kennedy's disease: expansion of CAG repeat in exon 1 of AR gene)
- Bulbospinal muscular atrophy *without* AR gene mutation (Paradiso et al., 1996)

Neuroacanthocytosis (syndrome of chorea with amyotrophy and areflexia, genetically heterogeneous; Rampoldi et al., 2002)
Polyglucosan body disease (Robitaille et al., 1980; McDonald et al., 1993; Bigio et al., 1997)
Algrove syndrome (Houlden et al., 2002)
Nyssen-van Bogaert syndrome (Larnaout et al., 1998)

2. Apparently sporadic (idiopathic) forms of motor neuron disorder:
Sporadic ALS (including limb and bulbar onset forms; PMA)
Primary lateral sclerosis
Distal sporadic focal spinal muscular atrophy – Hirayama syndrome
Distal SMA (heterogeneous forms; sporadic)
Atypical juvenile onset ALS in South India ('Madras' form of ALS)

Multisystem disorders in which anterior horn cells may be involved

Western Pacific and other similar forms of ALS (Guam, Kii peninsula, New Guinea)
Guadeloupe PSP-Dementia-ALS syndrome (Caparros-Lefebrve et al., 2002)
Progressive supranuclear palsy
Neurofilament inclusion body disease (Cairns et al., 2004)
Spinocerebellar atrophy (SCA) type 3; type 6 (Rosenberg and Fowler, 1981; Ohara et al., 2002)
Pantothenate kinase associated neurodegeneration (Vasconcelos et al., 2003)

3. Acquired forms of motor neuron disorder
Inflammatory and infectious disorders
Multifocal motor neuronopathy
Acute motor axonal neuropathy (AMAN)
Lewis-Sumner syndrome
HTLV-1 associated myelopathy (HAM)
HIV-associated ALS syndrome
Creutzfeld-Jacob disease (amyotrophic forms)
Acute poliomyelitis
West Nile fever
Lyme disease

Motor neuron disorders (modified from Kato et al., 2003)—*contd*

Toxins
Lead, mercury toxicity (Braff et al., 1952; Brown, 1954; Boothby et al., 1974)
Neurolathyrism (due to *lathyrus sativa,* containing fl–oxalyl-L-aminoacid, BOAA; Spencer, 1999)
Konzo (due to toxic cyanogenic cassava; Howlett et al., 1990)
Miscellaneous
Post-poliomyelitis muscular atrophy syndrome (PPMA)
Radiation radiculopathy (e.g. lumbo-sacral radiculopathy)
Autoimmune disorders (e.g. Sjögren's disease)
Endocrinopathy (hyperthyroidism, hyperparathyroidism, hypoglycemia)
Benign cramp/fasciculation syndrome

Some references have been provided for unusual conditions or rare associations. Other references are provided in the relevant part of the text.

malignancy, particularly of the thymus or lung, in a similar proportion (Hart et al., 2002).

In our experience, a more common (and entirely benign) syndrome is that of intermittent, repetitive muscle twitching. This is often described as fasciculation by those affected, who are usually health professionals, particularly medical students and doctors. Trains of twitches in part of a muscle may continue for several minutes. Such episodes draw the attention of the person affected to the area and the twitches vanish as unpredictably as they appear. They do not seem to be brought on by any factors other than exercise. Seldom does EMG examination reveal true fasciculations except sometimes in the calf muscles. Neurological examination reveals no abnormalities and on EMG denervation is absent. Repeated neurological and EMG examination may be required to reassure people with these symptoms that they do not have ALS.

Other diagnostic errors are not uncommon and regular review of diagnosis is a key aspect of specialist follow-up. In one survey over 40% of patients with a final diagnosis ALS were misdiagnosed initially and some had unnecessary operations (Belsh and Schiffman, 1990, 1996). In a population-based survey from Scotland (Davenport et al., 1996) diagnostic errors were recorded in about 10% of patients initially thought to have ALS. Some of these 'errors' included patients with ALS-dementia who did not fulfill the diagnostic criteria used in that study. It is arguable whether these cases should be considered as misdiagnoses, since it is now clear that ALS is a multi-system disorder (see above). 'True' misdiagnosis (i.e. diagnosis of ALS in patients with other diseases) occurred in 8% of the 552 patients included on the register and comprised patients with MFMN and other potentially treatable and certainly more benign conditions. In a survey of 437 referrals diagnosed initially as ALS in an Irish population-based study, 7% were considered to have other conditions (Traynor et al., 2000b). Twenty-two percent of these cases had MFMN and 13% had Kennedy's disease. The remainder comprised a miscellaneous group of cases. Most of the misdiagnosed cases were classified as suspected or possible ALS by the original El Escorial criteria, but a few (16% of the 32 misdiagnosed cases) met requirements for probable or definite ALS. The majority of misdiagnosed cases are LMN syndromes, although isolated bulbar syndromes also pose difficulties (Traynor et al., 2000b). In this context, Kennedy's disease is probably the condition most often diagnosed initially as ALS, although myasthenia gravis may occasionally mimic features of ALS, albeit lacking any UMN features (Visser et al., 2002). Kennedy's disease (see Chapter 8; Kennedy et al., 1968; Harding et al., 1982; Sinnreich and Klein, 2002; Lee et al., 2005) is usually easy to recognize as a LMN bulbar syndrome with prominent fasciculations of the tongue and lower facial muscles, sometimes resembling myokymia, but more coarse and irregular than true myokymia, postural upper limb tremor, gynecomastia, depressed or absent deep tendon reflexes and (usually evolving later in the progress of the disease) symmetrical proximal wasting and weakness. Because the disease evolves slowly, wasting of the tongue is often more marked than might be expected for the mild degree of dysarthria. Occasionally Kennedy's disease presents with distal weakness. EMG studies show widespread chronic partial denervation and sural nerve potentials are small or absent (Ferrante and Wilbourn, 1997). DNA analysis for the CAG repeat expansion in exon 1 of the androgen receptor (AR) gene is diagnostic. A similar syndrome of progressive bulbar and limb weakness associated with optic atrophy, electrophysiological changes akin to those of Kennedy's disease, but with no expansion of the CAG repeat in the AR gene, has been described in a brother and sister aged 61 and 58, respectively, at presentation (Paradiso et al., 1996).

Far less common even than Kennedy's disease are the Fazio-Londe syndrome and the Brown-Vialletto-van

Table 13.4

Diagnostic errors and most common 'ALS mimic syndromes' (modified from Kato et al., 2003, with permission)

Final diagnosis	Characteristic features	Distinguishing diagnostic features and investigations
Cerebral lesions	Focal motor cortex lesions very rarely mimic ALS, but frontal lesions with co-existent cervical or lumbo-sacral root damage may cause diagnostic confusion	MRI/CT; no EMG evidence of widespread chronic partial denervation (CPD) in limbs
Skull base lesions	Lower cranial nerve signs (bulbar symptoms and signs; wasting of tongue, often asymmetrical); seldom significant long tract signs unless foramen magnum involved in addition	MRI; CT with bone windows; no EMG evidence of CPD in limbs unless wasting of C8/T1 muscles (rare, but present in some lesions at foramen magnum or high cervical level)
Cervical spondylotic myelopathy	Progressive limb weakness. Asymmetrical onset; combined UMN and LMN signs in arm(s); spastic paraparesis; occasionally fasciculations in arms	Pain in root distribution, but pain may not be severe and may resolve quickly; often progression followed by clinical stabilization; no bulbar involvement; MRI evidence of spinal cord and root compression (NB: patients may have co-existent lumbo-sacral motor radiculopathy with lower limb denervation)
Other cervical myelopathies • Foramen magnum lesions • Intrinsic and extrinsic tumors • Syringomyelia	Progressive weakness; foramen magnum lesions and high cervical cord lesions may be associated with focal (C8/T1) wasting; syringomyelia usually associated with LMN signs and dissociated sensory loss	Usually involvement of cerebellar and/or sensory pathways; MRI of head and cervical spine reveal pathology
Conus lesions and lumbo-sacral radiculopathy	Progressive mixed UMN and LMN syndrome	Usually significant sensory symptoms if not signs; bladder involvement; MRI thoracic and lumbo-sacral region; EMG evidence of radiculopathy
Inclusion body myositis (IBM)	Progressive weakness; bulbar symptoms; sometimes respiratory muscle weakness	Characteristic wasting and weakness of deep fibers of flexor digiti communis and quadriceps femoris; EMG evidence of myopathy; muscle biopsy as definitive test (rimmed vacuoles)
Cramp/fasciculation/ myokymia syndromes	Cramps, undulating muscle contractions, ± weakness, stiffness (Isaac's syndrome; peripheral nerve hyper-excitability syndrome)	EMG evidence of myokymia; ~30% VGKC antibodies; ~20% associated with thymoma or lung cancer; association with other autoimmune diseases
Multifocal motor neuronopathy (MFMN)	Focal asymmetrical onset, often upper limb; pure LMN syndrome; may stabilize for months or years; M:F 4:1;	Conduction block on nerve conduction studies (NCS); weakness often out of proportion to wasting; improvement with intravenous immunoglobulin (IVIG) in ~70%
Kennedy's disease (X-linked bulbar and spinal muscular atrophy)	Males symptomatic; slowly progressive bulbar and limb weakness	Family history; fasciculations of facial muscles; gynecomastia; proximal symmetrical weakness in addition to foot drop; mild sensory neuropathy on NCS; positive DNA test for CAG repeat mutation in exon 1 of androgen receptor gene

Laere syndrome. Fazio-Londe disease is a rare (presumed) autosomal recessive or dominantly inherited form of progressive motor neuron degeneration with onset in infancy (McShane et al., 1992). The Brown-Vialetto-van Laere syndrome is heterogeneous clinically and genetically, with onset in childhood or adolescence, sensorineural deafness, progressive pontobulbar palsy and UMN and LMN signs in the limbs (Brucher et al., 1981; Hawkins et al., 1990; Megarbane et al., 2000; Sathasivam et al., 2000). Fazio-Londe syndrome and Brown-Vialetto-van Laere syndrome may overlap, as shown in one family in which four siblings born to consanguineous parents were variably affected by pontobulbar weakness, deafness and limb weakness (Dipti et al., 2005). Hexosaminidase deficiency (GM2 gangliosidosis) is also of early onset and usually associated with cognitive and neuropsychiatric changes in addition to an ALS-like syndrome (Johnson, 1982; Mitsumoto et al., 1985).

Monomelic amyotrophy (Hirayama's disease) is a syndrome of muscle wasting and weakness limited to one arm or less commonly to one leg, initially identified in Japan but subsequently recognized throughout Asia and less commonly seen in people not of Asian origin (Hirayama et al., 1959; Hirayama et al., 1963; Peiris et al., 1989; Gourie-Devi and Nalini, 2003a). Typically this syndrome affects young men aged between 15 and 40. The ratio of men to women is about 4:1. The disorder is sporadic, although rarely it may be familial (Nalini et al., 2004). The muscle wasting and weakness develop insidiously and in the arms is usually distal, affecting mainly the C8 and T1 innervated muscles. When the legs are affected the wasting and weakness is usually distal but may affect the whole limb. Although sensation is normal, pain and stiffness is precipitated or exacerbated by cold. The tendon reflexes are depressed. Weakness often progresses over 2 to 4 years and then stabilizes, although further weakness may evolve for up to 8 years (Peiris et al., 1989; Gourie-Devi and Nalini, 2003a). Although weakness may affect both arms, weakness remains asymmetrical in almost all. Seldom, if ever, does weakness spread to involve other parts of the body and UMN signs are absent. Pathological studies in two patients who died of coincidental causes showed atrophy of the affected region of the spinal cord with severe, asymmetrical, bilateral loss of anterior horn motor neurons (Hirayama et al., 1987; Araki et al., 1989). EMG studies show chronic partial denervation in the affected muscles and there may be evidence of denervation in the corresponding muscles of the contralateral limb. Motor and sensory conduction is normal. CSF examination is usually normal. Some have reported abnormalities of the cervical spinal cord on MRI. These changes comprise asymmetrical atrophy of the cord and forward displacement of the spinal cord on neck flexion. It has been suggested that this is the likely pathogenic mechanism through intermittent spinal cord compression and ischemia, but this has not been confirmed in other series (Hirayama, 1991; Schroder et al., 1999; Restuccia et al., 2003; Willeit et al., 2001). There is no evidence that trauma is implicated in the mechanism of neuronal damage. The pathogenic mechanisms underlying Hirayama's syndrome thus remain obscure. One case has been associated with a mutation in mitochondrial DNA (Fetoni et al., 2004).

A syndrome of early onset sporadic ALS was first recognized in Southern India in 1970 (Meenakshisundaram et al., 1970; Saha et al., 1997). The syndrome is that of ALS with bulbar and limb involvement. Although LMN signs predominate, especially in the cranial nerve territory, UMN signs are present and include brisk tendon reflexes and extensor plantar responses. The condition is twice as common in males as in females. Symptoms usually start in the second or third decades and most commonly comprise distal upper limb weakness with wasting, dysarthria, dysphagia and progressive sensorineural hearing loss. The latter is present in over half of affected patients, is progressive and is a characteristic feature distinguishing this syndrome from other forms of early onset sporadic ALS. Spasticity and distal leg weakness commonly occur and the course is of progressive disability but with long survival. EMG studies show chronic partial denervation. Other investigations serve to exclude alternative diagnoses. One case has been examined pathologically (Shankar et al., 2000). The spinal cord showed loss of anterior horn cells and brainstem motor neurons, neuronal loss and gliosis in the cochlear nucleus, and degeneration of the corticospinal tracts, with astrocytosis and microgliosis. There was also demyelination and axonal loss in the cochlear nerve. No comment was made on the presence or absence of ubiquitin immunoreactive inclusions.

There are many variants of hereditary spastic paraplegia, some of which (including at least one family found to have an *alsin* gene mutation) have lower motor neuron features, but all are slowly progressive predominantly upper motor neuron syndromes (Fink, 2001, 2003; Rowland, 2005). There is clinical overlap between HSP and PLS (Brugman et al., 2005; Strong and Gordon, 2005). Silver syndrome (Silver, 1966; Irobi et al., 2004a,b; Warner et al., 2004; see below) is a mixed UMN and LMN syndrome that overlaps HSP and hereditary motor neuropathy. Troyer syndrome (SPG20) associated with *spartin* mutations may exhibit both UMN and LMN signs (Auer-Grumbach et al., 1999; Patel et al., 2002).

Spinal muscular atrophy (SMA) presenting later in life can pose diagnostic difficulties. Patients with apparently sporadic LMN syndromes who survive more than 4 years from the onset of symptoms are likely to have

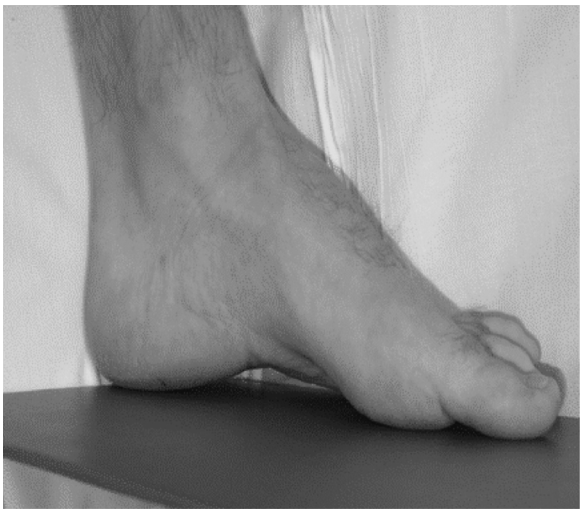


Fig. 13.4. Pes cavus and mild distal muscle wasting in patient with hereditary motor neuronopathy.

relatively benign forms of late onset SMA (van den Berg-Vos et al., 2003a,b). Some of the later onset predominantly proximal and symmetrical forms of spinal muscular atrophy are SMN gene related and, therefore, can be identified by genetic testing. Genetic testing is now becoming available for some forms of hereditary motor neuropathy (HMN), also variously termed distal spinal muscular atrophy and Charcot-Marie-Tooth disease type 2 (Irobi et al., 2004a). There is much phenotypic variation even within families with the same mutation. Clues to the diagnosis of HMN/SMA include slow progression, family history (although this may not be apparent without persistent enquiry), pes cavus and other features such as vocal cord involvement (Table 13.3; Fig. 13.4) and the genetic abnormalities most frequently associated with a pure or predominant motor disorder comprise mutations in heat shock protein (HSP) genes (*HSPB8/HSP22* and *HSPB1/HSP27*), the Berardinelli-Seip congenital lipodystrophy gene (*BSCL2*), the glycyl tRNA synthetase gene (*GARS*), the immunoglobulin μ-binding protein 2 gene (*IGHMBP2*) and the dynactin gene (*DCTN1*) (Irobi et al., 2004a,b; Sivakumar et al., 2005). There are at least five other loci for which the mutant genes are still unknown. ALS4, associated with senataxin gene mutations, is an autosomal dominant disorder with slowly progressive distal wasting and weakness starting in the lower limbs associated with pyramidal signs (Rabin et al., 1999; Chen et al., 2004), whereas the autosomal dominantly inherited form of HMN due to *GARS* mutations tends to affect distal upper limb muscles and may also have pyramidal signs. Several forms of HMN either present with or may develop vocal cord paralysis (Table 13.3). Examples include the LMN syndrome associated with *DCTN1* mutations and several forms for which the causative genes are as yet unknown (Irobi et al., 2004a).

Inclusion body myositis (IBM) may masquerade as a LMN form of ALS. IBM is probably the most common type of myopathy in people over 50 years (Griggs et al., 1995; Munshi et al., 2006). In a study of 70 patients with IBM, nine (13%) had originally been diagnosed as ALS (Dabby et al., 2001). Asymmetrical upper or lower limb onset of weakness and wasting, but absence of the characteristic weakness of finger flexion and quadriceps at presentation, preserved or even (apparently) brisk tendon reflexes, dysphagia, the presence of positive sharp waves, fibrillation and fasciculation potentials on EMG examination, with (in one case) apparent tongue involvement, and the lack of EMG evidence of a myopathic process, were all factors that led to misdiagnosis. The clue to the diagnosis of IBM in almost every case was the development of relatively selective weakness of finger flexors, although this was present at onset in only one case. Thus, IBM should always be considered in patients with a predominantly LMN syndrome and muscle biopsy is the only investigation that is likely to yield the diagnosis. Re-examination of patients in whom there is diagnostic uncertainty is vitally important. Although treatment of IBM is unsatisfactory, the outlook is certainly better than for ALS (Tawil and Griggs, 2002). Polymyositis may occasionally be mistaken for ALS. In a biopsy-proven case of polymyositis described by Ryan et al. (2003), clinical features included dysphagia, possible UMN signs and EMG evidence of fasciculations, with a normal creatine kinase level.

Other conditions that cause diagnostic confusion include focal mechanical lesions of the brain stem, spinal cord or nerve roots (Dagi et al., 1987; Visser et al., 2002; Kleopa et al., 2003). MRI combined with EMG and NCS will usually identify lesions such as mass lesions at the skull base, neurofibroma of the cervical spinal cord or cauda equina; syringomyelia, combined cervical and lumbar radiculomyelopathy, or focal lesions at the conus. Wasting of the small hand muscles may be associated with a lesion at the foramen magnum or in the high to mid-cervical spinal cord (Symonds and Meadows, 1937; Goodridge et al., 1987; Sonstein et al., 1996; Mathews, 1998; Sawaya, 1998), possibly due to vascular changes in the lower cervical expansion secondary to arterial compromise in a watershed territory or due to venous congestion and impaired perfusion. Most cases in which hand wasting is attributable to high or mid-cervical lesions also have neck pain and sensory symptoms (paresthesiae and/or numbness) in the hand. A large central cervical disk protrusion at C3/4 is probably the most common cause for the bilateral

"numb clumsy hand" syndrome but seldom is this mistaken for ALS – it is more often the indolently progressive lesions such as benign tumors at the foramen magnum or high cervical region that manifest initially as a predominantly motor syndrome, although hand wasting may also be associated with the Arnold-Chiari malformation in the absence of syringomyelia. Focal lesions at the lung apex, as with a pancoast tumor, cause wasting of intrinsic hand muscles, although there is also usually pain and sensory change. A cervical rib or band likewise may be considered in a patient presenting with hand wasting, but pain, sensory disturbances and electrophysiological and imaging studies will usually resolve the issue (Huang and Zager, 2004).

Multisystem disorders in which clinical (in addition to histopathological) manifestations of corticospinal tract and more rarely anterior horn cell involvement occur include some various forms of frontotemporal dementia (FTD; see below), forms of spinocerebellar atrophy including SCA6 and perhaps SCA3 (Ohara et al., 2002; Seilhean et al., 2004), multiple system atrophy (MSA), progressive supranuclear palsy and corticobasal degeneration (Neary et al., 1990; Kew and Leigh, 1992; Strong et al., 2003; Kertesz et al., 2005; Mott et al., 2005). Anterior horn cell degeneration in these disorders is always accompanied by prominent features of basal ganglia, cerebellar and/or cortical involvement appropriate to the predominant syndrome. With the exception of ALS with dementia, these are examples of multisystem disorders in which involvement of UMNs and LMNs is usually overshadowed by damage to other systems. The amyotrophic form of Creutzfeld-Jacob disease undoubtedly occurs, but is rare and was probably diagnosed more commonly before it was widely recognized that frontotemporal dementia is part of the spectrum of ALS (Allen et al., 1971; Salazar et al., 1983; Worrall et al., 2000).

HTLV1 associated myelopathy typically produces a slowly progressive spastic paraparesis (HTLV1-associated myelopathy, HAM; tropical spastic paraparesis, TSP) with early involvement of the bladder (Román et al., 1991). It may occasionally be associated with an ALS-like syndrome, with wasting and fasciculation of the tongue and limbs, pyramidal signs and EMG evidence of widespread denervation (Kuroda and Sugihara, 1991; Román et al., 1991; Ishida et al., 1997; Matsuzaki et al., 2000; Silva et al., 2005). In a survey of 606 HTLV-1 infected individuals from Brazil, 169 satisfied criteria for HAM and five patients had ALS. Two of these were El Escorial probable and three El Escorial definite (Silva et al., 2005). All had bladder symptoms and back pain. Autopsy was performed on one of the ALS patients. The brain and spinal cord showed loss of brainstem and spinal cord motor neurons, myelin loss in the corticospinal tracts, infiltration by inflammatory cells, but no Bunina bodies or ubiquitin-immunoreactive skeins. Thus, the molecular hallmark of typical ALS was lacking. In another autopsied case (Kuroda and Sugihara, 1991) the clinical features were compatible with ALS and there was pathological evidence of inflammatory infiltration associated with degeneration of motor systems. In a series of 15 patients with both UMN and LMN signs compatible with ALS, five had high viral load in their CSF similar to patients with HAM, whereas in 10 of the ALS cases the viral load was similar to that in HTLV-1 carriers (Matsuzaki et al., 2000). In the five ALS cases, CSF serology for HTLV-1 was positive. This does not, however, prove a causal link between HTLV-1 infection and an ALS-like syndrome. Nevertheless, it is important to consider HTLV-1 infection in the differential diagnosis, particularly if the individual originated from a country or community known to be at risk.

Likewise, a causal relationship between infection by the human immunodeficiency viruses (HIV-1, HIV-2) infection and ALS remains unproven (Verma and Berger, 2006) although reports that some patients with HIV infection and an ALS syndrome have improved with highly active anti-retroviral treatment (HAART) would support a pathogenic link (Jubelt and Berger, 2001; MacGowan et al., 2001; Moulignier et al., 2001; Calza et al., 2004). However, not all patients with HIV infection and ALS respond to HAART (Galassi et al., 1998; Verma and Berger, 2006) and the phenotype of the patients is not entirely typical of ALS, the patients being on the whole younger and with more rapidly progressive disease than is typical for ALS (Galassi et al., 1998; Jubelt and Berger, 2001). However, the flail arm syndrome (brachial amyotrophic diplegia) has been associated with HIV infection (Berger et al., 2005). There is no indication that the anti-retroviral agent indinavir is effective in ALS patients who are HIV negative (Scelsa et al., 2005). It is interesting that serum reverse transcriptase (RT) activity is increased in ALS (Andrews et al., 2000; Robberecht and Jubelt, 2005; Steele et al., 2005). Further evidence that HIV is a pathogenic factor for an ALS syndrome in this situation is required. It has been suggested that all patients with ALS should be tested for HIV infection (von Giesen et al., 2002). Certainly HIV testing should be considered in ALS patients with risk factors for HIV infection.

Despite the great successes of the polio eradication program, acute paralytic poliomyelitis probably remains the most common motor neuron disorder worldwide (Kidd et al., 1996; Wyatt, 1998; Howard, 2005; Paul, 2005). Vaccine-associated paralytic poliomyelitis (Kohler et al., 2002) is associated with transmission of live virus to unimmunized or immunocompromised individuals

following administration of the (oral) Sabin vaccine. There is no convincing evidence that infection with poliovirus or indeed with other viruses causes ALS (Martyn et al., 1988; Swingler et al., 1992; Jubelt and Lipton, 2004; Nix et al., 2004; Pamphlett et al., 2005).

According to Nollet and de Visser (2004) the first description of progressive weakness developing many years after acute paralytic poliomyelitis was made by Fulgence Raymond in 1875. Charcot considered this to be a case of PMA – what we would now call a LMN form of ALS. The coincidental occurrence of ALS on a background of paralytic poliomyelitis undoubtedly occurs, but the post-polio progressive muscular atrophy syndrome (PPPMAS) is quite different from ALS in its trajectory. PPPMAS can be differentiated from ALS by its development many years after an episode of acute paralytic poliomyelitis, by its very slow progression and by the lack of upper motor neuron signs, unless there are upper motor signs attributable to other types of pathology (Dalakas, 1995; Dalakas et al., 1986; Ramlow et al., 1992; Nollet and de Visser, 2004; Howard, 2005). It is not a life-threatening condition (Dalakas et al., 1986). It is usually regarded as a syndrome of motor decompensation related to ageing. Typically, several decades elapse between the acute paralysis and the onset of PPPMAS. Often no objective change in muscle strength can be detected on manual muscle testing over several years in patients who nonetheless complain of progressive weakness or of progressive difficulty carrying out tasks that formerly had seemed easy. The newly perceived fatigue, pain, cramps, wasting, weakness and functional deterioration develops in areas overtly or subclinically affected during the acute attack. Care must be taken to assess coincidental neurological disorders (e.g. pressure palsies; cervical or lumbar degenerative disk disease) and to identify orthopedic complications of longstanding weakness and limb dysfunction. PPPMAS may affect one third of patients who have had acute paralytic poliomyelitis (Ramlow et al., 1992; Ragonese et al., 2005).

Other acute viral infections can cause acute motor neuron damage. Enterovirus 71 has been linked to acute flaccid paralysis with encephalitis (Solomon and Willison, 2003) and many other viruses have also been associated with acute flaccid paralysis (Howard, 2005), as have infections with rickettsiae, mycoplasma and Borrelia (see below). Perhaps most consistently associated with acute flaccid paralysis is West Nile virus (Gadoth et al., 1979; Jeha et al., 2003; Leis et al., 2003; Madden, 2003; Park et al., 2003; Solomon and Willison, 2003). Infection with West Nile virus (WNV) is mediated by mosquito vectors, usually of the *Culex* genus. WNV is a single-stranded RNA virus of the genus *flavivirus*. First recognized in 1937 in Uganda, epidemic infection caused by WNV has been recorded in Russia, Israel, Romania and North America. The first known outbreak in the USA was in New York City in 1999. About 20% of people infected with the virus experience systemic symptoms and only 1% of those develop neurological complications. Most common are features of meningoencephalitis. Muscle weakness has occurred in 15–50% of symptomatic individuals in different epidemics. Paralysis of LMN type may be asymmetrical, mimicking acute anterior poliomyelitis or symmetrical, mimicking acute Guillain-Barré syndrome (Madden, 2003; Al-Shekhlee and Katirji, 2004; Bhangoo et al., 2005). It may be manifest as respiratory failure (10–25%). Other neurological complications include axonal polyneuropathy (~10%) and occasionally extrapyramidal syndromes, coma, seizures, ataxia, tremor, optic neuritis (Madden, 2003). The CSF shows the typical picture of viral meningoencephalitis with predominantly lymphocytic pleocytosis and normal glucose and protein. EMG studies reveal widespread acute denervation, even when weakness is localized (Al-Shekhlee and Katirji, 2004).

Lyme disease has been described as causing a progressive motor neuron disorder. Hemmer et al. (1997) described a 33-year-old man with a 15 month history of weakness of one hand and progressive gait disturbance, UMN and LMN signs in the limbs, and EMG evidence of denervation in the small hand muscles. There was no evidence of tick bite, erythema migrans or arthralgia, although he lived in an endemic area for Lyme disease. There was no evidence of peripheral neuropathy. Serum and CSF levels of IgG antibodies to *Borrelia burgdorferi* were raised and oligoclonal bands in CSF were specific for *Borrelia burgdorferi*. Total protein in the CSF was not raised and there was no pleocytosis. The patient was treated with doxycycline for 2 weeks soon after developing symptoms, followed by cefotaxim intravenously for 5 days. He did not improve with these treatments but, 6 months later, after further treatment with ceftriaxone for 2 weeks followed by prednisolone for 10 weeks, he gradually improved and 18 months after the onset of symptoms he was able to work, without physical impairment. Although suggestive, this case report does not prove that *Borrelia burgdorferi* infection was responsible for this man's symptoms and signs. It seems reasonable to consider Lyme disease as a possible cause of an ALS mimic syndrome in patients with possible exposure to tick bites or when CSF analysis unexpectedly reveals oligoclonal immunoglobulin bands. It must be remembered that antibodies to *Borrelia burgdorferi* are common in people living in endemic areas (Halperin et al., 1990).

About 5% of patients undergoing radiation therapy for breast cancer develop brachial plexopathy, but sensory changes and pain are almost always prominent

(Olsen et al., 1993; Jaeckle, 2004). EMG studies revealed a syndrome of myokymia and motor nerve conduction block was found in 11 out of 14 plexuses examined in patients with radiation-induced brachial plexopathy (Esteban and Traba, 1993). A syndrome of delayed progressive bulbar dysfunction with myokymia has been described (Glenn and Ross, 2000). Post-irradiation myelopathy can produce a pure lower motor neuron syndrome in the legs (Greenfield and Stark, 1948; Bradley et al., 1991; Lamy et al., 1991; Bowen et al., 1996) although lumbosacral radiculopathy due to para-aortic and midline irradiation for lymphoma or testicular cancer should no longer occur as radiotherapy regimes have been modified to avoid high radiation doses to cervical and lumbosacral nerve roots (Bowen et al., 1996). Post-irradiation lumbosacral radiculopathy comprises a syndrome of progressive weakness of the legs, with sensory symptoms but often without significant objective sensory signs and without loss of sphincter control or sexual function (Bradley et al., 1991; Bowen et al., 1996). The latency between radiotherapy and onset of symptoms varies between a few months and several decades. Wasting may not be apparent even in weak muscles, indicating that conduction block may contribute to weakness, although it is hard to demonstrate. MRI with gadolinium may show nodular enhancement of the conus and cauda equina (Bowen et al., 1996; Hsia et al., 2003). The CSF protein may be normal or elevated. Histopathology has shown a vasculopathy of the proximal spinal nerve roots, with preservation of anterior horn cells and spinal cord architecture (Bowen et al., 1996). The condition may progress for months or years, but may stabilize for periods of several years, and does not improve significantly with steroid therapy or intravenous human immunoglobulin.

ALS or an ALS-like syndrome may occur as a paraneoplastic syndrome (Brain et al., 1965; Barron and Rodichok, 1982; Evans et al., 1990; Younger et al., 1990; Rosenfeld and Posner, 1991). Epidemiological studies have failed to find a convincing association between ALS and most forms of cancer (Jokelainen, 1976; Evans et al., 1990; Rosenfeld and Posner, 1991; Freedman et al., 2005), although an association between ALS and melanoma has been mooted (Freedman et al., 2005). While a link between lymphoma, paraproteinemia or macroglobulinemia and ALS has been suggested (Younger et al., 1990, 1991; Rowland et al., 1995; Gordon et al., 1997), this has not been supported by epidemiological evidence and the apparent association may reflect a referral bias at one center. This does not exclude the possibility, however, that lymphoma and/or paraproteinemia may occasionally cause ALS or an ALS-like syndrome (Rowland et al., 1995). The range of lymphoproliferative disorders associated with ALS in the Columbia series (Gordon et al., 1997) included Hodgkin and non-Hodgkin lymphoma, macroglobulinemia, myeloma, POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes), Kikuchi necrotizing lymphadenitis, mycosis fungoides and other variants of lymphoma and paraproteinemia. Gericke et al. (1995) described a patient with ALS associated with multiple myeloma and a POEMS variant. Anti-Ma2 antibodies have been linked to an encephalitic illness with muscular weakness and wasting (Waragai et al., 2006). An ALS-like syndrome has also been associated with anti-Hu antibodies and breast cancer may be linked to an UMN syndrome without anti-Hu antibodies (Forsyth et al., 1997). Predominantly motor neuropathies or neuronopathies can be associated with anti-Hu antibodies (Graus et al., 2001) and a patient with progressive weakness and UMN and LMN signs had high titers of anti-Yo antibodies associated with a severe motor neuronopathy (Khwaja et al., 1998). In a survey of 92 patients with breast and gynecological cancer whose serum was tested for anti-neuronal antibodies, 63 patients had such antibodies. Two patients with breast cancer had ALS, but these patients did not have anti-neuronal antibodies (Rojas-Marcos et al., 2003). It is unlikely that benign monoclonal gammopathy of unknown significance (MGUS) is causally related to MND. Overall, a causative association between ALS and cancer cannot be regarded as proven, although examples of remission of motor syndromes similar to or even indistinguishable from ALS following treatment of cancer of various kinds suggests that ALS-mimics may be causally linked to cancer (Buchanan and Malamud, 1973; Rosenfeld and Posner, 1991; Rijnders and Decramer, 2000).

Autoimmune disorders such as Sjögren's disease and Systemic Lupus Erythematosus are described as mimicking ALS (Forns et al., 1992; Delalande et al., 2004). Autoimmune hyperthyroidism may present with muscle weakness, wasting and fasciculation – Basedow's disease (Rosati et al., 1980; Chotmongkol, 1999). Hyperparathyroidism may present with weakness and brisk reflexes and a causal relationship between hyperparathyroidism has been suggested (Patten and Pages, 1984), but is almost certainly coincidental (Jackson et al., 1998). Bulbar involvement in myasthenia gravis, the *mysthaenic* syndrome and botulism occasionally cause diagnostic confusion but this can be resolved speedily by the appropriate investigations.

13.6. Multisystem involvement in ALS: Dementia and ALS

Some aspects of multisystem involvement in motor neuron disorders have been mentioned above. There is

now abundant evidence that ALS is associated with dementia and that "Charcot" ALS represents one end of a spectrum of brain and spinal cord pathology linked by a common molecular pathology. ALS with dementia (ALS-D) is a frontotemporal dementia (FTD) associated with a characteristic molecular pathology and there is overlap with FTD lacking clinical features of motor neuron degeneration (Mitsuyama and Takamatsu, 1971; Mitsuyama and Takamiya, 1979; Hudson, 1981; Mitsuyama, 1984; Neary et al., 1990; Kew and Leigh, 1992; Okamoto et al., 1992; Wightman et al., 1992; Wilson et al., 2001; Mackenzie and Feldman, 2003, 2005; Strong et al., 2003; Bigio et al., 2004; Lipton et al., 2004; Johnson et al., 2005; Kertesz et al., 2005; Mott et al., 2005; Strong and Gordon, 2005). The molecular hallmark of ALS-D is the presence of intraneuronal, and in some cases intra-nuclear, ubiquitin-immunoreactive (UBIR) inclusions in the cortical areas most affected, with or without UBIR inclusions in brainstem and spinal motor neurons (Okamoto et al., 1992; Wightman et al., 1992). It has been suggested that ALS and ALS-D might be considered as a "gehrigopathy," "gehrig" being the hypothetical protein that forms complexes with ubiquitin in these inclusions (Ince and Morris, 2006). However, the situation is complicated by the fact that the clinical syndrome of FTD with ALS also comprises cases in which the molecular pathology is that of a tauopathy (Ince and Morris, 2006) and possible intermediate filament inclusion body disease (Bigio et al., 2003; Makenzie and Feldman, 2003). Such cases include familial FTD with tau gene mutations (17q-linked FTD and related syndromes) and other examples of Pick disease (Wilhelmsen et al., 1994; Ince and Morris, 2006). In the experience of most centers ALS-D is rare, affecting perhaps 5% of the clinic population. The criteria used in the studies that have identified a higher prevalence of dementia are sensitive but arguably non-specific (Lomen-Hoerth et al., 2003). Nevertheless, it is now clear that 20–40% of ALS patients who are not clinically demented show cognitive impairments of frontal type on formal neuropsychological testing (Kew et al., 1993; Abrahams et al., 2000; Ringholz et al., 2005). These abnormalities may be more common in patients with the pseudobulbar syndrome than in MND with mainly limb involvement (Abrahams et al., 1997). Some patients develop aphasia and some show apraxia of speech, although the latter is difficult to define in the presence of communication difficulties due to dysarthria. The syndrome of primary progressive expressive or non-fluent aphasia (PPA) and primary progressive semantic aphasia (PPSA) appears to be part of the ALS spectrum (Caselli et al., 1993; Bak and Hodges, 2004). Thus, there exists a spectrum of cognitive impairment associated with ALS encompassing normal cognitive function, barely detectable changes in executive and memory functions, PPA, PPSA and FTD. The clinical correlates of cognitive change often go unremarked in the clinic. The spouse or caregiver of an ALS patient may comment that he or she is "not the same person," manifesting subtle changes in character and behavior. Such changes may reflect frustration or depression and impaired communication compounds the difficulties of assessing this complex situation. Undoubtedly such apparently minor changes in character ('personality') have a negative impact on care and influence prognosis (Olney et al., 2005). Neuropsychological and neuropsychiatric evaluation is helpful in this context.

The syndrome of Western Pacific ALS remains a pathogenic conundrum more than 50 years after it was first recognized (Koerner, 1952; Kurland and Mulder, 1954; Hirano et al., 1961a,b; Rodgers-Johnson et al., 1986; Wiederholt, 1999; Galasko et al., 2000; Plato et al., 2002). The incidence of ALS (known by the indigenous Chamorro people in Guam as '*lytico*') was noted to be far higher on the island of Guam in the Mariana archipelago than elsewhere in the world, and high incidence foci have been identified in the Kii peninsula of Japan (Kuzahara and Kokubo, 2005) and in western New Guinea (Spencer et al., 2005). Clinically, ALS in these areas is often usually indistinguishable from "Charcot" ALS elsewhere although it is also associated with Parkinsonism and dementia (referred to as "*bodig*" colloquially), the Guam ALS-Parkinsonism-dementia complex (ALS-PDC). Pathologically, the Guam syndrome is a tauopathy, although neurofibrillary tangles are not present within motor neurons. Instead, as in typical ALS, UBIR inclusions are found in brainstem and spinal motor neurons (Oyanagi et al., 1994). The cause of the western Pacific ALS-PDC syndrome remains elusive and has been attributed to genetic and environmental factors (McGeer et al., 1997; Plato et al., 2002; Majoor-Krakauer et al., 2005). A syndrome of Parkinsonism and dementia with features of progressive supranuclear palsy in Guadeloupe has been attributed to toxins derived from fruit or leaves of the *annonaceae* family (Caparros-Lefebvre and Elbaz, 1999; Caparros-Lefebvre et al., 2002; Steele et al., 2002; Caparros-Lefebvre and Lees, 2005). The Guadeloupe syndrome is occasionally associated with ALS.

13.7. Investigations in the diagnosis of ALS

Unfortunately, there is no specific diagnostic test for ALS. The approach to investigation of ALS must balance pragmatism against the requirement to exclude other

possible causes for the ALS syndrome, particularly treatable causes (Andersen et al., 2005). Pragmatism has to be a factor in a situation where many of the causes of ALS mimics and other motor neuron disorders are very rare, and the tests required to exclude them are very expensive. Thus, it may be justifiable (indeed, mandatory) to exclude hexosaminidase deficiency (GM2 gangliosidosis) in a patient presenting at the age of 20–30, even in the absence of a family history and appropriate background (Ashkenazi Jewish origin; consanguinity), but it would require evidence of multisystem involvement and cognitive change, to trigger these tests in a patient over the age of 40. There will always be scope for clinical judgment and rigid guidelines are unlikely to be respected. The clinician can reasonably establish a set of "core" or "essential" investigations that aim to reassure the patient (and physician) that everything practical and reasonable has been done to exclude treatable or more benign causes of the clinical syndrome in question.

Electrophysiological studies (NCS and EMG) are essential in every patient, whereas many of the blood tests carried out are done for the sake of completeness, not in the real hope of altering the diagnosis. The creatine kinase (CK) serum level is often raised but is seldom more than four or five times the upper limit of normal. Examination of the CSF is seldom helpful, although the total protein may be somewhat raised. Most neurologists would examine the CSF in atypical cases, particularly those in which a motor form of chronic inflammatory demyelinating or axonal polyneuropathy (CIDP or CIAP) is suspected. As indicated in Table 13.5, neuroimaging is used to exclude other diagnoses. Although altered signal in the posterior limb of the internal capsule on T2-weighted MRI scans has been attributed to degeneration of the corticospinal tracts in ALS, this appearance can be seen in normal individuals. Hypointense signal change in the motor cortex on T2-weighted or FLAIR sequences can be detected in about 50% of ALS cases but is not a reliable diagnostic marker (Pioro, 2003). Research techniques such as MR spectroscopy, diffusion weighted imaging, diffusion tensor imaging, magnetization weighted imaging, PET and SPECT, have not yet found a place in clinical practice but hold promise for the future (Leigh et al., 2002; Pioro, 2003).

The management of ALS and the assessment of nutrition, respiratory complications and palliative care are discussed in detail in Chapter 20. The key factors in providing care for people affected by ALS management include sensitivity to the needs of the individual and family, the involvement of a multi-disciplinary team and a coordinated approach (Miller et al., 1999; Leigh et al., 2003; Andersen et al., 2005).

Table 13.5

Schema for investigations of ALS and motor neuron disorders

Essential investigations

- EMG and nerve conduction studies
- Full blood count, ESR, CRP
- Biochemical screen (liver and renal function, electrolytes, calcium, glucose, creatinine)
- Creatine kinase
- Thyroid function tests
- Protein electrophoresis for paraproteins
- Autoantibody screen
- Chest X-ray
- Respiratory function tests (forced or slow vital capacity; sniff nasal or mouth inhibitory pressure)
- MRI of brain and spine (depending on clinical syndrome)

Commonly performed additional investigations, depending on clinical features

- Muscle biopsy
- CSF analysis (protein, cells, glucose, oligoclonal bands, cytology)
- Serum B12 and folate
- Blood gases
- Serum electrolytes
- Plasma protein electrophoresis
- Anti-neuronal antibodies
- Anti-ganglioside (IgM GM_1) antibodies
- Anti-acetylcholine receptor antibodies/anti-MuSK antibodies
- Anti-voltage-gated K^+ channel antibodies
- Tumor markers
- Mammography
- Blood and/or urine analysis for toxins (lead; manganese)
- HIV, HTLV1 serological testing (blood *and* CSF, if real possibility)
- Lyme disease serology (blood *and* CSF, if real possibility)
- Viral culture, serology (West Nile, poliomyelitis. Other)

Investigations rarely required

- White cell enzymes (hexosaminidase deficiency)
- Very long chain fatty acids (adrenomyeloneuropathy)
- DNA analysis (e.g. SOD1 mutations, AR mutations, etc.)

13.8. Summary and conclusions

ALS is a well-delineated clinical syndrome. In the majority of cases ALS and its variants are easily recognized by physicians with some experience in neurology. The salient clinical features were brilliantly described and the fundamental features of pathology delineated by the pioneers of clinical neuroscience in the 19th century. A new era of clinical science starting in the 1960s and

1970s with the widespread use of electrophysiology and with the revolution in neuroimaging. These tools have substantially improved the diagnostic process, although we still lack a simple diagnostic test for ALS. Diagnosis can be difficult and is often delayed. Expertise is essential if serious errors are to be avoided and treatable conditions missed. Accurate and prompt diagnosis, sensitive communication of the diagnosis, close involvement of the patient and family and an active, positive care plan are prerequisites of good management. Riluzole has a small impact on survival and no perceptible effect on function or quality of life. Maintenance of good nutrition almost certainly improves quality of life. Non-invasive ventilation prolongs survival and improves or maintains quality of life. Arguably, the introduction of non-invasive ventilation represents the most significant advance in care since ALS was described more than 130 years ago. It should be far more widely available. The role of palliative care, broadly defined, is likely to expand and has the potential to improve quality of life, and quality of death, for many people affected by ALS. Many people affected by ALS are well-informed about, and wish to participate in, research. The aim of that research must be to halt disease progression and then to restore function. Although the ALS research community is on the threshold of exciting advances in clinical, molecular and cellular science, it is impossible to predict when the various strands will finally combine to yield the treatments so urgently needed.

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Chapter 14

Familial amyotrophic lateral sclerosis

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14.1. Introduction

14.1.1. Background

In this chapter we will critically review the epidemiology, clinical phenotype and genetic basis of the heritable forms of motor neuron disorder that have been classified as amyotrophic lateral sclerosis (ALS). All of these conditions are due to single gene defects that result in a progressive degeneration of upper motor neurons in the pre-central gyrus and/or lower motor neurons in the brainstem and anterior horn of the spinal cord. Some result in a clinical phenotype and rapid progression that is identical to classical ALS, while others are atypical with slow progression and a phenotype that reflects predominantly upper or lower motor involvement. The identification of mutations in the Copper/Zinc superoxide gene (SOD1) in both sporadic and familial ALS has blurred the distinction between familial and sporadic disease. It has resulted in the development of transgenic animal and cellular models that recapitulate many of the clinical and pathological features of ALS. These permit a detailed dissection of pathogenic pathways and informed drug screening but SOD1 mutations account for only ~5% of all ALS cases. Mutations in three other ALS genes have subsequently been discovered (ALS2/alsin, Senataxin and VAPB), as well as four new loci (ALS3, ALS6, ALS7 and ALS-FTD). Yet many of these genetic disorders are atypical and progression from locus identification to gene discovery has been slow. It is hoped that the application of new technologies will accelerate progress in this field so that more genes can be identified which will improve our understanding of the causes of ALS and accelerate the development of more effective therapies.

14.1.2. Epidemiology

The lifetime risk for sporadic ALS by age 70 is about 1 in 1,000 (Chancellor and Warlow, 1992; Traynor et al., 1999) and in the UK 1 in every 380 death certificates states motor neuron disease as the cause of death (Johnston et al., in press). About 1 in 10 affected individuals have a family history of ALS, typically of autosomal dominant inheritance with age-dependent and incomplete penetrance. In some of these kindreds penetrance is > 90% and the lifetime risk for ALS approaches 1 in 2, which places a huge psychological burden on every family member (Cudkowicz et al., 1997). Males are more commonly affected than females in sporadic ALS with a ratio of 3:2, but the gender ratio in familial ALS in most surveys is close to 1:1 (Kurland and Mulder, 1955; Strong et al., 1991). Apart from a few notable exceptions (e.g. homozygous D90A SOD1 ALS), the clinical presentation of most patients with autosomal dominant ALS are identical to those of sporadic disease (Andersen et al., 1996).

The incidence of adult-onset classical ALS is 1–2 per 100,000 person-years and the point prevalence is between 5 and 13 per 100,000 (Mulder and Kurland, 1987). Three populations have a much higher prevalence of ALS raising the possibility of a strong genetic founder effect. ALS in the Chamorro people of Guam and the Marianas Islands, the Japanese on the Kii peninsula of Honshu Island, and the Auyu and Jakai peoples of southern West New Guinea where it is associated with Parkinsonism and frontotemporal dementia (FTD) and known as the ALS/PD complex. In most cases the brain pathology is characterized by neuronal loss in the frontal and temporal lobes, substantia nigra and lower motor neurons in the

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brain and spinal cord, accompanied by neurofibrillary tau tangles in paired helical filaments, similar to Alzheimer disease and distinct from those seen in FTDP due to tau mutations. In Guam it is very uncommon for ALS to overlap with the other phenotypes and the prevalence has fallen from 70/100,000 in the 1960s to 7/100,000 in the 1990s (Chen, 1995). Although many reports have proposed a genetic basis for ALS/PDC in Guam it does not conform to Mendelian patterns of inheritance and no cases have developed in anyone born after 1945. This strongly implicates exposure to an environmental toxin or infection. Candidate gene studies in affected kindreds, including tau (MAPT) and a genome wide scan for linkage have been negative (Perez-Tur et al., 1999; Morris et al., 2004). "Muro disease," as it is known in the Kii peninsula, has a more predictable phenotype with progression from Parkinsonism at disease onset followed by dementia, muscular atrophy and corticospinal tract signs (Kuzuhara and Kokubo, 2005). The prevalence in Kii is around 50/100,000 and has been stable since the first surveys in the 1960s. The fact that 70% of patients report another family member as affected more convincingly implicates this form of PDC having a genetic etiology but again no mutations have been found in MAPT (Kuzuhara et al., 2001). Two pure ALS cases were shown to have the I113T SOD1 mutation but many other ALS/PDC cases were negative effectively excluding SOD1 as a cause of the high prevalence (Kikugawa et al., 1997).

14.1.3. History

Two French physicians are responsible for the first reports of ALS. The term ALS and the first classic description come from Jean-Martin Charcot in 1869, followed by a series of lectures based on 20 cases and five autopsies published in 1874 (Charcot and Joffroy, 1869). The first possible familial ALS case, however, was reported earlier by Aran in 1850. He described a syndrome of progressive muscular atrophy in a sailor who developed cramps in the upper limbs followed by generalized muscular weakness which was progressive and ultimately fatal (Aran, 1850). He ascribed this to a muscle disease but he noted that the sailor had a sister and two maternal uncles who were affected by a similar condition. Progressive muscular atrophy was later identified as a disease of lower motor neurons and is a phenotype seen in many familial ALS kindreds and can be associated with mutations in SOD1 (particularly A4V) and VAPB (P56S) so it is possible that Aran's case did represent FALS.

14.1.3.1. Onset and survival

The peak age of onset of all ALS in clinic-based studies is about 56 years (Strong et al., 1991), although population registers generally report a higher age at onset of around 70 (Chancellor and Warlow, 1992; Traynor et al., 1999). Some report that familial ALS presents on average about a decade earlier than sporadic ALS. About 25% of affected individuals present with bulbar onset of symptoms, but for affected older women, this rises to about 50%. Factors associated with a worse prognosis include older age of onset, a short interval between symptom onset and diagnosis (reflecting aggressive disease), reduced vital capacity and bulbar involvement, probably because this increases the risk of aspiration and pneumonia (Louwerse et al., 1997; del Aguila et al., 2003). Prognostic factors can be modeled to predict survival with reasonable accuracy, although a significant minority of individuals survive more than 10 years even with clinical features suggesting a poor prognosis (Turner et al., 2002, 2003).

14.2. Clinical features

14.2.1. Classical or 'Charcot' familial ALS

Familial ALS is essentially indistinguishable in its clinical phenotype from sporadic ALS. Symptoms of progressive muscular weakness and stiffness are accompanied by the clinical signs of upper and lower motor neuron degeneration. Motor neurons controlling eye movements as well as bladder and bowel sphincters are usually spared as are neurons subserving the sensory and cerebellar pathways. The El Escorial diagnostic criteria describe a hierarchy of diagnostic certainty based on the number of regions clinically affected and supplemented by neurophysiological tests confirming muscle denervation. An important difference is that the individual and their clinicians may not realize the significance of the early symptoms which commonly leads to a significant delay in the diagnosis. This is often not the case in at-risk individuals from familial ALS kindreds. They may live in dread that symptoms of cramp, muscle twitching, stiffness or weakness which may have a benign explanation actually heralds the onset of ALS. Early diagnosis is more common when the condition is familial, even in the absence of signs that fulfill the El Escorial criteria.

The presence of sensory symptoms is not uncommon, but sensory signs should lead to a search for other explanations. Postmortem examination of spinal cords has shown that involvement of sensory tracts does occur in familial ALS and some forms of the disease, such as those due to the D90A mutation of SOD1, may have autonomic and sensory involvement. In addition, evidence from neurophysiological studies (Bosch et al., 1985) and from individuals who have been ventilated invasively, shows that involvement of the motor system is not exclusive in ALS, but simply occurs earlier in the motor system. Most neuronal populations degenerate eventually, but, in most

cases, death has occurred before this occurs in the natural history of the disease.

Those presenting with bulbar symptoms typically have slurred speech, difficulty chewing and swallowing and nasal regurgitation of food. Lower motor neuron features include wasting of the facial musculature, poor palatal elevation and a wasted, fasciculating tongue, which may cramp. Upper motor neuron features include a brisk jaw jerk, brisk facial reflexes and a slow moving, spastic tongue, often associated with a spastic dysarthria and dysphonia. People who present with bulbar involvement that is predominantly lower motor neuron may be labeled as having Progressive Bulbar Palsy and upper motor neuron bulbar involvement as Pseudobulbar Palsy but most cases become generalized and eventually fall within the diagnostic rubric of classical ALS. Regardless of the part of the nervous system first involved, spread tends to be contiguous, to adjacent regions first. This interesting observation should provide clues to the mechanism of neuronal death and has yet to be explained.

14.2.2. Frontotemporal dementia

Frontotemporal dementia (FTD) is a neurodegenerative disorder of the frontal and anterior temporal lobes and is of interest because of a phenotypic overlap with ALS. Although ALS is classically described as a disease of the voluntary motor system, in about 5% of cases it occurs in association with significant dementia with the phenotype of FTD (Neary et al., 1990). Clinical and imaging studies show that more subtle frontal lobe involvement also occurs in a significant proportion of people with ALS, even in the absence of frank dementia (Massman et al., 1996; Abrahams et al., 1997). Similarly, in many cases, individuals with FTD have evidence of motor neuron involvement, although this may be subclinical.

Frontotemporal dementia (FTD) is characterized at the outset by a personality change and socially inappropriate behavior with relative preservation of memory and cognitive functions. Language defects are common and may present with a semantic dementia or nonfluent aphasia (Hodges and Miller, 2001). Eventually there is a more global cognitive deficit and death often due to immobility and respiratory infection. There are striking similarities to ALS in that the age at onset is 56 years (Chow et al., 1999) and survival of pathologically proven FTD is only 4 years (Rascovsky et al., 2005). Pathologically, neurons in the superficial frontal cortex and anterior temporal lobes degenerate. The molecular pathology of FTD has been divided into at least three groups: a taupoathy, with cytoplasmic neurofibrillary tangles; ALS-like, with ubiquitinated and neurofilamentous cytoplasmic (± intra-neuronal) inclusions and the remainder lacking distinctive histopathological features (Piguet et al., 2004). In ~40% of FTD cases, there is a family history of dementia indicating a significant genetic contribution (Rosso et al., 2003). In many sporadic and familial FTD cases there is clinical and pathological evidence of an overlap with ALS (Lipton et al., 2004). Up to 40% of families with autosomal dominant FTD may have mutations in the *MAPT* gene encoding microtubule-associated protein tau (Lynch et al., 1994). This gene was first implicated in chromosome 17q21.1-linked families in which amyotrophy, Parkinsonism and FTD occur together (FTDP-17, DDPAC). Pathological mutations in *MAPT* have not yet been described in an individual with pure ALS. In a detailed clinical and EMG study of 36 FTD patients who were not known to have ALS or have a family history of ALS, definite or probable ALS was diagnosed in seven and 11 others had fasciculations and swallowing problems without EMG evidence of denervation (Lomen-Hoerth et al., 2002). Phenotypic overlap between FTD and ALS is increasingly recognized and when their pathophysiology is better understood many cases of FTD and ALS may be viewed as existing within the same disease spectrum.

14.3. Diagnosis, investigation and management

There is no objective test capable of proving the diagnosis of ALS. It remains a clinical diagnosis based on evidence of upper and lower motor neuron degeneration and the exclusion of conditions that can mimic ALS. These include acquired conditions that result in compression of the brainstem, spinal cord nerve roots and peripheral nerves, generalized motor neuropathy, multifocal motor neuropathy with conduction block, neuromuscular junction disorders, myopathy, infections such as HTLV1, Polio and HIV (discussed in detail in Chapter 12). Inherited motor neuron disorders also need to be explored including such as the hereditary spastic paraplegias (HSP), adrenoleukodystrophy and its adult onset variant adrenomyeloneuronopathy (ALD), proximal autosomal recessive spinal muscular atrophies (SMA), distal spinal muscular atrophies (dSMA, also known as hereditary motor neuronopathy), spino-bulbar muscular atrophy (SBMA, also known as Kennedy's disease) and autosomal recessive GM1 gangliosidosis. It should be remembered that some of the acquired diseases can have very long latencies and affect several generations through vertical transmission (e.g. HTLV1 associated myelopathy) and many of the inherited motor disorders can only be distinguished from familial ALS by careful phenotyping.

14.3.1. Determining familiality

Evidence that the disease is likely to be familial may only come to light after exhaustive exploration of the

extended kindred. This may involve several interviews and several members of the family and the oldest female relative is usually the best source of information. A complete record of the age and cause of death of all second degree relatives back two or three generations and including cousins should be recorded. Where there is a suspicious death, for instance following a rapidly progressive gait disturbance, dysarthria or dysphagia, the death certificates and most recent photographs should be sought. Alternative and outdated diagnoses that could be indicative of ALS include "creeping paralysis, glosso-labial pharyngeal paralysis or motor neuropathy." One must also try to test the veracity of the diagnosis of Alzheimer's disease in a demented relative to determine whether it might have been FTD, particularly if the onset is less than 65 years. Symptomatic clues to FTD include early behavioral change that predates major memory loss, becoming either over-active or withdrawn, making socially inappropriate comments or actions, a loss of word-finding and language skills while retaining comprehension. Much of this "evidence" of familiality is likely to be subjective, but it is surprising how many sporadic ALS cases turn out to be familial when a detailed family history and documentation is obtained.

14.3.2. Genetic counseling

In our experience, genetic counseling has become a significant component of the discussion that follows an initial diagnosis. Many people with sporadic ALS seek genetic counseling once they become aware that ALS can be genetic and are better informed about gene testing. The lifetime risk of ALS occurring in the siblings and children of an individual affected by sporadic disease is greater than the general population risk (~1 in 500–1000) because the newly diagnosed individual might have developed a spontaneous mutation and FALS has an age-dependent and variable penetrance. Thus, mutation carriers in previous generations may not have lived long enough to develop disease. The risk is slightly higher if the index case is adopted or has little knowledge of their family history. We usually quote a figure of ~1–5%, although it is very difficult to derive an accurate statistic. We do not recommend SOD1 gene testing in sporadic ALS because the likelihood of detecting a mutation is only ~2–7% (Jones et al., 1995; Jackson et al., 1997; Shaw et al., 1998). Because SOD1 mutations are only detected in 20% of familial cases (Rosen, 1993) the absence of a mutation does not substantially decrease the possibility that it is familial. Thus, in our opinion, SOD1 gene testing in sporadic ALS can provide either bad news or false reassurance.

If there is convincing evidence of a first or second degree relative having ALS, then the diagnosis is very likely to be familial as the chance of two individuals in the same kindreds independently developing ALS by chance is between 1 in 250,000–1,000,000. The diagnosis of familial ALS must be relayed very sensitively and additional information in the form of information leaflets and counseling immediately made available to all those who wish to obtain further advice. The effects of this diagnosis may be likened to throwing a large rock into a still pond as the ripples of implied risk spread out through the family, creating a great deal of anxiety. If the disease is multigenerational and phenotype classical ALS then it is very likely to be autosomal dominant and due to a single gene mutation causing disease in the heterozygous state (to date no X-linked kindreds have been published). Even if the affected members are all siblings, or cousins in the same generation, the inheritance pattern is still likely to be autosomal dominant as most autosomal recessive kindreds have an onset in childhood or adolescence and a single affected generation is likely to reflect reduced penetrance. The risk of inheriting the mutated gene is 50%, but in families with reduced penetrance one can counsel that the risk of developing ALS may be considerably lower. This is certainly true for individuals heterozygous for the I113T and D90A mutations (see below). Even in families where the age of disease onset appears to be fairly consistent, the variable penetrance does not imply that older at-risk members have a greatly reduced risk as there is no upper age limit when disease can manifest.

14.3.3. SOD1 gene testing

In the first instance SOD1 gene testing should always be discussed with and offered to the affected index case. Although there is no direct benefit to that person it will inform and influence the counseling and gene testing offered to other members of the family. If a pathogenic SOD1 gene mutation is identified through a diagnostic test then other unaffected members can undergo predictive testing for the same mutation, should they wish. The decision to undergo diagnostic or predictive testing is intensely personal and there is no right decision. The genetic counselor should take an entirely neutral position and help the individual to make the decision that is right for them. Diagnostic testing for an affected individual can be done on the first visit following diagnosis but predictive gene testing in an unaffected at-risk person usually involves two initial counseling sessions followed by a 3 month "cooling off" period before formal consent obtained and blood is drawn for the gene test. The counselor(s) make a fixed follow-up appointment and will not review the test result until the day of the appointment. This facilitates neutral communication in the interim. The issue of familiality and gene testing

can be very divisive within families and the person being tested should be given the option of sharing or restricting news of the results with other family members. Someone wishing to have a predictive gene test must be made aware that if they are shown to harbor a high risk mutation then there may be consequences in terms of their employment, life assurance and ability to raise a mortgage. No one can be forced to have a gene test by another individual or organization, but once someone has voluntarily undergone gene testing they may be obligated to disclose the result to their employer and other financial agencies. We usually advise people to explore their options and make the necessary arrangements before they undergo gene testing. The likelihood of detecting a SOD1 mutation in most countries which are ethnically European is around 20%. Phenotype-genotype studies indicate that some mutations are highly penetrant and are associated with a rapid progression (e.g. A4V which accounts for ~50% of all SOD1 mutations in the US (Cudkowicz et al., 1997), while others have a much lower penetrance (e.g. D90A in the heterozygous state very rarely causes ALS (Andersen et al., 1997)).

14.3.3.1. Disease modifying and symptomatic treatments

At present, the only drug treatment known to prolong survival is the benzothiazole derivative, riluzole 50 mg taken twice daily by mouth. This has been shown in two randomized controlled trials (Bensimon et al., 1994; Lacomblez et al., 1996) and three retrospective case-control studies (Chio et al., 2002; Turner et al., 2002; Traynor et al., 2003). Meta-analysis of the randomized trials showed a relative reduction in hazard ratio for survival at 18 months of 17%. This corresponds to a hazard ratio of 0.88 (95% CI 0.75–1.02) (Turner et al., 2002). Including data on functional status (which was not generally a primary outcome measure), a small reduction in the rate of deterioration was observed. This is difficult to interpret because of the statistical techniques used. Both the retrospective studies, one clinic based and one population based, showed an improvement in survival with riluzole, even when taking into account other prognostic factors in a Cox regression analysis. Its mode of action is to inhibit glutamate release and thus decrease excitotoxicity. This may not be the mechanism, however, as other glutamate release inhibitors such as gabapentin which has good CNS penetrance have no beneficial effect on survival (Miller et al., 2001). The most common side effects include dizziness and vertigo (which may interfere with skilled tasks such as driving), nausea, lethargy and rash. An affected person taking riluzole will not feel any stronger, but on statistical grounds they are likely to experience a modest increase (~3 months) in survival. Lack of an objective measure of effect is one of the most common reasons given by those who discontinue it.

Symptomatic treatment strategies for FALS patients are essentially the same as for sporadic ALS and where possible genetic counseling should be part of the service provided by a multidisciplinary care team (discussed in detail in Chapter 13), The level of anxiety in familial ALS may well be a great deal higher because many individuals will have witnessed the relentless progression of disability and death in other family members.

14.4. Pathology

The pathology of classical autosomal dominant familial ALS is very similar to that described in sporadic ALS cases, but some authors report a greater propensity for the dorsal columns to be involved and that large hyaline inclusions are characteristic of mutant SOD1 cases (Ince et al., 1998). Although SOD1 aggregates have been identified in a few mutant SOD1 linked ALS cases there are no inclusions visible by light microscopy in the majority of cases (Shaw et al., 1997). All motor neuron groups are affected except those in the oculomotor and Onuf's nuclei, consistent with the clinical observation of sparing of the eye movements, bladder and bowel sphincters. The most frequent immunohistochemical finding is of ubiquitin-immunoreactive inclusions in the perikaryon and proximal axon of lower motor neurons which can be skein-like, globular or Lewy-body like (Leigh et al., 1991). They are often accompanied by neurofilament aggregates and may be prominent in mutant SOD1 cases (Rouleau et al., 1996). Bunina bodies are the only inclusion that is specific to and common in ALS (Bunina, 1962). To date they have not been described in familial ALS cases suggesting that there may be some as yet uncharacterized differences in their molecular pathogenesis (Shibata et al., 1996).

14.5. Hypotheses of causation

14.5.1. The excitotoxic hypothesis

Glutamate is the neurotransmitter used by the corticospinal tracts and some spinal interneurons. Arrival of the action potential at the presynaptic terminal results in the release of glutamate in a calcium dependent manner. This stimulates post-synaptic glutamate receptors which are of three types: NMDA, non-NMDA (both ionotropic receptors) and G-protein coupled metabotropic receptors. Glutamate is cleared from the synapse by excitatory amino acid transporters (EAAT) 1–4, found on astrocytes and neurons. The receptors and the transporters are modified at the transcriptional level so that

there are splice variants. Ionotropic receptor activation leads to a cascade of intracellular events mediated by calcium, nitric oxide, peroxynitrite and superoxide (Rothstein et al., 1996; Shaw, 1999). Excessively high levels of extracellular glutamate lead to necrotic or apoptotic cell death.

14.5.2. The free radical hypothesis

Free radicals are products of oxidative metabolism and are highly reactive species which attack cellular components such as proteins, nucleic acids, lipids and organelles. Superoxide is normally detoxified by Cu/Zn superoxide dismutase (SOD1) to produce hydrogen peroxide (Zhang et al., 2002). This can then be further detoxified by catalase to produce water and molecular oxygen. In the presence of reducing agents such as Fe(II) or Cu(I), however, hydrogen peroxide is converted to the dangerous hydroxyl radical. Nitric oxide synthase is upregulated by the glutamate pathway, leading to a greater production of the peroxynitrite radical. The excitotoxic hypothesis and the free radical hypothesis are therefore related.

14.5.2.1. The cytoskeletal hypothesis

The neuronal cytoskeleton consists of actin microfilaments, intermediate filaments known as neurofilaments and microtubules with their associated proteins. The cytoskeleton plays a role in maintaining the extreme shape of neurons, transport of subcellular organelles and proteins, signaling to glial cells and the growth of axons and dendrites. Defects in retrograde axonal transport are a feature of animal models of ALS (Al-Chalabi and Miller, 2003). Accumulations of neurofilaments are found in human ALS, but it is not clear if these are secondary to whatever causes ALS or if a primary cytoskeletal defect causes the accumulations and cell death.

14.6. Genetics

14.6.1. Background

Mendel was discovering the laws of genetics at about the same time as Charcot was describing ALS. In 1865 he presented his paper on the mating of sweet peas suggesting that "formative elements" were transmitted from parents to offspring and worked in pairs to determine their characters, with one formative element coming from each parent (Mendel, 1865). We now call the "formative elements" genes, the variants responsible for different characters "alleles" and the different "characters" phenotypes.

For any pair of alleles, one may be dominant to the other, but other genes and environmental factors may also influence the expression of the phenotype. One aspect of this is gene–gene interaction, also known as epistasis, and another is gene–environment interaction. These modifying genetic and environmental factors act to influence the likelihood a phenotype will manifest. The likelihood of a phenotype given a genotype is called penetrance. Familial ALS shows age-dependent penetrance which is incomplete. The older a disease-gene carrier is, the more likely they are to develop ALS but not everyone with the disease-gene will develop ALS.

Before continuing, it is important that we define what we mean by familial ALS. Where there is autosomal dominant ALS in multiple generations and high penetrance, the situation is straightforward. There are however families which have autosomal recessive ALS with an adult-onset (e.g. D90A SOD1 mutations (Andersen et al., 1996)) and some with childhood or juvenile ALS (see ALS2 and ALS5 loci below). No definitely X-linked kindred has yet been published. What is more difficult is the situation of familial clustering without a clear Mendelian pattern of inheritance. For example, an individual and a first cousin both affected are unlikely to be coincidental, but the heritability of that trait is difficult to determine. Some of these cases and some apparently sporadic individuals are heterozygous for SOD1 mutations so these kindreds may reflect low penetrance and blur the distinction between familial and sporadic ALS.

14.6.2. Genetic and phenotypic classification of familial ALS

The classification of the different forms of familial ALS was initially constructed on the basis of age at disease onset and clinical phenotype. As distinct loci, and more recently disease-associated gene mutations, have come to light the numbering system has evolved. The one used here is the most current at the time of writing. Although several of the familial conditions described as "Familial ALS" do have upper and lower motor neuron degeneration they are quite different and distinctive from the familial form of adult-onset ALS as originally described by Charcot. Where possible we will define the relevance of the genes identified in atypical forms of ALS to the sporadic disorder. Although the discovery of FALS loci was initially slow, there has recently been a flurry of activity and several new loci and genes have been identified in patients with upper and lower motor neuron degeneration described broadly as ALS. A great deal of work is underway to characterize the effects of these gene defects, but as yet no final common pathogenic ALS pathway has been implicated.

14.6.3. ALS1: Cu/Zn superoxide dismutase (SOD1)

The first successful linkage study on familial ALS was published in 1991. A genome-wide scan was undertaken

in 23 multigenerational, autosomal dominant ALS kindreds. Five families showed linkage to chromosome 21q21-22 with a collective maximum multipoint LOD (logarithm of the odds) score of 5.03 (Siddique et al., 1991). Two years later mutations were identified in the gene encoding *Cu/Zn superoxide dismutase* (*SOD1*) (Deng et al., 1993; Rosen, 1993). *SOD1* is a relatively small gene comprising only five short exons and it is translated into a protein of 153 amino acids with a molecular weight of ~22 kDa. SOD1 is a metallo-enzyme which has one copper and one zinc ion bound per molecule. SOD1 functions as a homodimer and serves to catalytically convert potentially toxic superoxide radicals to oxygen and hydrogen peroxide (H_2O_2), which is then converted to water by glutathione peroxidase or catalase through the Fenton reaction (see Fig. 14.1) (McCord and Fridovich, 1969; Fee and Gaber, 1972).

Mutations in *SOD1* can be identified in 20% of the familial ALS patients and 3–7% of sporadic ALS cases (Rosen, 1993; Shaw et al., 1998). To date more than 112 different mutations have been identified in *SOD1* affecting 70 different codons (Orrell, 2000; Andersen et al., 2003), but not all have yet been logged in the international database detailing the genotype and phenotype of SOD1-linked ALS cases (www.alsod.org). The mutations are scattered across all five exons, with relative sparing of exon 3 (Shaw et al., 1998). There are 94 missense mutations leading to a single amino acid

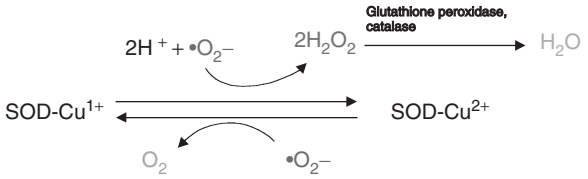

Fig. 14.1. The superoxide dismutase activity of Cu/Zn SOD protein is a bi-directional event involving copper in both its oxidized (Cu^{2+}) and reduced (Cu^{1+}) states.

substitution, 11 nonsense mutations leading to the generation of a truncated protein, three insertions and four deletions. All mutations shown to be pathogenic are within the coding region of SOD1 and alter the amino-acid sequence but they do not show regional clustering within the protein (Fig. 14.2).

One substitution mutation, N19S, has been reported in a single sporadic ALS case but was also found in seven of their unaffected relatives and 1/268 controls suggesting that it might be a benign variant or be of very low penetrance in isolation (Mayeux et al., 2003).

All mutations are associated with familial or sporadic ALS in heterozygotes and the majority are detected in autosomal dominantly inherited ALS kindreds. One mutation, D90A in exon 4, is associated with ALS in heterozygotes from several dominant kindreds and sporadic ALS cases, but more commonly it appears to

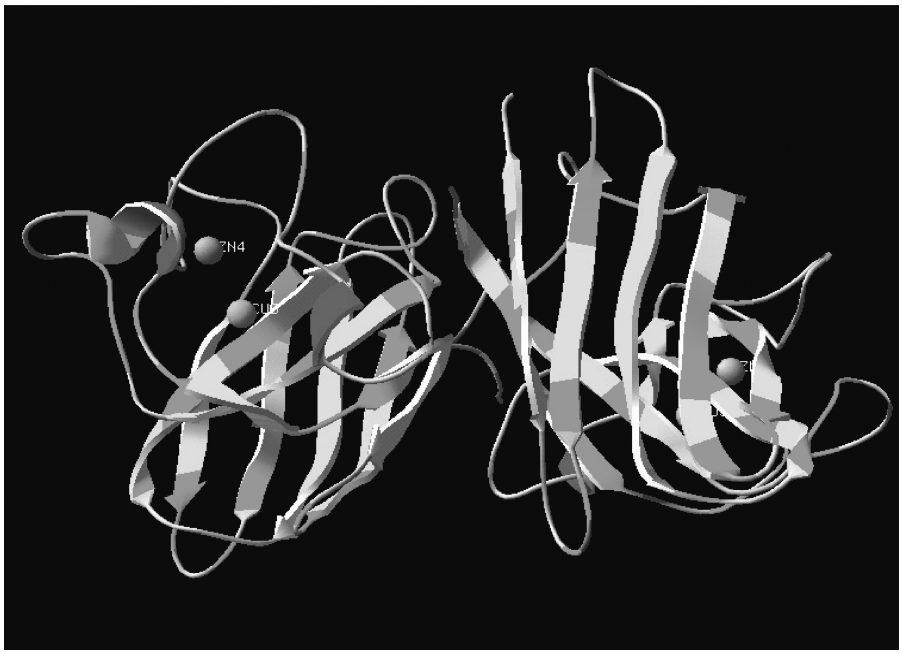

Fig. 14.2. For full color figure, see plate section. SOD1 protein, showing the amino acids subject of mutation in green. Mutations do not show functional clustering and are distributed throughout the protein.

be recessive. ALS patients homozygous for D90A are relatively common in Scandinavia (Andersen et al., 1995), but have also been found throughout Europe (Andersen et al., 2003). Haplotype studies of markers around SOD1 point to a single ancient founder for D90A cases of any inheritance (Parton et al., 2002).

Mutation of SOD1 is thought to lead to the mutant protein acquiring a novel toxicity, rather than disease being caused by a loss of enzymatic activity. This is supported by the observations that individuals with a single copy of chromosome 21 (monosomy) do not develop ALS (Ackerman et al., 1988) and there is no relationship between superoxide activity and clinical phenotype of ALS patients (Borchelt et al., 1994; Ratovitski et al., 1999). Mice knocked-out for SOD1 and those over-expressing wild-type SOD1 develop normally (Gurney et al., 1994; Reaume et al., 1996). While mice genetically engineered to over-express any of six different SOD1 mutants all develop progressive limb and diaphragmatic paralysis including the human mutants G93A (Gurney et al., 1994), G37R (Wong et al., 1995), G85R (Bruijn et al., 1997b), D90A (Gurney et al., 1994) and 127X (Jonsson et al., 2002) and one mouse mutant G86R (Ripps et al., 1995). Furthermore, the pathological features of motor neuron degeneration in transgenic mice have many characteristics in common with post-mortem studies of human ALS cases (Wong et al., 1995; Tu et al., 1997).

What is remarkable is that all mutations in a ubiquitous protein like SOD1 should be toxic to such a select population of cells. Various hypotheses have been put forward as to what the novel function might be, including enhancement of protein nitrosylation, enhanced peroxidase activity, exposure of toxic copper at the active site, accumulation or aggregation of abnormal protein and mutant SOD1-induced mitochondrial damage.

14.6.3.1. Hypotheses involving aberrant catalysis by mutant SOD1 protein nitrosylation

Peroxynitrite (-ONOO) is a toxic intermediate generated non-enzymatically by the interaction between superoxide anions and nitric oxide. Its subsequent catalysis by SOD1 could lead to the nitration of tyrosine residues within a large number of proteins (Fig. 14.3) (Beckman et al., 1993).

Several groups have presented data supporting this theory, showing high levels of nitrotyrosine in the spinal cord tissue of mutant SOD1 transgenic mice (Bruijn et al., 1997a; Ferrante et al., 1997; Andrus et al., 1998) and human ALS patients (Beal et al., 1997; Tohgi et al., 1999). Nitric oxide is generated by type I nitric oxide synthase (NOS). When mutant SOD1 transgenic mice were crossed with mice null for neuronal NOS their motor neuron degeneration was not ameliorated (Facchinetti et al., 1999). Furthermore, the administration of

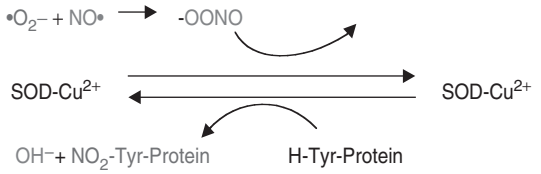

Fig. 14.3. Peroxynitrite hypothesis.

inhibitors of neuronal NOS does not alter the disease course in these mice (Upton-Rice et al., 1999). Thus, although this is a plausible hypothesis, the evidence suggests that this is not the mechanism.

14.6.3.2. Enhanced peroxidase activity

The other main aberrant substrate proposed for mutant SOD1 is hydrogen peroxide, which is usually the end product of normal SOD1-Cu^{+2} activity. The reduced form of SOD1 (SOD1-Cu^{+1}) can catalyse H_2O_2 as a substrate to generate hydroxyl radicals by a process of 'peroxidative catalysis.' Hydroxyl radicals are highly reactive agents and could lead to a cascade of lipid and protein peroxidation and cellular injury (Fig. 14.4).

One study of in vitro catalysis reported a two- to four-fold increase in the use of hydrogen peroxide by A4V and G93A mutants when compared to WT SOD1 (Wiedau Pazos et al., 1996). In vivo studies in transgenic mice have presented conflicting evidence. Some have detected products consistent with peroxidative damage in G93A SOD1 transgenic mice (Andrus et al., 1998; Hall et al., 1998), while others could not find evidence in the G85R mouse (Bruijn et al., 1997a).

14.6.3.3. Copper-mediated toxicity

Conformational change induced by SOD1 mutation can expose the copper ion at the active site of SOD1, which is toxic. Because of this toxicity, intracellular free copper is maintained at less than one atom per cell (Rae et al., 1999). Spinal motor neurons isolated from G93A transgenic mice have a 46% worse survival than wild type motor neurons, but the addition of copper chelators improves their survival by over 200% with no effect on the wild type cells (Azzouz et al., 2000).

The strongest evidence refuting a role for aberrant copper-mediated catalysis comes from transgenic studies

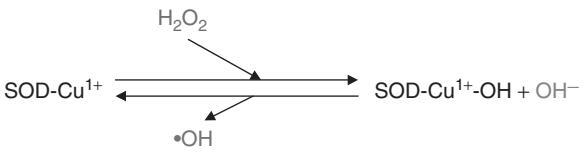

Fig. 14.4. Peroxidase hypothesis (adapted from Weidau Pazos et al., 1996).

that sought to disrupt copper binding. Copper is normally inserted into SOD1 by the copper chaperone for SOD1 (CCS). Mice knocked out for CCS are healthy and if mutant SOD1 toxicity is copper dependant then crossing CCS–/– mice with mutant SOD1 mice should rescue the phenotype. When this experiment was performed using G37R, G85R and G93A transgenic lines all developed progressive paralysis and their life-expectancy was unchanged (Subramaniam et al., 2002). However, CCS is not essential to copper loading of SOD1 (Carroll et al., 2004) and the CCS–/– mice still had 20% SOD1 activity on biochemical assays so this residual function could be implicated in pathogenesis.

Another approach is to physically disrupt copper binding to the four histidine residues. Mice were generated that transgenically expressed two FALS-associated mutations at copper binding residues (H46R and H48Q). SOD1 catalytic activity was effectively abolished but the mice developed progressive paralysis and death (Wang et al., 2002). Subsequently, the same group reported that mice transgenic for all four copper binding histidines (additionally mutating the His63 and His120 residues) resulted in motor neuron degeneration and death (Wang et al., 2003).

Furthermore, the G86R mutation of the mouse SOD1 gene (analogous to the human G85R mutant) incorporates no copper into its active site, has no dismutase activity but is still toxic (Ripps et al., 1995). However, when catalytically inactive G86R mice are crossed with a copper transport deficient mutant, Mobr, survival is prolonged (Kiaei et al., 2004). Thus copper itself, and/or some form of aberrant catalysis, may make a minor contribution to the pathogenesis of ALS.

14.6.3.4. Hypotheses involving the aggregation of mutant SOD1

Many neurodegenerative diseases are characterized by protein aggregates including Alzheimer, Huntington, Parkinson and the prion diseases. The discovery of SOD1 aggregates in the cytoplasm of motor neurons and astrocytes of mutant SOD1 transgenic mice and some FALS patients (Shibata et al., 1996; Bruijn et al., 1997b) promoted the idea that SOD1 aggregation itself may be pathogenic. In a study in which cDNA for WT and mutant SOD1 were microinjected into primary neurons in cell culture, only mutant SOD1 protein aggregate and was toxic (Durham et al., 1997). Furthermore, mutant SOD1 expression caused cell death only in motor neurons but not in sensory or hippocampal neurons, suggesting that aggregate formation and toxicity is cell-type specific. Detergent resistant high-molecular weight forms of mutant SOD1 have been identified from the spinal cord of mutant G93A mice well before clinical signs develop, suggesting that they may play a primary role in pathogenesis (Johnston et al., 2000). Several hypotheses have been proposed to explain how mutant SOD1 and other protein aggregates might cause motor neuron degeneration (see Fig. 14.5).

14.6.3.5. Sequestration of essential proteins

One hypothesis suggests that the aggregates themselves are not directly toxic but that they sequester a set of cellular proteins into insoluble complexes. This critically reduces their availability for essential homeostatic functions (Durham et al., 1997; Bruijn et al., 1998). One family of proteins that might be essential are the chaperones required to fold and refold the mutant SOD1 proteins (Bruening et al., 1999; Shinder et al., 2001). Reduced chaperone activity would have an immediate effect on the function of other cellular processes and may contribute to motor neuron death. Experiments in which the levels of the protein folding chaperone HSP70 or the ubiquitin E3 ligase Dorfin were elevated showed a decrease in the aggregate formation in cultured motor neurons and decreased toxicity (Bruening et al., 1999; Niwa et al., 2002).

14.6.3.6. Saturation or damage to the proteasome machinery

The most common and characteristic aggregates in the motor neurons of ALS patients at postmortem are immunoreactive to ubiquitin (Leigh et al., 1988, 1991; Mather et al., 1993). They are also a striking feature in mutant SOD1 transgenic mice where ubiquitin positive inclusions are an early feature of disease (Bruijn et al., 1998). Mutant or damaged proteins are bound by polyubiquitin chains and directed to the proteasome for degradation and recycling (Ciechanover, 1998). The proteasome may be overwhelmed by the large amount of mutant protein targeted for disposal. Although ubiquitin containing aggregates are a frequent feature of ALS at postmortem, they rarely contain SOD1, even in the individuals with known SOD1 mutations (Ince et al., 1996). The absence of a very strong heat shock protein response in motor neurons may also contribute to their susceptibility to proteasomal dysfunction and make this an attractive theory of pathogenesis.

14.6.3.7. Toxicity of mutant SOD1 oligomers

High-molecular weight forms of SOD1 have been detected in spinal cord tissues of transgenic mice and transfected cells (Johnston et al., 2000) and more recently in the spinal cord of a single ALS patient with the G127X mutation (Jonsson et al., 2004). Given that the mutant SOD1 protein is expressed ubiquitously and, at very high levels in many tissues, it is interesting to note that the SOD1 oligomers are only detected in neural tissues and predominate in the site of pathology,

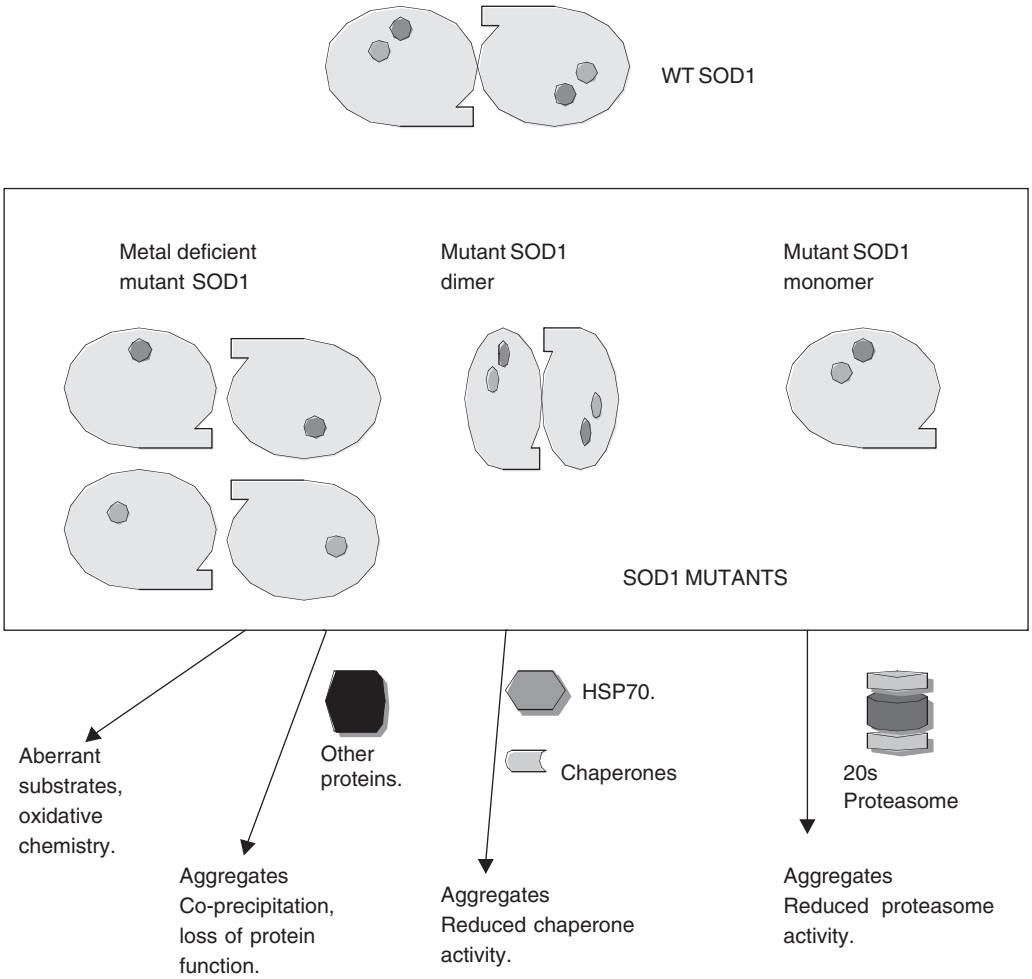

Fig. 14.5. Hypotheses for SOD1 mediated toxicity derived from altered structure or aggregation (adapted from Cleveland and Liu, 2000).

the spinal cord. Mutant SOD1 may form macroaggregates when their concentration exceeds the proteasome capacity. Although the association between mutant SOD1 oligomer/aggregate formation and motor neuron degeneration is still circumstantial it does align with mechanisms of pathogenesis in many other neurodegenerative disorders. WT SOD1 protects the edges of its β-sheet strands by blocking self-association with its well ordered electrostatic and zinc loops (Valentine and Hart, 2003). Recent crystallographic studies show that SOD1 mutants possess very disordered zinc and electrostatic loops (Strange et al., 2003) and can form amyloid-like filaments and water filled nanotubes (Elam et al., 2003).

14.6.3.8. Mutant SOD1-induced mitochondrial damage

In mice carrying mutant SOD1, mitochondrial degeneration and dysfunction occur early in the disease process, and both wild-type and mutant SOD1 is found in the mitochondria, which are vacuolated. While similar changes have not been definitively shown in human ALS, vacuolation was a feature in Charcot's original description. Enzyme activities of the mitochondrial electron transport chain in spinal cords from mice at different disease stages are decreased in the spinal cord, particularly in the ventral horn. This starts in early disease stages and persists throughout the course of the disease (Jung et al., 2002).

14.6.4. Genotype–phenotype correlation

Although SOD1-mediated ALS is usually inherited as an autosomal dominant trait with age-dependent penetrance, in about 2% of cases mutations may be found in individuals with no family history (Jones et al., 1993; Jackson et al., 1997; Andersen et al., 2003). In one case this is known to be a result of new mutation (Alexander et al., 2002), but, in general, this is because of reduced

penetrance (Andersen, 2001). The two most commonly found mutations showing this property are D90A and I113T. The D90A variant typically only causes ALS when found in homozygous individuals (Andersen et al., 1995), suggesting that two doses of mutant SOD1 are worse than one. Three reports of inbred families with SOD1 mutations (L84F, N86S and L126S) and affected homozygous individuals suggest that this is a common theme, with at least two of these associated with an aggressive form of disease (Hayward et al., 1998; Kato et al., 2001).

In general, although different ALS-causing genes may be associated with different phenotypes, for SOD1-mediated ALS there is a poor correlation between specific mutations and phenotype (Andersen et al., 2003). Most SOD1 mutations are associated with a predominantly lower motor neuron pattern of ALS, and none with a predominantly upper motor neuron pattern or dementia at presentation. Except for a few variants, nearly all SOD1 mutations are associated with a poor prognosis, and survival is often less than 3 years. The commonest mutation in the USA, A4V, seems to be particularly aggressive, with survival sometimes measured in months. SOD1-mediated ALS may also be associated with extra-motor symptoms, such as paresthesiae, shooting pains, bladder disturbance or occasionally autonomic failure (Karlsborg et al., 2003; Rezania et al., 2003).

Some variants are possibly associated with slow progression. These include homozygous D90A, G93D and E100K, all found in exon 4. This is not to say that all exon 4 variants are associated with slow progression however, as D90V and I112T are associated with aggressive disease. Similarly, there is a poor correlation between the position of a mutation within the gene and its penetrance. Again, using exon 4 as an example, heterozygous D90A and I113T have low penetrance, but recessive D90A, F108V and G114A have almost complete penetrance.

Symptom onset with lower limb weakness is seen with G41D, H46R, D76V, homozygous D90A and E100K. This is usually followed by a slowly ascending weakness and, as with all SOD1 mutations, begins with a predominantly lower motor neuron pattern. One mutation of SOD1 is particularly interesting and deserves more detailed assessment: the D90A mutation.

14.6.4.1. D90A

SOD1 was first identified in 1967, when it was known as indophenoloxidase. Electrophoretic variants of the protein were known to be rare in humans but a variant was found to be common on the Orkney Islands and in Sweden. We now think this represents the D90A variant of SOD1, which shows a different electrophoretic mobility from wild-type SOD1, but is polymorphic in parts of Scandinavia (Beckman, 1973; Roychoudhury and Nei, 1988).

The D90 mutation is an enigmatic variant of the SOD1 gene. Although some families show a typical autosomal dominant pattern of inheritance of ALS, some individuals with no family history of ALS also carry the D90A mutation (Robberecht et al., 1996; Khoris et al., 1997; Jonsson et al., 2002). This has been attributed to reduced penetrance. SOD1-mediated ALS associated with a single copy of D90A has been found in the UK, Belgium, France, the USA and Belorussia (Slominsky et al., 2000; Skvortsova et al., 2001). Such individuals have a phenotype indistinguishable from classical Charcot ALS. Taken worldwide, however, this is not the normal pattern for the D90A mutation. In parts of Scandinavia it is polymorphic, with up to 2.5% of the population carrying a mutated SOD1 gene with no ill effects (Andersen et al., 1995, 1996). Because of the high rate of heterozygosity, homozygotes are not uncommon, and this effect is exacerbated by the relative isolation of some communities in which this mutation is prevalent. Whereas heterozygotes are not usually affected, homozygotes develop a characteristic relatively benign phenotype of ALS with onset in the legs and a slow ascent of weakness, often with extramotor features. This is consistent and distinct enough that an experienced clinician may make the genotypic diagnosis from the phenotype alone. The D90A mutation is therefore found in heterozygous individuals with no family history of ALS, heterozygous individuals from families consistent with autosomal dominant inheritance and in families with autosomal recessive inheritance. Families tend to breed true, so that heterozygous individuals from recessive families are never affected. The homozygous D90A variant with its classic phenotype has now been found in Russia, France, Germany, Italy, Canada, the USA and possibly Australia.

Founder studies have shown there is a single founder for all cases and, from the descendants of this single founder, one has given rise to all the recessive families (Al-Chalabi et al., 1998; Parton et al., 2002). One explanation is that a protective factor, tightly linked to the SOD1 gene, has been passed on to the descendants of the recent founder. This explains the need for homozygosity before disease is manifest, and also explains the fact that families breed true. Such a factor has yet to be found. Another explanation can be found within the liability threshold model of diseases with complex inheritance. This proposes that the liability to a disease is normally distributed within a population and only individuals with liability above a particular threshold will develop disease. Under this model, a single copy of the D90A variant would increase liability to ALS to more than 50% of the threshold required to develop disease,

but not beyond 100% as would usually be the case for SOD1 mutations. A further copy of D90A would then be sufficient to result in ALS because the 100% threshold would be passed, and homozygotes would therefore be affected. In affected heterozygotes, the model would suggest that some other factor or factors (genetic or environmental) have increased the liability to beyond the threshold. This is consistent with the case of two siblings who had compound heterozygosity for SOD1 mutations, carrying both one copy of D90A and another apparently low penetrance mutation, D96N (Hand et al., 2001). They therefore each had two doses of a liability factor for ALS and, although each factor alone was below the threshold required for disease, consistent with the liability threshold model, both had ALS with a phenotype similar to that of homozygous D90A cases. This dose effect model is also consistent with the evidence from a girl who developed ALS at age 13 and was found to be homozygous for the D86S mutation. She died within 7 months of symptom onset (Hayward et al., 1998).

14.6.4.2. ALS2 due to mutations in ALS2/alsin

ALS2 was the phenotypic description given to a form of autosomal recessive, juvenile onset, motor neuron disorder first identified in consanguineous Tunisian families (Ben Hamida et al., 1990). Three phenotypic groups were described and, in two of these, linkage was demonstrated to two loci, chromosome 2q33 and chromosome 15q15 (Hentati et al., 1994, 1998). The chromosome 2-linked families (phenotypically classified as RFALS type 3) had predominantly upper motor neuron disease, with onset in the first or second decade of life, and survival of many decades. The gene at this locus was identified as a novel protein, named Alsin (Hadano et al., 2001; Yang et al., 2001). The *ALS2* gene has 34 exons with at least two splice variants, AlsinLF and AlsinCF. The long variant (AlsinLF, 6,394 nucleotides) is expressed in various tissues with highest expression in the brain. It is not clear that the short transcript is translated to protein, and it may play a regulatory role at the RNA level. The protein shows similarity to three domains (RCC1, Pleckstrin-DB1, VPS9) and to membrane occupation and recognition nexus repeat motifs, which are characteristic of various guanine exchange factors (Ran, Rho and Rab, respectively). It is now known to be a Rab5 and Rac1 guanine nucleotide exchange factor and probably plays a role in endosomal dynamics. Recent evidence in transfected primary neurons indicates that AlsinLF overexpression increases neurite outgrowth through the RAC and PAC family kinases (Tudor et al., 2005).

Other families with homozygous truncation mutations of ALS2 have been reported in kindreds described as having "infantile ascending hereditary spastic paraparesis" and "juvenile primary lateral sclerosis" (Eymard-Pierre et al., 2002; Devon et al., 2003; Gros-Louis et al., 2003; Lesca et al., 2003). Essentially they are all upper motor neuron syndromes of very early onset and only one family has been reported to have any lower motor neuron features, with distal amyotrophy in the upper limbs (Hentati et al., 1994). Because these mutations probably act by a loss of function and are recessive, they could be a cryptic cause of apparently sporadic ALS, or of young onset, predominantly upper motor neuron, slowly progressive disease. Two independent studies have now excluded coding mutations or haplotype variants across the ALS2 locus being associated with disease susceptibility or clinical phenotype of ALS (Al-Chalabi et al., 2003; Hand et al., 2003).

14.6.5. ALS3 linked to chromosome 18q21

In 2002, a large European pedigree was found to be linked to chromosome 18. The family had typical adult onset, autosomal dominant ALS, with 20 affected individuals (eight able to provide DNA), and a total of 51 DNA samples were available for analysis permitting the recreation of five further affected cases. A maximum LOD score of 4.5 was found which is close to the maximum achievable of 5.1 generated by simulation analysis. Haplotype analysis revealed two separate recombination events which were present in two individuals and defined a conserved region of 7.5 cM which contains 13 known and ~ 37 predicted genes (Hand et al., 2002). To date no mutation segregating with disease has been reported. The clinical phenotype is of classical ALS without any atypical features. Onset was on average at 45 years and mean survival was 5 years. The phenotype was reasonably homogeneous with weakness beginning in the lower limbs in the majority of cases which became generalized to involve the bulbar musculature with upper and lower motor neuron signs and diagnostic classification of Clinically Definite ALS by the El-Escorial Criteria (Hand et al., 2002).

14.6.6. ALS4 due to mutations in SETX/senataxin

In 1998 an 11-generation Caucasian American family with a form of autosomal dominant, juvenile onset, slowly progressive motor neuron disorder was linked to a 5 cM interval on chromosome 9q34 (Chance et al., 1998), subsequently classified as ALS4. In 2004, missense mutations were detected in the gene SETX, encoding Senataxin, in the original kindred and two other kindreds from Austria and Belgium who had a similar phenotype (Chen et al., 2004). The disorder in the American kindred had an onset before age 25 with distal limb weakness

and amyotrophy, accompanied by upper motor neuron signs in the limbs. Bulbar muscles and cognition are spared and the life expectancy is normal. Although symptomatically and on electrophysiological testing there was no sensory involvement, autopsy showed degeneration of the dorsal columns and dorsal root ganglia, as well as degeneration of anterior horn cells and corticospinal tracts.

Nonsense and missense mutations were earlier reported in SETX, an unrelated autosomal recessive disorder, ataxia-oculomotor-apraxia type 2 (AOA2) (Moreira et al., 2004). The phenotype includes ataxia and neuropathy but no upper or lower motor neuron signs. Interestingly the majority of the mutations result in truncation of the protein and none of the heterozygous mutant carriers in AOA2 families have been reported to develop motor neuron dysfunction. Senataxin contains a DNA/RNA helicase domain with homology to IGHMBP2 which encodes for an RNA processing protein. Mutation of IGHMBP2 is seen in the autosomal recessive infantile-onset motor neuron disorder spinal muscular atrophy and respiratory distress type 1 (SMARD1) (Grohmann et al., 2001). The phenotype of ALS4 is very different from classical ALS and the mechanism by which missense mutations in Senataxin cause motor neuron degeneration is unknown.

14.6.7. ALS5 linked to chromosome 15q15

In contrast to ALS2, which is predominantly an upper motor neuron disorder, those linked to chromosome 15 and defined as ALS5, present with lower motor neuron features (Hentati et al., 1998). Autosomal recessive kindreds from Tunisia and Germany develop a progressive gait disturbance with an onset between 8 and 18 years. They have evidence of significant muscle wasting in the limbs, exaggerated reflexes and an extensor plantar response. After 3–4 years they develop dysarthria due to bulbar muscle atrophy and spasticity and survival is between 10–25 years from symptom onset. Linkage to Chromosome 15q 15–22 was identified in a genome wide scan of 151 markers in one large Tunisian kindred and supported by intra-familial analysis of markers at this locus which demonstrated homozygous haplotype sharing in two other Tunisian kindreds and one from Germany (Hentati et al., 1998). Thus, the phenotype is closer to classical adult-onset ALS and fatal, but no gene has yet been identified.

14.6.8. ALS6 linked to chromosome 16q12

In 2003, three families of European ancestry with typical autosomal dominant, adult-onset ALS were independently linked in genome-wide scans to the same region of chromosome 16q12 (Abalkhail et al., 2003; Ruddy et al., 2003; Sapp et al., 2003). The strongest linkage was demonstrated in a UK family with a maximal multipoint LOD score of 3.85, but there was little recombination and the haplotype spanned 42 Mb (Ruddy et al., 2003). A North American family was linked to a neighboring marker with a multipoint LOD score of 3.29, but again the region was very large with a haplotype that spanned 37 Mb (Sapp et al., 2003). Two other UK families were reported to show linkage to 16q12 but with much weaker data; one with a two point LOD score of 1.51 (maximal achievable 3.8, Abalkhail, 2003), and the other with a two point LOD score of 1.84 (maximal achievable 2.04, Ruddy, 2003). Haplotype analysis of all four families shows that the overlapping region is ~ 4 Mb and contains 18 known and 70 predicted genes. These have been screened and no pathogenic mutations have yet been identified (C.E. Shaw, personal communication). The clinical phenotype in all but one family was of classical limb onset ALS with rapid disease progression. Several members with ALS from the family with the weak linkage had psychological and behavioral symptoms suggestive of fronto-temporal dementia. Two previously unaffected members of this kindred subsequently developed ALS and do not share the Chromosome 16 haplotype, effectively excluding the ALS6 locus (C.E. Shaw, personal communication). Although there is strong evidence of linkage to Chromosome 16, only one family now defines the small overlapping region.

14.6.9. ALS7 linked to chromosome 20p13

In the same publication that reported linkage to chromosome 16 in a North American family, linkage to chromosome 20 was described in another kindred with typical ALS (Sapp et al., 2003). A genome-wide scan was conducted on 16 different families with ALS. One kindred comprised a sibship of 15 siblings where only two members had developed ALS in that generation. Two members of a previous generation had developed ALS but DNA was not available from them or their offspring. The peak multipoint LOD score was 3.64 at marker D20s103. The two affected individuals share exclusively only a 1 Mb region on chromosome 20p13 (i.e. none of the other siblings share this minute chromosomal region). The LOD score is highly dependant on the figures used for penetrance in the linkage analysis of this kindred which are based on familial ALS population statistics. DNA was not available on either parent, neither of whom manifested ALS in their lifetime, and only 2/15 siblings were symptomatic, thus penetrance is likely to be incomplete. Linkage to this locus is only secure if no other member of this sibship develops

ALS, as a result we think the evidence for linkage to 20p13 in this kindred is tentative.

14.6.10. ALS8 due to a mutation in vesicle associated membrane protein B (VAPB)

In 2004, a four generation Brazilian family of Portuguese ancestry with a lower motor neuron disorder was linked to Chromosome 20q13 (Nishimura et al., 2004a). The kindred included 28 affected members and the age of onset was between 31 and 45 years with a very slow disease progression lasting many decades. DNA was available on 11 affected members and in a genome-wide scan yielded a maximal multipoint LOD score of 7.45 for a marker at chromosome 20q. Recombinant events narrowed the region to just 2.7 Mb containing 17 known and seven predicted genes. A mutation was subsequently identified in exon 2 of the vesicle associated membrane protein B (VAPB) that segregated with disease in this kindred (Nishimura et al., 2004b). This mutation was also found in six other Brazilian kindreds with a similar clinical phenotype. The mutation results in the substitution of proline for serine at codon 56. Recent haplotype analysis of all the kindreds suggests that they share the same Portuguese founder (Nishimura et al., 2005).

VAPB is expressed in all tissues and localizes to the endoplasmic reticulum and Golgi apparatus (Nishimura et al., 2004b). It is involved in intracellular trafficking of vesicles and may interact with microtubules. VAPB has three domains and exists as a homo-dimer. The major sperm protein domain is common to all VAP proteins, a central coiled-coil domain predicted to mediate protein–protein interaction, and a hydrophobic transmembrane domain required for membrane anchoring. The Pro56Ser mutation occurs within a highly conserved motif and is predicted to disrupt the beta-pleated sheet structure. Primary cortical neurons transfected with wild-type VAPB show a homogeneous pattern of expression while those transfected with mutant VAPB rapidly develop aggregates (Nishimura et al., 2004b).

14.6.11. ALS-FTD linked to chromosome 9q21

A set of five families from a consortium of US researchers were reported in which ALS and frontotemporal dementia (FTD) occurred independently within the pedigree or sometimes concurrently in the same individual. The ALS in these families was typical of classical ALS, and the FTD manifested by socially inappropriate, impulsive behavior and a general inability to perform daily tasks. Linkage was found in two families and subsequently replicated in the other three (Hosler et al., 2000). The linked region is on chromosome 9q21-q22 and spans 17 cM.

14.7. Other genes implicated in the pathogenesis of ALS

14.7.1. DCTN1

A family with a slowly progressive and distinctive lower motor neuron disorder was linked to chromosome 2p13 and a missense mutation subsequently identified in exon 2 of the p150 subunit of dynactin, DCTN1 (Puls et al., 2003). The mutation results in the Gly59Ser substitution and was identified in all affected members of the pedigree but not unaffected members, nor was it detected in 200 European controls. The disorder begins at an average age of 34 years (range 23–39), with either stridor and dysphonia due to vocal cord paresis, or weakness in the hands (Puls et al., 2005). Weakness and wasting typically involves the thenar muscles, sparing the hypothenar muscles, but does slowly progress to involve all limbs, bulbar and facial muscles causing dysarthria, dysphagia and aspiration. The weakness is always more marked distally than proximally and is more severe in the upper limbs than the lower limbs. No one from this kindred became wheelchair bound but some did require tracheostomy. Sensory nerve conduction was normal and, although the amplitude of compound muscle actions potentials were reduced in affected muscles, the motor nerve conduction velocities were preserved. Postmortem analysis of a 76-year-old individual revealed that the brain and corticospinal tracts were unaffected but there was a loss of motor neurons in the hypoglossal and anterior horn of the spinal cord with accumulation of neurofilaments and aggregates of dynactin and dynein.

The phenotype of the DCTN1 kindred is different from ALS but, in a screen of 250 individuals with ALS and 150 controls, DCTN1 mutations were identified in six people, one with apparently sporadic ALS (Thr1249Ile), one with familial ALS (Met571Thr), and in a single pedigree in two affected and two unaffected individuals (Arg785Trp) (Munch et al., 2004). A further missense mutation resulting in a Arg1101Lys substitution has recently been described in an autosomal dominant kindred with ALS and frontotemporal dementia by the same group (Munch et al., 2005). Two brothers, one with ALS and one with FTD, were shown to have the mutation. However, no unaffected individuals were genotyped and two other missing affecteds could not be recreated. Furthermore, only 100 controls were screened for this mutation so one cannot confidently say the mutation segregates with disease in this kindred and is pathogenic. The detection of DCTN1 mutations in ALS cases is of great interest but it is not proof of pathogenicity which will only come from biological studies of the in vivo effects of these mutations in cellular and animal models.

The dynactin-dynein complex is a major retrograde axonal motor and the authors postulate that the mutation disrupts axonal transport leading to motor neuron degeneration (Puls et al., 2005), as has been described in mutant SOD1 mediated ALS (Williamson and Cleveland, 1999). Mutations in the dynein gene have been found to cause a motor neuron disorder in the legs at odd angles (Loa) (Hafezparast et al., 2003) but, to date, no dynein gene mutations have been reported in ALS patients (Ahmad-Annuar et al., 2003).

14.7.2. NEFH

Although mutations in the heavy neurofilament subunit gene (NEFH) have been detected in nine sporadic ALS cases and one familial ALS case they were also detected in ~ 2/1,000 controls (Figlewicz et al., 1993; Tomkins et al., 1998; Al-Chalabi et al., 1999). Unfortunately the other affected family member in the familial case could not be examined or genotyped, so there is no proof that the mutation segregates with disease. Furthermore, a comprehensive survey of the light, middle and heavy neurofilament subunit genes failed to find evidence of disease segregation in 200 familial ALS kindreds (Garcia et al., 2006).

14.7.3. Angiogenin

One group has reported allelic association between a particular SNP variant in the angiogenin gene (ANG) and susceptibility to ALS, as well as two different missense mutations in two patients with sporadic ALS (Greenway et al., 2004).

14.8. Conclusion

Familial ALS is clearly heterogeneous and overlaps with the sporadic form of the disease. Of the eight ALS loci described, so far only SOD1 mutations cause classical ALS. It is interesting to note that in most surveys as many people with ALS and SOD1 mutations have sporadic as have familial disease. Given that SOD1 mutations are often detected in the most highly penetrant kindreds it seems likely that other familial ALS genes with lower penetrance may be even more frequent in sporadic cases.

The importance of taking an extensive and detailed family history cannot be overstated. Many individuals thought to have sporadic disease subsequently turn out to be familial and the reassurance regarding recurrence risk to their children has been false. If familial disease is suspected then every effort should be made to track down an accurate history and medical records of the other affected individual to be as certain as possible of the historical diagnosis. Equally important is the phenotype of any relatives with dementia. Symptoms of behavioral or personality change and language deficits early in the course of dementia should raise the suspicion of frontotemporal dementia and a blanket diagnosis of Alzheimer disease in the demented elderly person should not be accepted at face value. Death certificates are notoriously unreliable and corroborating medical record evidence should be sought.

The implications of SOD1 gene screening should always be discussed with the family in detail prior to testing. Consent and DNA from an affected individual should be tested first before predictive testing in at risk individuals is considered to confirm that mutation of SOD1 is relevant to this family. The penetrance of different SOD1 mutations varies greatly and must be taken into consideration when ascribing risk to a particular gene carrier. The carrier of heterozygous D90A mutation has a < 5% lifetime risk of developing ALS, whereas a homozygous D90A carrier or a heterozygous carrier of F108V has close to 100%. The burden of anxiety on ALS families is huge and they require long-term counseling and support.

Although the mechanism by which mutant SOD1 is toxic to motor neurons is not well understood it is not due to aberrant copper-mediated catalysis. Evidence is mounting that aggregation of mutant SOD1 protein, perhaps within mitochondria, may initiate motor neuron degeneration. Although protein aggregation within motor neurons is a feature of all ALS cases the identity of most of these proteins is still unknown. Mutation studies in candidate genes have so far been disappointing, with promising leads that fail to be replicated in subsequent studies. The most recent gene of interest is DCTN1. Missense mutations have been detected in three familial and one sporadic ALS cases but biological evidence that these mutations are pathogenic is still awaited.

The other ALS loci (ALS2, ALS4, ALS5 and ALS8) cause progressive motor neuron disorders that have significant phenotypic differences from ALS and no overlap between these genes and typical ALS has yet been demonstrated. To date, gene hunting efforts at the more typical ALS loci (ALS3 and 6) have failed to identify pathogenic mutations. Furthermore, the limits of the ALS6 locus are not as robust as was originally thought and the evidence for linkage to ALS7 is not yet convincing. There is growing evidence that a significant number of kindreds contain individuals who have phenotypes typical of ALS, or frontotemporal dementia, and sometimes both. Linkage to Chromosome 9p21 has been detected in five families and more work is needed to explore the clinical and biological links between these two disorders.

Although great progress has been made in defining the genetic basis of many motor neuron disorders,

20 years of research has yielded only one gene, SOD1, that when mutated causes typical ALS. Mutant SOD1 accounts for only ~5% of all cases and more clues are urgently needed if we are to gain a better understanding of what causes motor neuron degeneration in ALS. It is only by mapping out common pathogenic pathways that we can develop a sensible therapeutic strategy and develop therapies that might arrest or reverse the disease process. When more ALS genes are identified and a truly effective therapy is developed then we will be in a position to offer mass screening of the population to determine who is at risk and offer the ultimate intervention, preventative treatment.

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Chapter 15

Juvenile amyotrophic lateral sclerosis

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15.1. Summary

Several forms of genetically defined juvenile amyotrophic lateral sclerosis (ALS) have now been characterized and discussion of these conditions will form the basis for this chapter. ALS2 is an autosomal recessive form of ALS with a juvenile onset and very slow progression that mapped to chromosome 2q33. Nine different mutations have been identified in the *ALS2* gene that result in premature stop codons, suggesting a loss of function in the gene product, alsin. The alsin protein is thought to function as a guanine–nucleotide exchange factor for GTPases and may play a role in vesicle transport or membrane trafficking processes. ALS4 is an autosomal dominant form of juvenile onset ALS associated with slow progression, severe muscle weakness and pyramidal signs, in the absence of bulbar and sensory abnormalities. Mutations in the *SETX* gene cause ALS4, and the *SETX* gene product senataxin may have DNA and RNA helicase activity and play a role in the regulation of RNA and/or DNA in the cell. A third form of juvenile-onset ALS (ALS5) is associated with slowly progressing lower motor neuron signs (weakness and atrophy) initially of the hands and feet, with eventual bulbar involvement. Progressive upper motor neuron disease becomes more obvious with time. ALS5 has been linked to a 6 cM region of chromosome 15q15.1-q21.1, but the causative gene mutation for ALS5 has yet to be identified. The high degree of clinical and genetic heterogeneity in the various forms of juvenile ALS can make differential diagnosis difficult, other genetic disorders that must be considered include: spinal muscular atrophy, hereditary spastic paraplegia, SBMA, GM2 gangliosidosis and the hereditary motor neuronopathies/motor forms of Charcot–Marie–Tooth disease. Acquired disorders that must also be considered include heavy metal intoxications (especially lead), multifocal motor neuropathy, paraneoplastic syndromes, vitamin deficiencies (B12) and infections (HTLV-II, HIV and poliomyelitis).

15.2. Introduction

ALS (also known as Lou Gehrig's disease) is the most common form of motor neuron disease. ALS is characterized by selective degeneration of the upper and lower motor neurons in the central nervous system involved in voluntary movement and respiration, leading to progressive weakness and often to complete paralysis. In ALS muscle weakness generally spreads in an anatomically contiguous manner to eventually involve the entire body and usually leads to death within 5 years of disease onset. In the vast majority of cases, ALS occurs late in life and appears to be a sporadic disorder, but approximately 5–10% of ALS is considered to be familial or likely genetic in origin.

In general familial forms of ALS have an earlier onset of symptoms and are associated with slower disease progression than sporadic ALS. To date, seven loci (three genes) have been associated with forms of familial ALS (ALS1–7) of which ALS2, 4 and 5 are juvenile onset forms. Approximately 20% of identified familial patients have mutations in the ALS1 gene encoding Cu/Zn superoxide dismutase 1 (*SOD1*) on chromosome 21. Well over 100 disease-causing mutations in *SOD1* have been identified to date (Bruijn et al., 2004) and mutations of SOD1 are also found in approximately 3% of sporadic ALS cases tested (Jackson et al., 1997;

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Table 15.1

Genetic loci for juvenile onset ALS

Locus	Inheritance pattern	Chromosomal locus	Gene (protein) mutated
*A*LS2	autosomal recessive	2q33	ALS2 (alsin)
ALS4	autosomal dominant	9q34	*SETX* (senataxin)
*A*LS5	autosomal recessive	15q15.1-q21.1	unknown

Shaw 2001). In addition, there have been a few reports of juvenile onset ALS cases caused by point mutations in *SOD1* (Kawamata et al., 1997; Hayward et al., 1998; Rezania et al., 2003), but these appear to be very rare, and will not be covered further in this chapter.

Juvenile onset ALS (defined here as an age of onset less than 25 years of age) is a very rare form of motor neuron disease. Clinically, the juvenile forms will all eventually develop evidence for both upper and lower motor dysfunction, but phenotypic variability is the norm rather than the exception in these disorders, often making accurate diagnosis difficult. Signs of upper motor neuron (UMN) dysfunction include the extensor plantar response (Babinski sign), clonus and increased tone and hyper-reflexia. Lower motor neuron (LMN) signs include muscle atrophy, decreased motor power or weakness and fasciculations. Generally, cognitive functions are spared and sensory symptoms are not prominent features. Emotional lability and inappropriate crying or laughing (pseudobulbar signs) occur in the juvenile forms of ALS as well as the adult-onset forms. Little is currently known about the specific neuropathology of the defined genetic forms of juvenile ALS, as no detailed published information is available.

Juvenile onset ALS is more frequently thought to be genetic in origin than the adult onset-forms. It can be autosomal dominant, for which one locus has been mapped (ALS4), but more often it is autosomal recessive, with the symptoms often manifesting in siblings with consanguinity in the family or living in small isolated populations (Garg and Srivastava, 1968; Staal and Went, 1968; Gragg et al., 1971; Myllyla et al., 1979; Ozge et al., 2002). Three clinical forms of autosomal recessive juvenile familial ALS (RFALS) were described in 1990 in 17 families from Tunisia (Ben Hamida et al., 1990). Type I, the most common form, was characterized by upper limb amyotrophy with bilateral pyramidal involvement. Type II was characterized by spastic paraplegia and peroneal atrophy, and individuals with type III exhibited a spastic pseudobulbar syndrome with spastic paraplegia. Using these families, two loci for RFALS have now been mapped, ALS5 for type I RFALS and ALS2 for type III. Known genetic loci for juvenile ALS are shown in Table 15.1.

The high degree of clinical heterogeneity in juvenile ALS implies that there are more genetic loci remaining to be mapped. Unfortunately the majority of case reports are of single individuals or small families, which have insufficient power for genetic linkage analysis. It is also virtually impossible to predict candidate genes for mutation analysis in the absence of linkage, due to the diversity in function of genes implicated in ALS thus far (from cellular detoxification to vesicle trafficking to DNA or RNA processing). To complicate the situation further, genetic heterogeneity may exist between seemingly identical clinical descriptions and, conversely, two individuals with very different courses of illness may both harbor mutations in the same gene. Different mutations within a gene may lead to diverse clinical outcomes, and these differences may be exacerbated by the effect of modifier genes.

15.3. ALS2 (OMIM 205100)

ALS2 is a clinically heterogeneous form of familial juvenile ALS characterized by early onset of limb and facial muscle weakness, usually accompanied by bulbar or pseudobulbar symptoms. Motor symptoms generally progress slowly during childhood; eventually, deficits become static, and are not associated with decreased long-term survival. The predominant clinical phenotype in most families is suggestive of UMN dysfunction, but LMN signs and symptoms may also develop, or may be recognized when detailed electrodiagnostic evaluations are performed (Ben Hamida et al., 1990). Individuals with this form of autosomal recessive ALS have also been given the clinical diagnosis of infantile-onset ascending hereditary spastic paraplegia (OMIM 607225) and/or juvenile primary lateral sclerosis (OMIM 606353). ALS2 is a very rare form of motor neuron disease with only nine reported families world-wide to date. No gender bias has been identified for ALS2.

The large Tunisian family (pedigree 1212) that formed the basis of the description of type III RFALS

(Ben Hamida et al., 1990) was used to map the ALS2 locus. This family included 12 affected individuals over five generations; all the affected cases were the offspring of first-cousin marriages. Hentati et al. (1998) performed genetic linkage analysis on 24 individuals from this family, including 10 affected members, with 75 markers across the genome. Significantly positive LOD scores (> 3) were obtained for the chromosome 2 markers D2S72, D2S116, D2S117 and D2S155. A maximum LOD score of 8.28 was obtained for D2S72, located on chromosome 2q33–35. On the basis of informative recombinations within affected individuals, the critical ALS2 region was placed in an 8 cM region between D2S115 and D2S155.

To assist in the identification of candidate genes in the ALS2 region, two groups constructed a yeast artificial chromosome contig across the region. The 8 Mb contig constructed by Hadano et al. (2001) spanned the region between D2S115 and D2S155 and contained 52 expressed sequence tags (ESTs) or known genes. Hosler et al. (1998) genotyped members of the same Tunisian family with additional chromosome 2q markers and considerably narrowed down the ALS2 minimal genomic region to 1.7 cM between D2S116 and D2S2257. Hosler et al. (1998) then generated a YAC contig across this 3 Mb region.

Initial attempts to identify mutations in candidate genes proved fruitless. These authors screened individuals from the Tunisian family for mutations in the coding sequence of two candidate genes (CD28 and CTLA4) but did not detect any sequence variants that segregated with affected status. Later, Hadano et al. (2001) similarly screened six candidate genes (ALS2CR1, ALS2CR2, ALS2CR3, CFLAR, CASP10 and CASP8) and did not detect pathogenic mutations. Both groups were also able to PCR amplify every marker, EST or gene exon from affected individuals and controls, indicating that ALS2 patients do not have extensive deletions of genomic DNA in this region.

The cloning of the *ALS2* gene was reported in two manuscripts published back to back in 2001 (Hadano et al., 2001; Yang et al., 2001). Hadano et al. (2001) identified 395 exons from the 42 transcripts they had previously mapped, and sequenced each one along with its flanking intron-exon boundaries in 14 ALS2 affected individuals from the Tunisian pedigree 1212 and 10 normal controls. A total of 77 DNA sequence variants were detected, among which was a homozygous single nucleotide deletion (261delA) in exon 3 of a novel gene, now designated *ALS2*. This deletion was predicted to change the reading frame of the encoded protein, thereby resulting in a premature termination codon. It segregated perfectly with the ALS2 phenotype in the family, and was not present in 553 normal controls. In a similar study, Yang et al. (2001) detected the same deletion in the same family following the saturation sequencing of 250 exons from 43 transcripts in the ALS2 critical region. Additional confirmatory evidence was provided in both papers by the detection of two further deletion mutations in the same gene: of 2 bp of exon 5 (1548delAG) in a Kuwaiti family with juvenile primary lateral sclerosis or JPLS (Hadano et al., 2001) and of 2 bp of exon 9 (1990delCT) in a Saudi Arabian family also affected by JPLS (Yang et al., 2001). Both of these deletions also segregated perfectly with affected or carrier status in the family and were not found in large numbers of normal controls. A total of nine families have now been shown to harbor mutation in the *ALS2* gene. The known ALS2 gene mutations are shown in Fig. 15.1.

The families are of varied geographical origin (North African, Arabian, South-East Asian and European) and the mutation is different in each. All the families exhibit juvenile onset of symptoms, but, interestingly, only the original Tunisian family exhibits lower motor neuron symptoms and was diagnosed with ALS initially. Two families exhibit JPLS as described above, and the remaining six are diagnosed with infantile onset ascending hereditary spastic paraplegia (IAHSP). This illustrates well the clinical heterogeneity associated with *ALS2* gene mutations. At least eight other published families with indistinguishable syndromes have been screened but are negative for *ALS2* gene mutations: a Saudi Arabian JPLS family (Yang et al., 2001), IAHSP families from Libya, France and Italy (four families) (Eymard-Pierre et al., 2002) and two Turkish brothers with juvenile ALS as described by Ozge et al. (2002) (R.S. Devon, unpublished results).

Each mutation in the *ALS2* gene is homozygous in affected individuals and heterozygous in carriers, consistent with an autosomal recessive pattern of inheritance. In all but two cases, there is recognizable consanguinity in the family; in the remaining two families the homozygous nature of the mutation is strongly indicative that both chromosomes are derived from the same ancestral origin. Eight of the mutations are short deletions (1 to 10 bp) and the ninth is a nonsense mutation, resulting in a termination codon. All the mutations are predicted to result in premature truncation of the encoded protein and complete loss of function. Consistent with this is the observation that the phenotype does not vary according to the length of the intact protein.

The *ALS2* gene is comprised of 34 exons and the detected mutations are spread throughout, the most proximal being in exon 3 and the most distal in exon 32. Two transcripts, of 6.5 kb and 2.6 kb, can be detected by northern blot. The 6.5 kb mRNA (NM_020919 in

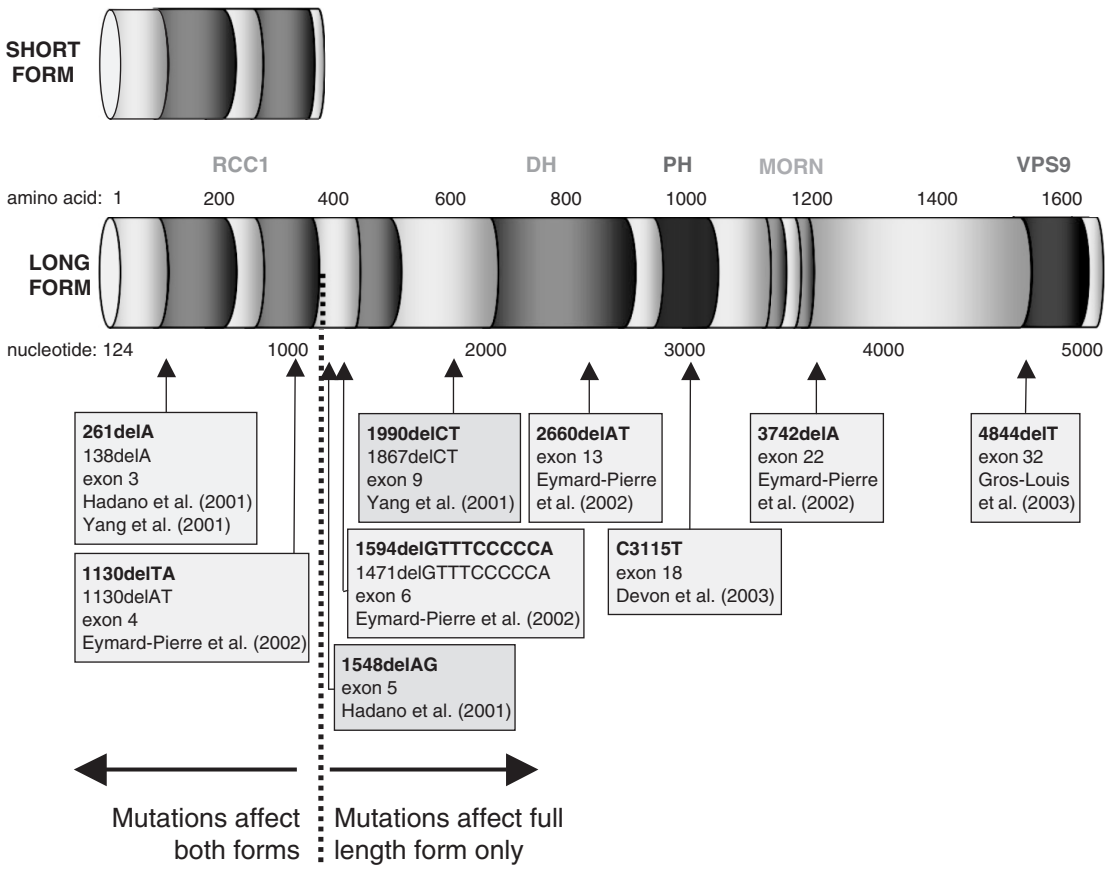

Fig. 15.1. For full color figure, see plate section. Predicted protein domains of the alsin mutation and location of published mutations. RCC1: Regulator of Chromatin Condensation 1; DH: Dbl Homology; PH: Pleckstrin Homology; MORN: Membrane Occupation and Recognition Nexus; VPS9: Vacuolar Protein Sorting 9. The dotted vertical line indicates the position of the C-terminus of the short form of alsin. Mutations indicated in bold typeface are consistent with the sequence position in RefSeq entry NM_020919; alternative names (as reported in previous publications) are shown below. The mutation in a yellow-filled box was detected in ALS2, those in blue-filled boxes were detected in IAHSP and those in lilac-filled boxes were detected in JPLS.

the RefSeq database) is predicted to arise from transcription of the full length gene, and the 2.6 kb mRNA from transcription and splicing of exons 1 to 4 only, followed by read-through into intron 4 after failure to recognize the exon 4 splice donor site. It has been suggested that the 261delA mutation, affecting both the short form and the full length form of *ALS2*, can provide an explanation for the presence of lower motor neuron degeneration in the Tunisian family 1212 that is absent in the other families (Shaw, 2001; Yang et al., 2001; Leavitt, 2002). However, the discovery of the 1130delTA mutation, also predicted to affect both transcripts, in an IAHSP family (Eymard-Pierre et al., 2002) calls this hypothesis into question. In addition, the short form of *ALS2* appears not to be translated into protein (Otomo et al., 2003; Yamanaka et al., 2003; Devon et al., 2005) and its existence in other species is not proven, whereas the full length transcript is highly conserved among vertebrates (Devon et al., 2005). Overall, the role of the short form of *ALS2* in motor neuron disease is unknown and warrants further investigation.

The full-length *ALS2* gene is translated into a 1657 amino acid (185 kDa) protein called alsin (NP_065970). The *ALS2* gene (and its mouse orthologue *Als2*) and alsin protein are widely expressed (Hadano et al., 2001; Yang et al., 2001; Otomo et al., 2003; Yamanaka et al., 2003; Devon et al., 2005). In the adult mouse, gene expression is predominantly in neurons (but not glial cells) of the CNS, with high levels found in the alpha motor neurons of the spinal cord and, particularly, the granule cell neurons of the cerebellum. The alsin protein is likely to be trafficked into the granule cell axons, which project into the cerebellar molecular layer. During development, *Als2* is also widely expressed, and brain expression only becomes predominant in early

postnatal life, suggesting that *Als2* may play different roles in the embryo and in the adult (Devon et al., 2005). Data regarding expression in humans suggest that it is expressed in motor neurons but not glia of adult motor cortex (Otomo et al., 2003) and in motor neurons in the spinal cord and possibly in some sensory neurons of the dorsal root entry zone and the column of Clarke (Bros et al., 2004). These latter data derive from a single post-mortem patient sample, however, and have yet to be replicated by other groups. Both groups reported localization of alsin to neuronal cell-bodies and dendrites.

The protein displays marked conservation amongst mammals showing 92 and 91% identity between human and mouse and human and rat, respectively (Devon et al., 2005) and contains several functional domains (Hadano et al., 2001; Yang et al., 2001). An N-terminal region contains five repeats of consensus sequences for Regulator of Chromatin Condensation 1 domains, and is thus referred to as the RLD or RCC1-like domain (Otomo et al., 2003; Topp et al., 2004). RCC1 is a guanine-nucleotide exchange factor (GEF) for Ran, a small nuclear Ras-related GTPase. Of over 90 known proteins with RCC1 domains, however, RCC1 itself is the only one with Ran GEF activity (Topp et al., 2004), and alsin does not show significant GEF activity for Ran (Otomo et al., 2003). Instead, the seven-bladed propeller structure that the N-terminal portion of alsin is likely to adopt may simply comprise a protein–protein interaction domain.

A C-terminal domain (residues 1360–1657) shows high homology to yeast Vps9p, a GEF for Vps21p, which is a small GTPase involved in vesicle trafficking. The few mammalian proteins with Vps9p-homology domains so far examined have all shown GEF activity toward Rab5 family members which are known to regulate some early endosome functions in mammalian cells. Both yeast two-hybrid and mammalian tissue culture pull-down experiments have shown interaction between alsin and Rab5 that is dependent on the presence of the Vps9p domain of alsin (Otomo et al., 2003, Topp et al., 2004). Furthermore, both Otomo et al. and Topp et al. demonstrated GEF activity of alsin toward Rab5 proteins. There was disagreement between the two groups, however, as to the role of a MORN domain (Membrane Occupation and Recognition Nexus) which is found slightly N-terminal of the Vps9p domain (in residues 1018–1351), Otomo et al. claiming this was required for Rab5 GEF activity, and Topp et al. showing evidence to the contrary. Both groups agreed, however, that GEF activity was specific for Rab5 proteins, showing little or no activity toward Rab4 and Rab11 GTPases, which also have early endosome-related functions. Endogenous alsin in tissue culture fibroblasts (Topp et al., 2004) and exogenous alsin in cultured rat cortical neurons (Otomo et al., 2003), co-localized with Rab5 and EEA1, markers of early endosomes. Over-expression of the Vps9p domain of alsin produced visible changes in early endosomes like those produced by expression of mutant constitutively active Rab5 in cultured neurons and fibroblasts (Otomo et al., 2003; Topp et al., 2004).

A central region of alsin (residues 685–1026) contains Dbl-homology (DH) and pleckstrin homology (PH) domains, which together comprise the signature of GEF activity toward members of the Rac/Rho family of GTPases. In vitro pull-down experiments revealed interaction between alsin and Rac1, but not the related Rac3, Rho or cdc42 GTPases, and GEF activity toward Rac1 was also demonstrated (Topp et al., 2004; Kanekura et al., 2005). In fibroblasts, alsin was observed to partially co-localize with activated Rac in membrane ruffles and lamellipodia. Rac1 activation is associated with changes in the cellular cytoskeleton, and actin remodeling in particular, a process that is required for trafficking events in neurons, such as clustering of post-synaptic receptors, recycling of endocytic vesicles and transport and fusion of exocytic vesicles, and the formation of synapse and dendritic spines and synapses (Topp et al., 2004; and references therein), see Fig. 15.2.

In view of the biochemical activities of alsin so far demonstrated, alsin is likely to function in endocytosis. Topp et al. (2004) speculate that interaction of the N-terminal RCC1 repeat region with as yet unknown proteins regulates early endosome trafficking activity of alsin, and that failure of these functions underlies ALS2. Possible molecular mechanisms for this include loss of the Rac1 dependent functions mentioned above, and failure of glutamate receptor endocytosis leading to overabundance of cell-surface glutamate receptors and excessive neuronal stimulation and excitotoxicity. Additionally alsin may be required for trafficking of "signaling endosomes" which may be essential for moving trophic factor survival signals from distal dendrites and axons to the nucleus in order to maintain neuronal viability (references within Topp et al., 2004). A further interesting speculation of Topp et al. (2004) is that the Rac1 exchange activity of alsin may be involved in activation of phosphatidyl inositol 3 kinase, which in turn activates an anti-apoptotic pathway involving Akt. This theme is further explored by Kanekura et al. (2005) who show that both constitutively active Rac1 and exogenous alsin can rescue a neuronal cell line from death induced by over-expression of mutant SOD1. These authors further showed, by RNAi-mediated knockdown, dominant-negative constructs and pharmacological inhibition, that the rescuing activity of alsin was dependent on Rac1, PI3kinase and Akt3 in

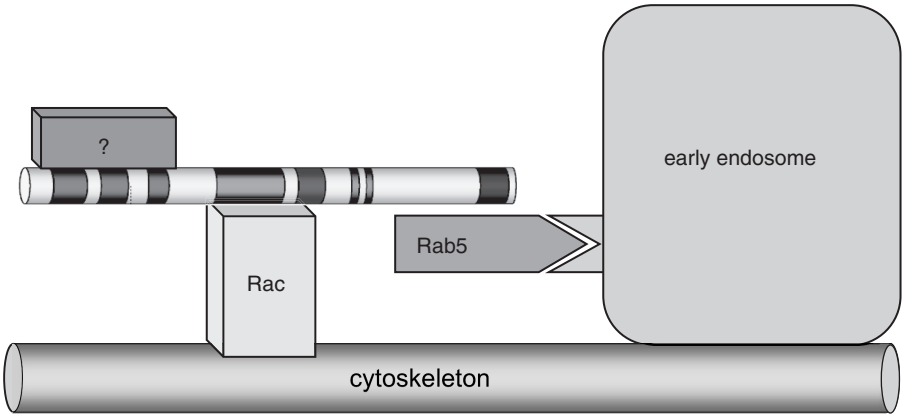

Fig. 15.2. Hypothetical schema of the role of alsin in endocytosis (not to scale). Movement along microtubules is essential for recycling or down-regulation of cell-membrane receptors and for delivery of some neurotrophic receptor signals to the nucleus. Activation of Rab 5 via the C-terminal domain of alsin may control functions of the early endosome, allowing it to interact with cytoskeletal microtubules. Activation of Rac through the DH-PH domain of alsin may promote movement of endosomes by stimulating polymerization of cytoskeletal components. These functions of alsin may be regulated by as yet unidentified proteins that interact with the N-terminal RCC1 domain.

these cells. Kanekura et al. (2005) have also published data suggesting the possibility of physical interaction between mutant, but not wild-type SOD1 and the Rac1 GEF domain of alsin, hypothesizing that mutant SOD1 may in fact impede some aspect of alsin function. Given, however, that within the CNS, alsin is only expressed in neurons, whereas mouse model evidence suggests that mutant SOD1 must be present in both neurons and glial cells for mice to develop ALS1-like disease (reviewed in Bruijn et al., 2004), it seems unlikely that such a model could explain the entire mechanism of mutant SOD1-mediated ALS. The finding of interaction between SOD1 and alsin has yet to be replicated by other groups.

Several groups have created alsin knockout mice, but the details of the phenotype of these mice have not yet been published. One investigator recently reported a subtle decline of motor function in alsin-deficient mice, and loss of axons in L5 ventral nerve roots by the age of 12 months (Julien et al., 2005).

15.4. ALS4 (MIM 602433)

Affected individuals with the autosomal dominant form of juvenile ALS (ALS4) exhibit a clinical phenotype of symptoms of motor neuron dysfunction that has early onset (often less than 6 years), slow progression, severe muscle weakness and pyramidal signs, but absence of bulbar and sensory abnormalities. This form of juvenile ALS is also known as "distal hereditary motor neuronopathy" with pyramidal features or dHMN.

A family affected by autosomal dominant juvenile onset ALS was described by Chance et al. (1998). Originally described as having Charcot-Marie-Tooth disease (Myrianthopoulos et al., 1964), this remarkable 11-generation pedigree (K7000) had been traced back to 17th century England, and included 52 affected persons living in southern Maryland and neighboring states. The inheritance pattern was clearly dominant; X-linkage was excluded by demonstrating male to male transmission.

Chance et al. (1998) investigated whether the disorder in pedigree K7000 was genetically linked to chromosomal loci previously associated with other forms of ALS and related motor neuron syndromes. Using DNA from 107 individuals (52 affected and 37 at risk), these authors excluded linkage to the ALS1 and ALS2 regions with markers D21S223 and D2S72, respectively. Additionally, they did not detect linkage with markers from the spinal muscular atrophy (SMA) loci on chromosomes 5 and 7, and no mutation was identified in the survival motor neuron (SMN) gene itself. These negative findings suggested that the ALS documented in this family was genetically distinct from previously described forms, and it was therefore designated ALS4.

To identify the genetic locus responsible for the ALS in this family, Chance et al. (1998) performed a genome-wide linkage scan using 150 markers spaced at ~ 10 cM intervals throughout the genome. This study resulted in highly significant positive LOD scores for markers on chromosome 9q34 (D9S158: $Z_{max} = 6.12$; D9S915: $Z_{max} = 4.82$) and, when additional markers in this region were tested, the highest LOD score was obtained with marker D9S1847 ($Z_{max} = 18.84$). These findings were supported by a multi-locus analysis, with

a remarkably high peak LOD score of more than 20 in the vicinity of markers D9S1847 and D9S1847. Recombinations within informative individuals placed the ALS4 locus a 5 cM interval of chromosome 9q34 between D9S1831 and D9S164.

Blair et al. (2000) carried out further linkage analysis with 14 chromosome 9q34 markers in pedigree K7000 following controversy over the diagnosis of a key individual used in the original study. This resulted in a refinement of the ALS4 locus to between D9S149 and D9S1198, a genetic interval of 0–3 cM, and a physical distance of only ~ 500 kb.

Two years later, De Jonghe et al. (2002) described three further families, from Belgium, Austria and Australia, with positive linkage to the ALS4 locus. The patients in these families were diagnosed with distal hereditary motor neuronopathy (dHMN) with pyramidal features, but, in light of the linkage results and clinical similarity, the authors suggested that it was the same disorder as ALS4. Linkage and recombination events within the three families placed the ALS4 locus within a 3.5 Mb interval between D9S64 and D9S164, encompassing the small region previously implicated by Blair et al. (2000).

The gene for ALS4, senataxin (*SETX*), was cloned in 2004. Chen et al. (2004) performed mutation screening of 340 exon fragments from 19 candidate genes in the ALS4 region. All the fragments could be PCR amplified as expected, suggesting that there were no gross deletions, insertions or other rearrangements of the 9q34 region in these families. However, DNA sequencing of the same exons revealed a heterozygous mutation (1166T/C) in exon 10 of the *SETX* gene in all affected members of the K7000 pedigree but none of 100 controls. This missense mutation would be predicted to cause a L389S amino acid substitution in the encoded protein.

Chen et al. (2004) also detected *SETX* mutations in two of the three dHMN families. These families also carried heterozygous missense mutations, namely 6407G/A (predicted to cause a R2136H substitution) and 8C/T (T3I substitution). No mutation was detected in the third family, and upon re-evaluation and extension of the linkage data in this family, no positive linkage was found and no haplotype was found to segregate with disease.

The *SETX* gene is comprised of 26 exons and is transcribed into two mRNAs of 11.5 kb and 9.0 kb, as a result of the use of alternative polyadenylation signals. Both transcripts were detected in all tissues tested by northern blot (Chen et al., 2004; Moreira et al., 2004), namely: adrenal gland, bladder, bone marrow, brain, heart, kidney, liver, lung, lymph node, mammary gland, prostate, skeletal muscle, placenta, spinal cord, stomach, thyroid, trachea and uterus). There are, as yet, no published data describing the expression of senataxin at the protein level.

The open reading frame encodes a large protein (2677 amino acids) with a predicted molecular weight of 302.8 kDa (NP_055861). The initiating ATG is in exon 3. Examination of the human (NM_015046/AY352728), rat (XM_342400) and mouse (NM_198033) cDNAs shows that the missense mutations documented by Chen et al. (2004) are all in residues that are identical across these species. The protein shows considerable homology with members of the yeast Sen1p family of RNA/DNA helicases, from which the name ‘senataxin’ was derived (Moreira et al., 2004). In particular, the N-terminal 466 residues of senataxin show approximately 20% similarity to the N-terminus of *S. pombe* Sen1p2. No other known functional domains or structural motifs have been found in the first 1930 amino acids.

The helicase domain encoded by amino acids 1931–2456 shows 85% identity between human and rat, and 90% identity between human and mouse orthologs, and is highly homologous to the helicase domain of the yeast Sen1p proteins. This domain also shares significant similarity with two other members of the DExxQ-box family of helicases: Rent1/Upf1, an RNA helicase involved in nonsense-mediated decay, and IGHMBP2, initially identified as a DNA-binding protein with transcriptional activity (Mizuta et al., 1993). The domain includes an ATP/GTP binding site motif (P-loop) that has been shown to be required for DNA unwinding activity in mammalian fibroblast and epithelial cell lines (Molnar et al., 1997). Interestingly, IGHMBP2 is mutated in spinal muscular atrophy with respiratory distress (OMIM 604320; Cox et al., 2001). Senataxin residues 2070–2087 are predicted to contain a bipartite nuclear localization signal (Chen et al., 2004).

It is noteworthy that loss of function (nonsense) mutations in the *SETX* gene have been detected in a prima facie unrelated autosomal recessive disorder, ataxia-oculomotor apraxia type 2 (AOA2, MIM 606002, Moreira et al., 2004). Both ALS4 and AOA2 cause a slowly progressing peripheral motor neuropathy. It is possible that a toxic gain of function in the SETX protein causes the motor neuron pathology of ALS4, whereas loss of SETX function leads to AOA2, a disorder with more widespread pathology (Chen et al., 2004).

To date, there are no published biochemical data that speak directly to the function of senataxin, nor any animal models of senataxin mutation. The homologies to the proteins outlined above, however, suggest that senataxin may function in RNA biogenesis, stability or translation, and/or in DNA repair, replication, recombination or transcription (Tanner and Linder, 2001; Chen et al., 2004). Moreira et al. (2004) speculate that, like

the yeast protein Sen1p1, senataxin may have both DNA and RNA helicase activity, and that ALS4, like ataxia-telangiectasia, AOA1, ataxia-telangiectasia-like disorder, and spinocerebellar ataxia with peripheral neuropathy 1 may be due to aberrant DNA repair activity. AOA2 may, on the other hand, be due to loss of RNA splicing related activity, like spinal muscular atrophy and spinal muscular atrophy with respiratory distress.

15.5. ALS5 (OMIM 602099)

The locus designated ALS5 was mapped using families affected by the most common form of autosomal recessive ALS, designated type I by Ben Hamida et al. (1990). This form is characterized by onset in the first or second decade of life of a slowly progressing weakness and atrophy that first affects the hands and feet, then later the tongue and pharynx. Upper motor neuron involvement becomes more apparent as the disease progresses.

Hentati et al. (1998) performed linkage analysis using seven families, from Tunisia, Pakistan and Germany. In total, DNA samples were collected from 64 individuals including 22 affected persons. Each family met the clinical criteria for type I ALS and exhibited an inheritance pattern consistent with an autosomal recessive trait. Linkage to the ALS2 locus on chromosome 2, and the ALS1 locus (*SOD1*) on chromosome 21 had previously been excluded, and no mutations in *SOD1* had been detected (Nijhawan et al., 1995).

Hentati et al. (1998) genotyped 161 markers across the genome in the largest family, of Tunisian origin. Allele sharing was detected for the chromosome 15 markers D15S118 and D15S117 among affected individuals but not unaffected siblings. Informative recombination events in this family also placed the ALS5 locus between these two markers. To confirm these findings, all seven families were genotyped with additional markers in this region of chromosome 15. Significant LOD scores (> 3) were obtained from two families, one family gave suggestive evidence of linkage (LOD > 2 for one marker) and a fourth gave weakly positive LOD scores. The remaining three families did not show evidence of linkage to chromosome 15, suggesting further genetic heterogeneity among this form of ALS. Cross-over events within the three most strongly linked families were used to refine the ALS5 region to a 6 cM interval of chromosome 15q15.1-q21.1, between D15S146 and D15S123. No cross-overs were detected between D15S778 and ALS5 in any family, suggesting that the gene may be located close to this marker.

The gene for ALS5 has yet to be identified. The 6 cM minimal region comprises 7.9 Mb of DNA and contains approximately 285 known genes (R.S. Devon, unpublished results). One promising candidate, tropomodulin 2, that is involved in the capping of actin filaments, was found to be negative for mutations in ALS5 patients (Cox et al., 2001).

15.6. Differential diagnosis

Making an accurate clinical diagnosis of ALS can be very difficult in cases with juvenile onset, and a broad differential diagnosis needs to be considered (see Table 15.2). The diagnosis of juvenile ALS should be based on the development of a clinical syndrome that meets diagnostic criteria for ALS with an age of onset less than 25 years (World Federation of Neurology Guidelines: www.wfnals.org – *Revised Criteria for the Diagnosis of ALS*). In general, the juvenile onset forms of familial ALS have less rapid progression and much longer survival times than adult onset ALS. Phenotypic variability within the specific forms of juvenile ALS may delay diagnosis or may mimic other disease entities. Some of the original cases clinically described as familial primary lateral sclerosis were found to have linkage to the *ALS2* gene critical region on chromosome 2 (Hadano et al., 2001; Yang et al., 2001). The described PLS phenotype in these cases was very similar to a slowly progressive form of ALS, except that the patients have isolated UMN signs and symptoms without any initial evidence of denervation on EMG studies, or other signs of LMN degeneration. With the recent identification of the specific disease gene mutation responsible for ALS2 and familial PLS, we now know that these apparently distinct disease phenotypes are

Table 15.2

Differential diagnosis of juvenile ALS

- Spinal Muscular Atrophy (SMA)
- Hereditary Spastic Paraplegia (HSP)
- Spinal and Bulbar Muscular Atrophy (SBMA)
- Adrenoleukodystrophy (ALD/AMD)
- GM2 Gangliosidosis (Tay-Sachs disease)
- Hereditary Motor Neuronopathy (HMN/CMT)
- Infections (Poliomyelitis, HTLV-I, HIV)
- Multifocal Motor Neuropathy (MMN)
- Heavy metals (Lead)
- Vitamin deficiencies (B12)
- Demyelinating disorders (Multiple sclerosis)
- Myasthenic syndromes

caused by allelic mutations within the *ALS2* gene. The onset of the full spectrum of LMN signs and symptoms may be significantly delayed or in some cases early and severe LMN signs may mask more subtle UMN findings. The occasional co-existence of mild sensory symptoms may mimic a hereditary polyneuropathy.

15.6.1. Spinal muscular atrophy (SMA)

Spinal muscular atrophy is a common and devastating autosomal recessive disease that affects approximately one in 10,000 live births. SMA is caused by a deficiency of the survival of motor neuron protein encoded by the SMN genes, *SMN1* and *SMN2* (reviewed in Ogino and Wilson, 2004). SMA is characterized by muscle weakness and atrophy resulting from progressive degeneration of LMNs. Disease onset ranges from fetal to early adulthood onset. Progressive symmetric weakness and atrophy of the skeletal muscles without significant signs of UMN disease (required for ALS diagnosis) are the clinical hallmarks of SMA. Molecular genetic diagnosis is now available that can detect the homozygous absence of SMN genes in most patients with clinically typical SMA.

15.6.2. Hereditary spastic paraplegia (HSP)

Hereditary spastic paraplegia (HSP) is a complicated and heterogeneous group of hereditary neurodegenerative conditions characterized by progressive, generally severe, lower extremity spasticity and weakness (paraplegia). HSP is also sometimes referred to as familial spastic paraplegia (FSP) or Strumpell-Lorraine syndrome. The genetics of HSP is complicated with over 21 known genetic loci that have been mapped for various forms of HSP, and causative mutations have been identified in 10 different genes (Fink, 2003). HSP is clinically divided into two major subtypes. Spastic paraplegia in the absence of other findings is known as "uncomplicated" or "pure" HSP. 'Complicated' HSP is characterized by progressive spasticity and muscle weakness in association with ataxia, mental retardation, visual and/or hearing impairment and/or other abnormalities.

15.6.3. Spinal and bulbar muscular atrophy (SBMA)

This is also known as Kennedy disease and is an X-linked recessive disorder caused by a CAG trinucleotide repeat in the androgen receptor (La Spada et al., 1991). Patients with this disorder can develop signs of motor neuron disease in early adolescence (Sperfeld et al., 2002) and be misdiagnosed as having ALS. A family history of transmission consistent with X-linked recessive inheritance (males only affected) and a relatively mild progression in the presence of the usual ancillary clinical features of gynecomastia, testicular atrophy and oligospermia suggest this diagnosis. Given the relatively good long-term prognosis of SBMA, compared to ALS, this diagnosis should be definitively assessed by molecular genetic testing prior to making the diagnosis of ALS.

15.6.4. Adrenoleukodystrophy (ALD/AMD)

Adrenoleukodystrophy and adrenomyeloneuropathy are rare inherited disorders of peroxisomes, characterized by the accumulation of very-long-chain fatty acids (VLCFA) in brain, spinal cord, adrenal glands and testes (Moser et al., 2004). In young patients, ALD may be confused with juvenile ALS, as symptoms typically include progressive spastic paralysis of the lower extremities, cognitive impairment and ataxia. The diagnosis of ALD should be suggested by the presence of adrenal insufficiency and hypogonadism in addition to the progressive neurological symptoms. The diagnosis of ALD and AMD is usually established by measurement of absolute levels of VLCFA.

15.6.5. GM2 Gangliosidosis (TSD or Tay-Sachs disease)

A heterogeneous group of neurodegenerative disorders caused by various forms of hereditary hexosaminidase A deficiency resulting in lysosomal storage of the glycosphingolipid, GM2 ganglioside (Vellodi, 2005). The juvenile variants of hexosaminidase A deficiency often develop slowly progressive LMN weakness with hyperreflexia and sustained ankle clonus, a phenotype which can mimic ALS, although often associated features such as progressive dystonia, spinocerebellar degeneration, cognitive impairment and presence of a cherry red spot in the retina often make the clinical diagnosis clear. The definitive diagnosis of TSD relies on genetic analysis or on the demonstration of dramatically decreased or absent hexosaminidase A enzymatic activity in blood or tissues.

15.6.6. Hereditary motor neuronopathy (HMN/CMT)

A variety of hereditary motor neuronopathies can mimic the early stages of ALS and make diagnosis difficult. CMT2A and CMT2D are two forms of Charcot-Marie-Tooth hereditary polyneuropathy that can present without significant sensory deficits and with a primary motor neuron phenotype (Berciano and Combarros, 2003). Over time the absence of UMN signs and distinctive findings on electrodiagnostic

studies should distinguish cases of HMN/CMT clinically from the juvenile forms of ALS.

A number of acquired disorders in childhood may rarely mimic juvenile ALS. In addition to genetic testing for the above disorders, the work-up should include where indicated by the clinical picture: appropriate electrodiagnostic testing (EMG/NCS) and possibly a nerve/muscle biopsy, imaging of brain and spinal cord (MRI/CT scan), heavy metal screening (lead), complete blood counts, vitamin B12 and folate levels, HIV and HTLV-I testing and serum VLCFA. Multifocal motor neuropathy (MMN) is a very important diagnosis to consider as it has very effective and specific treatment (high dose intravenous immunoglobulin therapy). The identification of multifocal conduction blocks in motor nerves, through nerve conduction studies and serum antibodies against ganglioside GM1, make the diagnosis in MMN. The diagnosis of juvenile ALS is essentially one of exclusion.

When the work-up is negative for other genetic or acquired disorders and the clinical setting is appropriate, it is now possible to pursue genetic testing for mutations in the *ALS2* and *ALS4* genes, but testing is only performed on a research basis (www.GeneTests.org). A limitation facing genetic testing for ALS2, and many other recessive diseases, is that the current testing only detects mutations within exons and splice junctions that are likely to affect protein sequence. Since the sequences critical to promoter and enhancer functions in these genes are generally not well characterized, it is possible that mutations occurring in these regions could cause disease through insufficient protein expression in required tissues, and nevertheless escape detection by these methods. Since for many diseases of the CNS it is impossible to obtain a biopsy for assay of appropriate protein expression in the appropriate tissue, firm correlation between CNS expression and expression in some more readily available sample tissue (e.g. peripheral blood) should be established for each disease, where possible, and assay of protein level in the relevant tissue could then be added to current sequence-based testing methods.

15.7. Conclusion

The relevance of these rare genetic forms of juvenile motor neuron disease to the more common sporadic forms of ALS or to other neurodegenerative disorders remains to be proven, but it is likely that a greater understanding of the specific disease pathogenesis in these rare conditions will provide important information about more common disorders. This understanding of disease pathogenesis will arise from ongoing studies of the basic functions of these genes in vitro and in vivo, by revealing the effects of the disease causing mutations on gene function, and through the development of model systems and model organisms in which to study these processes. Currently, riluzole is the only approved therapy for ALS and, given the limited success of efforts to develop novel therapeutic approaches over the last decade, the recent discovery of the genetic causes for two forms of juvenile ALS (A*LS2* and *ALS4*) is encouraging. The identification of these and additional ALS genes should lead to the elucidation of novel therapeutic targets and strategies for ALS, and an eventual treatment for this devastating disease.

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Appendix: Useful websites for juvenile ALS information

Please note that due to the ever-changing nature of the world-wide web, the publishers cannot guarantee that these web addresses will still be valid after going to press.

- WFN Guidelines for ALS Diagnosis: http://www.wfnals.org/guidelines/1998elescorial/elescorial1998criteria.htm
- OMIM - Online Mendelian Inheritance in Man: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
- The GeneTests/GeneReviews Website: http://www.Genetests.org
- ALS Society of Canada: http://www.als.ca/
- The ALS Association (United States): http://www.alsa.org/
- International Alliance of ALS/MND Associations: http://alsmndalliance.org/
- International Organizations, Motor Neuron Disease Association (UK): http://www.mndassociation.org

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Chapter 16

Primary lateral sclerosis

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16.1. Introduction

About a decade following Charcot's original description of amyotrophic lateral sclerosis (ALS) (Charcot, 1865; Goetz, 2000), Erb (1875) described a disorder characterized by exclusive involvement of the corticospinal tract which he named "spastic spinal paralysis." Several cases given the name of "lateral sclerosis" were described even earlier, and four of these were familial. In retrospect they most likely represented some form of hereditary spastic paraparesis, or one of the recently described infantile ALS syndromes (Lerman-Sagie et al., 1996; Devon et al., 2003). It appears that Charcot's first case of ALS was in fact a case of PLS (Charcot, 1865).

Primary lateral sclerosis (PLS) is a rare disorder. Most large ALS centers have recorded less than 20 cases over a decade or two, but there is no accurate information regarding the incidence or prevalence of PLS. It is estimated that worldwide there are less than 600 cases. Clinical criteria for PLS were initially proposed by Stark and Moersch in 1945 (Stark, 1945) and included insidious onset of a pyramidal syndrome, of otherwise undetermined cause, with slow disease progression and absence of muscle wasting. A duration of more than 5 years was a requisite; however, this duration is entirely arbitrary. Many years elapsed before additional criteria were added by Pringle et al. (1992). These included adult onset; although infantile and childhood cases have been reported (Grunnet et al., 1989; Gascon et al., 1995). Some of these have been familial (Kuruvilla and Joseph, 2002; Lesca et al., 2003) and were associated with the recently discovered ALSIN gene (Eymard-Pierre et al., 2002) or a variety of hereditary spastic paraplegia (Fink, 2001). It is therefore doubtful that true familial or infantile cases of PLS exist and absence of a family history, nearly symmetrical and bilateral pyramidal involvement including the face commencing in adulthood are the accepted characteristics of PLS. Pringle et al. (1992) also recommended the 5-year disease duration in the absence of lower motor neuron features be reduced to 3 years. This too is arbitrary and many patients are seen before that and require information about the prognosis. They can be informed that the diagnosis of PLS does not have the same lethal implications that ALS has, but that they will not be totally free of that risk for decades. The longer they go without lower motor neuron signs, the less likely they will ever show signs of ALS.

In recent years it has become apparent that PLS is heterogeneous in clinical presentation and that degeneration is not restricted to the central motor system (Rowland, 1999; Le Forestier et al., 2001a,b). Associated dementia, particularly of the fronto-temporal type, has been well documented (Tan et al., 2003; Mackenzie and Feldman, 2004; Mochizuki et al., 2004; Yoshida, 2004). The heterogeneity is further exemplified by a recent report describing patients who clinically mimicked Parkinson's disease, but turned out to have PLS (Mabuchi et al., 2004). They had bradykinesia, frozen gait and severe postural instability, as well as slowly progressive spinobulbar spasticity. MRI of the head showed precentral gyrus atrophy, central motor conduction was very prolonged or failed to evoke a response and positron emission tomography (PET) showed significant reduction of [18F]fluoro-2-deoxy-D-glucose uptake in the area of the precentral gyrus extending to the prefrontal, medial frontal and cingulate areas. However, no abnormalities were seen in the nigrostriatal system with PET using [18F]fluorodopa or [11C]raclopride or with proton MR spectroscopy.

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Thus, widespread prefrontal, medial and cingulate frontal lobe involvement can be associated with the Parkinsonian symptoms in PLS (Mabuchi et al., 2004).

Cases of PLS can convert to a more classic form of Charcot ALS even after long intervals of time (Bruyn et al., 1995), and most authorities agree that PLS is unlikely to be a specific entity, but more likely reflects one end of a spectrum of ALS (Swash et al., 1999), in which the other end is represented by an initial presentation with pure lower motor neuron involvement. The debate as to specific nosology will undoubtedly continue until the entire motor neuron disease genome has been unraveled (Veldink et al., 2004). For the purposes of this chapter PLS is referred to as a sporadic disorder of unknown etiology, characterized by a slowly progressive symmetrical pyramidal deficit occurring in the absence of muscle wasting and in which no other cause for the neurological dysfunction can be established. The syndrome as thus defined and recognized clinically, remains an unsatisfactory diagnosis-of-exclusion. Furthermore, there have been so few autopsy cases that a reliable clinico-pathological correlation is not possible and one must rely on clinical series to define the variations of the syndrome (Rowland, 1999).

16.2. Clinical description

16.2.1. Demographic characteristics

The mean age of symptom onset has varied with different series reported. Many indicate an older age of onset, about 60 years (Pringle et al., 1992; Hudson et al., 1993; Norris et al., 1993; Preux et al., 1996; Le Forestier et al., 2000, 2001a,b). However, it is apparent that a younger average age of onset may pertain and certainly patients in their late thirties and early forties are well recognized (Kuipers-Upmeijer et al., 2001). Like Classsic Charcot ALS men are affected slightly more frequently than women (Lerman-Sagie et al., 1996; Kuipers-Upmeijer et al., 2001; Le Forestier et al., 2001a).

By definition disease duration is 3–5 years or more and it is justifiable that, if after 5 years, there has been no development of lower motor neuron disease, the risk of ALS decreases and the possibility for a diagnosis of PLS increases. However, worldwide there have been too few cases reported to comment on average survival, but certainly it can be a long time. Many patients are aware of long periods in which the disease does not seem to progress and some even consider there has been improvement, sometimes several years after the onset of symptoms (Stark, 1945).

16.2.2. Motor system symptoms and signs

Upper motor neuron symptoms and signs are the hallmark of the PLS. Muscle stiffness leading to overt and progressive spasticity without associated muscle weakness or atrophy typifies the disorder. In about 2/3 of patients there is an ascending pattern with spasticity spreading in a rather stereotyped fashion from the legs to the arms and finally to involve the bulbar musculature (Zhai et al., 2003). Bulbar onset occurs in one third to one half of patients with PLS, a slightly higher incidence than reported for ALS (Pringle et al., 1992; Le Forestier et al., 2000, 2001a,b). In other cases there is a less well defined pattern of progression and symptoms can begin in any region (bulbar, cervical and lumbar) (Pringle et al., 1992; Le Forestier et al., 2000, 2001a; Zhai et al., 2003). Irrespective of the site of onset, the disease eventually progresses to produce marked disability in bulbar, cervical and lumbar regions. Spasticity can become very disabling and is often associated with severe pain, a combination of symptoms that is challenging to treat.

Dysarthria may occur early in the disease, as a presenting feature, and later become severe. In contrast, swallowing frequently remains normal, so much so that many patients with PLS gain weight during the course of their disease; this is in marked contrast to Classical ALS, in which dysarthria and dysphagia with weight loss frequently coexist (Eisen and Krieger, 1999).

Virtually all patients develop emotional lability and with disease progression inappropriate laughing and crying become obvious. This may not be entirely due to loss of downstream inhibitory influences and may partially reflect fronto-temporal dysfunction. There are no apparent differences in familial versus non-familial fronto-temporal dementia (Piguet et al., 2004). In addition to emotional lability loss of facial expression, restricted to the lower half of the face and probably related to supranuclear facial diplegia with dissociation of voluntary versus automatic (reflex) movement, occurs fairly frequently (Gastaut et al., 1988; Gastaut and Bartolomei, 1994). Bulbar signs and symptoms may correlate with a later age of onset (Kuipers-Upmeijer et al., 2001; Le Forestier et al., 2001b).

Urinary urgency, which is rather uncommon in ALS, is quite frequent in PLS and is probably related to detrusor hyperreflexia and a spastic internal sphincter (Russo, 1982; Le Forestier et al., 2001a,b). However, sphincter dysfunction is not a universal feature of PLS (Stark, 1945; Pringle et al., 1992; Kuipers-Upmeijer et al., 2001; Le Forestier et al., 2001a,b).

16.2.3. Neuropathological findings

Neuropathological studies in PLS are limited in number and extent and few, if any, studies have reported sizable series with most reports based on one or two cases (Beal and Richardson, 1981; Eisen et al., 1992; Pringle

et al., 1992; Hudson et al., 1993; Konagaya et al., 1995; Mascalchi et al., 1995; Le Forestier et al., 2000; Komine, 2001; Mochizuki et al., 2004). The first pathologically verified case of PLS in the modern era was a man who developed progressive spinobulbar spastic paresis at the age of 67 years (Fisher, 1977). Four years later a case of a 67-year-old woman who presented with spastic dysarthria, followed by dysphagia, pseudobulbar palsy and spastic tetraparesis was reported by Beal and Richardson (1981). Pathologic study showed severe atrophy of the precentral gyrus which microscopically showed a complete absence of Betz cells. There was loss of myelin throughout the corticospinal system, yet complete preservation of anterior horn motor neurons. Some patients have not shown a loss of Betz cells in the precentral gyrus; many of these cases showed degeneration of myelin in the posterior limb of the internal capsule (Younger et al., 1988).

However, much of the cited literature subsequently exploring clinical-pathological correlations in PLS and its difference from ALS is based on a single case report by Pringle et al. (1992). The neuropathologic features (including morphometric analysis) showed selective involvement of the motor cortex. There was complete absence of Betz cells from layer 5 of the precentral cortex and the remaining pyramidal cells were significantly smaller than those seen in normal controls, particularly in layers 3 and 5, with a laminar gliosis. These areas are key regions for the cortical organization and execution of voluntary movement and explain the marked motor disability seen in PLS (Pringle et al., 1992).

More recent clinical (Engel and Grunnet, 2000; Tan et al., 2003; Mackenzie and Feldman, 2004; Yoshida, 2004) and radiological evidence (see later) clearly indicates that pathology in PLS must extend beyond that reported up to the present. Recent neuropathological reports have shown marked atrophy of the frontal and temporal lobes with Betz cells completely absent, and moderate atrophy of the neostriatum. The spinal cord and nerve roots appeared normal. Immunohistochemically, ubiquitin-positive but tau-negative intraneuronal inclusions were found in the frontal and temporal cortices, including the precentral cortex and the hippocampal dentate gyrus, and the neostriatum (Konagaya et al., 1998; Mochizuki et al., 2004). As well as mild dementia of the fronto-temporal type this patient had features reminiscent of Parkinson's disease but did not respond to carbidopa-levodopa. Similar cases have been recently reported in the absence of neuropathology (Mabuchi et al., 2004). In addition to these, a large family with severe corticospinal involvement but otherwise fairly typical Alzheimer's disease has been described, highlighting the overlapping nature of neurodegenerative diseases as a whole (Eisen and Calne, 1992; Verkkoniemi et al., 2000, 2001).

16.2.4. Clinical evidence for lower motor neuron disease

The lack of lower motor neuron involvement in PLS, although considered a hallmark of the disease, differentiating it from classical ALS, is relative. Nevertheless, at onset and during subsequent years patients do not meet El Escorial criteria for definite or probable ALS because of the lack of clinical lower motor neuron features (Brooks, 2000; Brooks et al., 2000; Le, 2004). These, as with most research diagnostic criteria, remain controversial and will require ongoing modification (Belsh, 2000; Forbes et al., 2001; Beghi et al., 2002).

There is ample evidence that during the course of the disease there are clinical and electrophysiological observations indicating lower motor neuron involvement (Russo, 1982; Kuipers-Upmeijer et al., 2001; Weber et al., 2002; Zhai et al., 2003). Features suggestive of lower motor neuron diseases included cramps, fasciculations and/or mild amyotrophy. While these features are classic symptoms of ALS, all have been described in PLS (Younger et al., 1988; Norris et al., 1993; Bruyn et al., 1995; Kuipers-Upmeijer et al., 2001; Le Forestier et al., 2001a,b). Furthermore, post-mortem studies have shown loss affecting anterior horn cells (Beal and Richardson, 1981; Younger et al., 1988; Pringle et al., 1992; Bruyn et al., 1995; Hainfellner et al., 1995). Konagaya et al. (1998) reported Bunina bodies immunoreactive for cystatin-C and skein-like ubiquinated inclusions in the anterior horn cells. Both are typical of ALS.

16.3. Electrophysiology

16.3.1. Needle EMG

Many authors describe PLS patients with overt electrophysiological signs of denervation implying involvement of the lower motor neuron (Russo, 1982; Younger et al., 1988). In a recent prospective study (Le Forestier et al., 2001a), 14 patients had fibrillation potentials, positive sharp waves, abnormal recruitment and a reduction in the number of motor unit potentials recorded at variable times during the course of their disease. There was EMG evidence for chronic reinnervation in 12 patients. Muscle biopsies in 13 patients showed changes indicating denervation and/or reinnervation (Le Forestier et al., 2001a). Some reports suggested that fibrillation potentials and denervation activity only occur at later stages of PLS (Brown et al., 1992; Pringle et al., 1992; Norris et al., 1993). These findings are consistent with periods of denervation followed by periods of reinnervation throughout the course of the disease.

16.3.2. Motor unit estimates

Further support for lower motor neuron disease in PLS has been recently derived from motor unit estimates (MUNE). Stewart et al. (2002) used the mutiple point stimulation technique (MPS) to estimate the number of motor units in the thenar complex of 18 PLS patients (see Fig. 16.1). This method relies on recording of threshold responses, with the median nerve stimulated at very low intensities, sufficient to elicit a threshold response. The stimulating electrode is then moved along the nerve and the process repeated. Ten to 12 morphologically distinct single motor unit potentials (SMUPs) are recorded and, from this sample, the mean peak to peak amplitude is calculated, and this value when divided into the maximum compound muscle action potential yields the MUNE (Albrecht and Kuntzer, 2004; Shefner, 2004).

There was a significant MUNE reduction (mean value 123) in PLS patients that was intermediate between controls (mean value 180) and ALS patients (mean value 86). MUNE have recently been reported to be modestly reduced in Parkinson's disease, which too is not associated with lower motor neuron disease (Caviness et al., 2000, 2002). The pathophysiological process(s) underlying the reduction of MUNE in PLS is speculative. It is very possible that it reflects dysfunctional motor units (functional loss) rather than actual demise of these neurons.

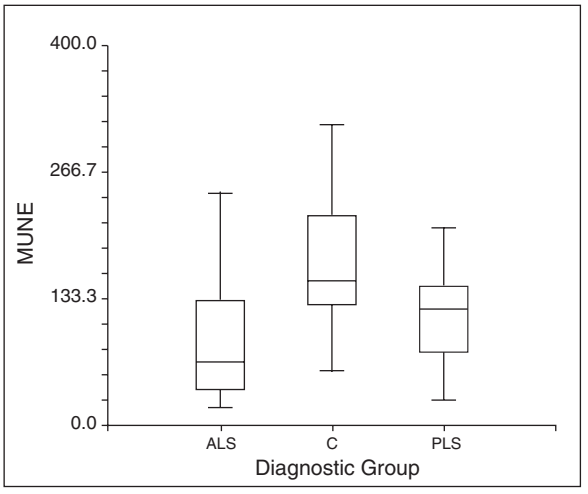

Fig. 16.1. Box and whisker plots of MUNES in ALS, Controls (C) and patients with PLS. MUNE was from the thenar complex and recorded using the multiple point stimulation method. The mean value in PLS (123) was intermediate between controls (180) and ALS (86). Some of the reduction in PLS may be due to functional impairment of motor units rather than actual loss of anterior horn cells.

Hara et al. (2004) recently reported that MUNE, measured by the F-wave method, is significantly decreased in the hemiparetic thenar complex subsequent to a stroke. The motor unit reduction correlated with severity of the stroke and the reduction remained 1 year after onset. Trans-synaptic degeneration secondary to the upper motor neuron lesion was considered as the likely cause of the reduced MUNE. But, spinal anterior horn cells have been assessed in patients dying with post-stroke hemiplegia and no differences were seen in anterior horn cell populations or diameter and size distribution between affected and unaffected sides or between patients and control subjects (Terao et al., 1997). This argues against anterograde transneuronal degeneration of lower motor neurons after upper motor neuron damage, at least in stroke patients (Terao et al., 1997). However, MUNE values represent the number of 'functioning' motor units in the muscle and this number could be reduced by loss of the trophic effect from corticomotoneurons (and other pyramidal neurons) without actual death of anterior horn cells (Eisen and Weber, 2001). Functional versus actual loss of anterior horn cells implies potential reversibility of damage. Although this may not be directly applicable to PLS because of its slow progression it could have great potential in ALS. Some of the much greater MUNE reduction in ALS could equally be functional in nature.

16.3.3. Motor evoked potentials

The characteristic loss of Betz cells in PLS readily explains the increase in cortical threshold as measured by transcranial magnetic stimulation. This is a frequent finding, but is not specific to the disease. In many PLS patients cortical threshold is so high that a motor evoked potential (MEP) cannot be elicited (Brown et al., 1992; Salerno et al., 1996; Le Forestier et al., 2001b; Stewart et al., 2002; Weber et al., 2002). Using peristimulus time histograms (PSTHs) which allow unitary recording of cortically evoked MEPs in 12 PLS patients, Weber et al. (2002) found the cortical threshold of single motor units to be 73.6% and the duration of the primary peak, a measure of corticospinal desynchronization, was significantly longer than normal and most cases of ALS (see Fig. 16.2).

These abnormalities are indicative of severely compromised descending corticomotoneuronal influences and could readily be associated with decrease or loss of normal trophism to the anterior horn cell causing a functional loss of motor units as described above. Several reports suggest that there is significant prolongation of MEP latency (Brown et al., 1992; Pringle et al., 1992; Kuipers-Upmeijer et al., 2001; Le Forestier et al., 2001a,b). However, when cortical threshold is

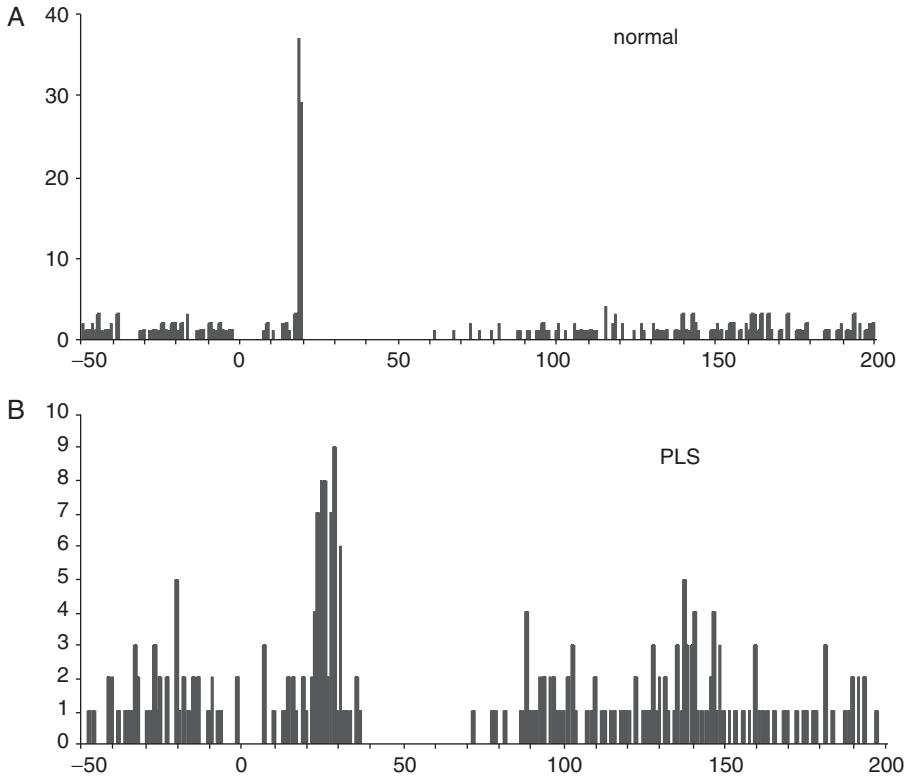

Fig. 16.2. Peristimulus time histograms in a control subject (top trace) and a patient with PLS (lower trace). The x-axis indicates the time scale (ms). It shows events occurring for 50 ms before and 200 ms after transcranial cortical magnetic stimulation which was applied at time 0 ms. The apparent short inhibition of activity immediately following the stimulus, lasting 5 ms, is due to stimulus artifact suppression built into the program. Following the primary peak there is a variable period of inhibition. Events were collected in 1 ms bins. Note that the scales for the y-axes (bin count) are different in the control and patient. The onset latency of the primary peak is similar in both examples (about 20 ms) and the major abnormality is the marked desynchronization and increased duration of the primary peak in PLS. Similar findings have been recorded in ALS.

high, requiring a strong stimulus intensity to be applied, the evoked MEP is usually very small in amplitude and this makes delineation of its onset difficult.

16.4. Imaging studies

16.4.1. Magnetic resonance imaging (MRI)

There are no specific MRI changes in PLS and the abnormalities seen occur equally in classic ALS (Peretti-Viton et al., 1999; Kalra and Arnold, 2003; Lee et al., 2003; Cabello et al., 2004). The imaging abnormalities do not appear to correlate with disease severity or duration. An interesting finding is that of hyperintensities of the corticospinal tracts bilaterally, extending from the internal capsule to the brain stem – producing a "wine glass" appearance on coronal sections (see Fig. 16.3). This is best visualized on coronal T2 FLAIR images (Kuruvilla and Joseph, 2002; Zhang et al., 2003). The hyperintensities may extend into the cervical spine, possibly suggesting antegrade degeneration (Mascalchi et al., 1995).

Serial MRI over several years demonstrates progressive atrophy of the premotor, parietal and primary sensorimotor cortex, but sparing of the temporal lobe, occipital lobe and cerebellum (Smith, 2002). Although the pattern of cerebral atrophy is restricted, it is more extensive than expected from sensorimotor cortex pyramidal cell loss described in literature autopsy reports of PLS.

Newer MR tools, such as diffusion tensor imaging, magnetization transfer imaging and functional MR imaging have substantial promise as scientific and clinical tools in both PLS and ALS (Chan et al., 2003). MR diffusion tensor imaging has been recently reported in PLS. All of seven patients showed decreased diffusion anisotropy and increased diffusion constant in the posterior limb of the internal capsule. This was not seen in normal controls, but was seen in patients early in the course of the illness (less than 3 years) (Ulug et al., 2004). Similar changes are seen in ALS and may occur in the absence of clinical upper motor neuron signs (Toosy et al., 2003; Sach et al., 2004).

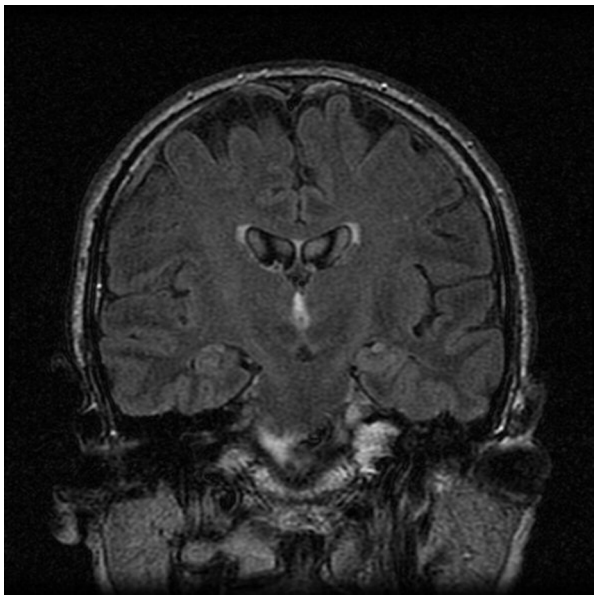

Fig. 16.3. MRI-T2 coronal Flair image from a 59-year-old lady meeting criteria for PLS. Her symptoms had begun 4 years prior to the MRI, which shows bilateral, symmetrical hyperintensities along the corticospinal tracts. Similar findings also occur in ALS.

16.4.2. PET studies

Using [11]-Flumazenil PET imaging in nine patients with PLS, Le Forestier et al. (2001a) found five patients with abnormalities in both benzodiazepine receptor density and regional distribution of cerebral blood flow (rCBF), mainly in the fronto-opercular (precentral gyrus) and anterior cingulate cortices. In ALS, PET studies clearly indicate widespread disease involvement and it is likely that, as further studies are reported in PLS, the same diffuse abnormalities will be encountered (Kew et al., 1994; Abrahams et al., 1995, 1996; Lloyd et al., 2000; Kalra and Arnold, 2003; Turner et al., 2004).

16.5. Biochemistry

CSF analysis is normal in PLS. Negative oligoclonal banding is important to rule out spinal multiple sclerosis. Tests should be performed to rule out syphilis and human T lymphocytic virus 1–2. Desai and Swash (1999) reported an IgM paraproteinemia in a patient with PLS. Recent publications underscore the need to screen patients suspected of PLS for systemic inflammatory disease and for cancer (Forsyth et al., 1997; Rowland, 1997).

A raised creatine kinase occurs in PLS. Kuipers-Upmeijer et al. (2001) found slightly increased levels in four of their nine patients.

16.6. Differential diagnosis

The diagnosis of PLS is determined clinically, being essentially a disease of exclusion. One can never be totally confident that conversion to Classic Charcot ALS will not occur even several years after symptom onset. The main diseases to consider that can mimic the major aspects of PLS are listed in Table 16.1. Most of these entities can be eliminated with appropriate imaging techniques or specific genetic or biochemical markers. Nevertheless, some cases of multiple sclerosis or sporadic hereditary spastic paraplegia may defy diagnosis, and not be discovered until autopsy.

The presence of cervical spondylosis or partial Chiari malformations are common findings and can contribute to diagnostic confusion, and occasionally lead to unnecessary operations. Although most cases of PLS start with gait difficulty accompanied by clinical evidence of a spastic paraparesis, others begin with spastic dysarthria so that the differential diagnosis also needs to include this symptom.

Cervical spondylotic myelopathy is very common, and its manifestations can mimic early features of PLS resulting in a spastic myelopathy with lower limb spasticity and hyper-reflexia (Davis, 1997; Kaptain et al., 2000; Sakaura et al., 2003). Radiological changes, including MRI changes indicative of some degree of cervical cord compression, are also common in the age group of PLS. This makes it difficult to determine to what extent if any the radiological findings are relevant.

Human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II) are closely related retroviruses with similar biological properties and common modes of transmission. Both are associated with a number of neurological diseases (Khan et al., 2001; Araujo and Hall, 2004). The distribution of HTLV-1-associated neurological disease is worldwide. In endemic areas, up to 30%

Table 16.1

Differential diagnosis of PLS

1. Progressive spinal multiple sclerosis
2. HTLV-1 myelopathy
3. HTLV-2 myelopathy
4. Spondylotic myelopathy
5. Vascular malformations of the spinal cord
6. Charcot ALS
7. D90A – SOD1 mutation of ALS
8. Other genetic forms of ALS (ALSIN)
9. Hereditary spastic paraplegias

of the population may be infected with HTLV-1, of which only a small percentage of infected persons develops neurological disease. A chronic progressive myelopathy with a resulting spastic paraparesis is the commonest and best recognized complication of these viruses. The paraparesis is frequently associated with sphincter disturbances, paresthesia and/or lumbar pain. Other neurological manifestations occur including a myopathy, encephalopathy and an ALS-like syndrome without upper motor neuron findings (Leite et al., 2003). These diseases are readily confirmed by serologic evidence of infection with reactive ELISA tests for HTLV-I or II, confirmed by positive Western blot assays.

Of the now well over 100 superoxide dismutase (SOD1) mutations (Andersen, 2003), the D90A mutation is phenotypically characteristic and early manifestations are atypical of sporadic ALS and other forms of FALS (Andersen et al., 1996, 1997; Andersen, 2001). There is an insidious pre-paretic phase in 2/3 of cases consisting of lower extremity stiffness and cramps, unsteadiness or clumsiness and general fatigue. Approximately half of patients complain of burning or aching pain in the lower back, buttocks, hips and/or legs. The pre-paretic phase can last from a few months to 7 years, during which time the neurological and neurophysiological studies are normal. The paretic phase is stereotyped with slowly ascending, usually asymmetric weakness with predominant upper motor neuron findings including spastic tone, brisk deep tendon reflexes and bilateral Babinski signs. Urgency of micturition and/or difficulty initiating urination are also common. This picture is very similar to PLS in its early stage. Later, features more characteristic of classical ALS, such as muscle wasting and fasciculations appear.

Late onset multiple sclerosis, defined as the first presentation of clinical symptoms in patients over 50, is not as rare a phenomenon as was previously thought (Martinelli et al., 2004). The prevalence ranges between 4% and 9.6% in different studies. The course of the disease is typically primary progressive with frequent pyramidal (or cerebellar) involvement, starting as a spastic paraparesis. Clinical characteristics, magnetic resonance imaging (MRI) pattern of abnormalities, evoked potential studies and cerebrospinal fluid (CSF) oligoclonal band analysis are of high diagnostic yield in these patients and can usually clarify the diagnosis.

The hereditary spastic paraplegias are a complex group of disorders dealt with in detail in Chapter 17 of this volume. More than 20 different gene loci have been identified (and 11 genes) with resulting recessive, dominant and sex-linked variants. They all have in common the clinical constellation of a spastic, hyperreflexic progressive paraparesis as a central feature of the syndrome complex. Early symptoms of gait difficulty, muscle stiffness, cramps and sometimes urinary dysfunction can readily be confused with a very similar constellation typical of PLS. Cases that are recessive or sex-linked may lack a good family history making the correct diagnosis more challenging.

16.7. Therapeutic strategies

There is no specific treatment for PLS and prospective longitudinal studies have not yet been carried out; largely because individual experience with this disorder is so limited. Treatment strategies therefore remain largely symptomatic. A multimodal approach as has been successfully applied in ALS and multiple sclerosis (MS) could prove effective in PLS (Crayton et al., 2004; Miller et al., 2005). Certain symptoms in PLS occur regularly, although variably, and treating these is critically important to maintaining quality of life. Symptoms that require treatment include spasticity, fatigue, sexual dysfunction, bladder and bowel dysfunction, pain, depression, inappropriate laughing and crying and dysarthria.

A multimodal approach requires effective communication, patient education, physical modalities and activities, occupational and other therapies and pharmacologic interventions. Individualizing treatment for each patient involves gaining control of symptoms as early as possible. There are a variety of agents in use to treat spasticity. They include, amongst others, tizanidine, dantrolene, baclofen, diazepam and gabapentin. A recent review on the efficacy of these medications in slowly progressive neurological disease concluded that the evidence favoring their use was weak and did not include evaluation of patients' quality of life (Montane et al., 2004). If any, efficacy was marginal and adverse drug reactions were common. Muscle cramping and spasms often respond to quinine sulfate, but when they become painful this is often inadequate and specific pain medication will be required.

Excessive laughing and crying may respond to tricyclic antidepressants, some of which (trazodone, amitriptyline) are also helpful for insomnia, sometimes a prominent symptom in PLS. Selective serotonin reuptake inhibitors are also effective for emotional lability. Oxybutynin is useful for treating urinary urgency or frequency. Augmentative-alternate communication, any method of communication that supplements or replaces speech is frequently required for patients with PLS. They can range from "low-tec" devices such as a communication board to "high-tec" type and speak devices.

16.8. Conclusion

Many arguments favor the hypothesis that PLS is not a discrete nosological entity but represents part of

a continuous spectrum of motor neuron diseases and in particular ALS. The distinction between ALS and PLS relates primarily to the degree and stability of lower motor neuron involvement. More pertinent to the argument as to whether or not PLS is a distinct entity is its benign, slow course compared to classical ALS and the typically prolonged protection of anterior horn cells and what possible protective mechanisms might underlie these differences. It is not clear that the mechanism(s) that account for the modest anterior horn cell involvement in PLS are the same as those that contribute to their dramatic loss in ALS.

The unifying concept can be taken a stage further in that the motor neuron diseases might be considered as a member of a family of neurodegenerative diseases with no absolute boundaries between them. This concept has emerged in recent publications (Bigio et al., 2003; Ross and Poirier, 2004). Further longitudinal studies of PLS, as well as more systematic electrophysiological and morphological studies (neuroimaging, post-mortem study) could help to understand its common abnormalities and differences compared with other neurodegenerative diseases and contribute to further elucidation of their underlying physiopathology.

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Chapter 17

Hereditary spastic paraparesis

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The term hereditary spastic paraparesis (HSP) represents a group of conditions in which the prominent feature is a progressive spastic paraparesis. There have been no recent epidemiological studies but previously the incidence has been reported as between 1 in 10,000 to 1 in 100,000 (Polo et al., 1991; Filla et al., 1992; Leone et al., 1995; Silva et al., 1997). Although genetically diverse with 28 genetic loci for HSP identified, it is often difficult to separate the disorders on clinical grounds. This phenotypic uniformity perhaps reflects a final common pathway in the disease process which results in degeneration of the corticospinal tracts. Advances in recent years identifying the genes at 11 of these loci have suggested that disruption in any of the following: axonal transport, cytoskeleton regulation, mitochondrial function, myelin maintenance and assembly and neuronal migration may cause axonal damage in HSP.

17.1. Classification and clinical features

The most useful way of classifying HSP is genetically to one of the current HSP gene loci. There are currently 28 spastic paraplegia (SPG) loci. The genes have been identified at 11 of these loci. Table 17.1 details the currently known SPG loci.

HSP can also be classified clinically according to mode of inheritance, age of onset or clinical phenotype. HSP is inherited most often as an autosomal dominant trait, with autosomal recessive and X-linked inheritance occurring rarely and very rarely, respectively. Identifying the mode of inheritance is complicated by an increasing recognition of reduced penetrance, most often described in spastin related HSP.

Two broad HSP phenotypes, pure HSP and complicated HSP, have been used to clinically classify HSP (Harding, 1981). Pure HSP, perhaps usefully considered uncomplicated HSP, is used to describe a phenotype in which the spastic paraparesis is the only major feature. However it is still helpful to consider pure HSP as an umbrella term, as other symptoms and signs such as sphincter disturbance, dorsal column sensory loss, distal amyotrophy (in prolonged disease) and pes cavus may all be seen. The typical patient with pure HSP will have a progressive spastic paraparesis. The age of onset can vary from infancy to the eighth decade. Often the first symptoms will be of gait disturbance (including toe walking, inability to run, difficulty with sports, frequent tripping), excessive shoe wear or leg stiffness. The classical findings on examination are of disturbed gait (spastic scissoring, toe walking), increased tone, hyperreflexia, extensor plantar responses, vibration and/or joint position sense impairment and distal amyotrophy (disease duration over 10 years). Weakness may be seen but rarely is this the major cause of the disability. A common scenario is to find a patient who is wheelchair bound due to severe spasticity with normal power when examined. Upper limb involvement is not uncommon but usually consists of brisk deep tendon reflexes and less often a spastic increase in tone. Prominent involvement of the upper limbs is not commonly seen in pure HSP. Other diagnostic alerts against pure HSP include bulbar signs or symptoms, prominent amyotrophy occurring early in the disease, asymmetry, ataxia, extrapyramidal signs and signs of peripheral neuropathy. Table 17.2 summarizes the suggested diagnostic criteria for pure HSP.

Whereas in pure HSP the spastic paraparesis is the prominent feature, in complicated HSP the spastic paraparesis occurs with one or more additional clinical features contributing to a broader phenotype. There are many reports in the literature of various clinical features occurring in addition to a spastic paraparesis

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Table 17.1

Currently identified SPG loci

Genome database designation	Chromosome	Inheritance	Gene
SPG1	Xq28	X-linked	L1CAM
SPG2	Xq22	X-linked	PLP
SPG3	14q11.2	AD	Atlastin
SPG4	2p22	AD	Spastin
SPG5	8p12-q13	AR	
SPG6	15q11.1	AD	NIPA1
SPG7	16q24.3	AR	Paraplegin
SPG8	8q24	AD	
SPG9	10q23.3-24.2	AD	
SPG10	12q13	AD	KIF5A
SPG11	15q13-15	AR	
SPG12	19q13	AD	
SPG13	2q24-q34	AD	Hsp60
SPG14	3q27-q28	AR	
SPG15	14q22-q24	AR	
SPG16	Xq11.2	X-linked	
SPG17	11q12-q14	AD	BSCL2
SPG18	Pending		
SPG19	9q33-q34	AD	
SPG20	13q12.3	AR	Spartin
SPG21	15q22.31	AR	Maspardin
SPG22	Xq21	X-linked	
SPG23	1q24-q32	AR	
SPG24	13q14	AR	
SPG25	6q23.3-q24.1	AR	
SPG26	12p11.1-12q14	AR	
SPG27	10q22.1-q24.1	AR	
SPG28	14q21.3-q22.3	AR	

including: amyotrophy (Fig. 17.1), cardiac defects, cerebellar dysfunction, sensorineural deafness, dementia, endocrine dysfunction, epilepsy, dystonia, choreathetosis, icthyosis, optic atrophy, retinal degeneration, neuropathy, cataracts and skeletal deformities. Some are isolated reports making it uncertain whether the occurrence is a chance phenomenon. Similarly, chance may be all that is responsible when a common condition such as epilepsy is occurring in association with HSP. However, more recently certain complicated phenotypes have been linked to specific HSP loci suggesting that some reported associations may be robustly linked to HSP. Table 17.3 outlines some of the more interesting complicated HSP phenotypes which the genetics have elucidated and they are discussed later in the relevant genetic section.

Table 17.2

Clinical features that aid diagnosis of pure HSP

	Clinical feature
Common	Brisk lower limb reflexes Progressive gait disturbance Spasticity of lower limbs Family history Brisk upper limb reflexes Extensor plantar response Mild paresis of lower limbs Sphincter disturbance Mild dorsal column disturbance Pes cavus Loss of ankle jerks Mild terminal dysmetria
Uncommon	Late distal amyotrophy Paresis of upper limbs
Diagnostic alert	Bulbar involvement Asymmetry Paresis greater than spasticity Prominent or early amyotrophy Prominent upper limb involvement Peripheral neuropathy Ataxia Extrapyramidal signs

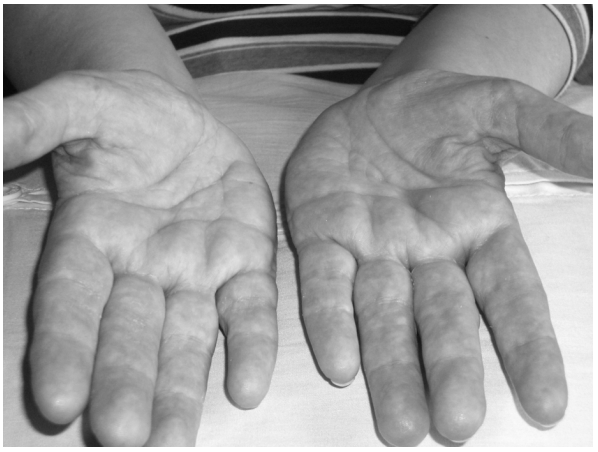

Fig. 17.1. Severe amyotrophy of the hands in complicated HSP SPG17 (Silver syndrome) caused by mutation in BSCL2.

Table 17.3

Additional features observed in complex HSP phenotypes

Inheritance	Additional features	Syndrome	GDB classification	Gene
AD	Cognitive impairment or dementia	—	SPG4	Spastin
	Prominent distal amyotrophy	Silver	SPG17	BSCL2
	Cataracts, esophageal reflux, axonal neuropathy, amyotrophy	—	SPG9	—
AR	Cerebellar signs ± optic atrophy ± amyotrophy, peripheral neuropathy	—	SPG7	Paraplegin
	Thin corpus callosum ± diffuse white matter change, dysarthria, tremor, dysphagia, neuropathy	—	SPG11	—
	Neuropathy, mild mental retardation	—	SPG14	—
	Retinal degeneration ± amyotrophy, mental retardation	Kjellin	SPG15	—
	Amish population, amyotrophy, spastic dysarthria, developmental delay, skeletal abnormalities, cerebellar dysfunction, choreoathetoid movements, deep white matter signal change on MRI	Troyer	SPG20	Spartin
	Amish population, dementia, extrapyramidal movement disorder, generalized cerebral atrophy and demyelination on MRI	Mast	SPG21	Maspardin
	Skin and hair pigmentation defects ± neuropathy	—	SPG23	—
	Susceptibility to intervertebral disc herniation ± neuropathy	—	SPG25	—
	Dysarthria, distal amyotrophy, emotional lability	—	SPG26	—
X-linked	Mental retardation, congenital skeletal abnormalities, aphasia, hydrocephalus, corpus callosum hypoplasia	CRASH/L1	SPG1	L1CAM
	Cerebellar syndrome, mental retardation optic atrophy, microcephaly, white matter change on MRI	—	SPG2	PLP
	Dysmorphic face, skeletal abnormalities, muscle hypoplasia, severe mental retardation, dysarthria, athetoid movements	Allan-Herndon-Dudley	SPG22	—

HSP was previously classified according to age at disease onset (Harding, 1981). Type 1 HSP indicated an onset of younger than 35 years, whereas type 2 HSP indicated an onset of older than 35 years. It was proposed that the type 2 late onset group had a more rapid and severe disease course compared to the earlier onset group. Later studies following the identification of the SPG loci have not supported age of onset as a predictor of disease severity. The marked inter- and intrafamilial variation in disease severity remains unexplained.

17.2. Investigation and diagnosis

The large number of genes and even greater number of loci linked with HSP can make establishing a positive diagnosis of HSP a somewhat daunting task. When a case of HSP is suspected, it is important to clinically evaluate as many members of the family as possible. Relying simply on a family history from an index case is unwise as this is limited by patient memory, patient acceptance of other possibly erroneous diagnoses in family (e.g. multiple sclerosis or arthritis) and will miss asymptomatic relatives. It is important to consider the differential diagnosis of spastic paraparesis in the index case at an early stage, particularly if the diagnostic alerts suggested in Table 17.2 are present. Diagnoses which may be amenable to intervention (e.g. MS, structural spinal lesions, dopa responsive dystonia, B12 deficiency etc.) or those which have a poor prognosis (e.g. amyotrophic lateral sclerosis) are important to exclude. In addition to neuro-imaging, more detailed investigation, depending on the presentation may include: HTLV1 serology, HIV serology, syphilis serology, vitamin E levels, very long chain fatty acids and white cell enzymes. If at this stage HSP is still the favored diagnosis, then we suggest the following

Table 17.4

Genetic loci and genes identified for pure HSP

Inheritance	Distinguishing features	Genome database designation	Gene
AD	Young onset	SPG3	Atlastin
	—	SPG4	Spastin
	—	SPG6	NIPA1
	—	SPG8	—
	Young onset	SPG10	KIF5A
	Young onset, rapid progression	SPG12	—
	Severe functional handicap	SPG13	Hsp60
	Late onset, benign course	SPG19	—
AR	—	SPG5	—
	—	SPG7	Paraplegin
	—	SPG11	—
	—	SPG24	—
	—	SPG27	—
		SPG28	
X-linked	White matter change on MRI	SPG2	PLP
	—	SPG16	—
	—	SPG22	—

process for proceeding with targeted genetic testing. It is first useful to classify the phenotype as either pure or complicated as discussed earlier. Having classified into pure or complicated HSP consult either Tables 17.3 or 17.4 for each phenotype, respectively. Within each table by using the mode of inheritance and/or any distinguishing features of the phenotype, the number of possible loci is reduced. This approach may help in deciding which gene or group of genes to test for in a particular family, given the increasing availability of genetic testing for these disorders.

17.3. Neuropathology

The major neuropathological feature of pure HSP is a length dependent axonopathy within the CNS (Schwarz, 1952; Schwarz and Liu, 1956; Behan and Maia, 1974; Kramer, 1977; Sack et al., 1978; Bruyn, 1992). The terminal portions of the long axons of the descending corticospinal tracts and ascending dorsal columns are most severely affected. Consequently axonal loss is greater in the lumbar region of the corticospinal tracts and in the cervical region of the dorsal column pathways (particularly in the longer fasiculus gracilis). Degeneration is also seen in the spinocerebellar tracts in approximately one half of reported cases. Dorsal root ganglia, posterior roots and peripheral nerves are usually normal. In a quantitative study of six HSP cases, corticospinal tract area, axonal density and axon number were observed to be reduced at all levels (Deluca et al., 2004). In the lumbar region axon number was estimated to be reduced by 56% compared to control cases. The ratio of brainstem (medulla) and lumbar total axonal number was significantly greater in HSP cases compared to controls, suggesting greater axonal loss distally. Similar findings were demonstrated in the sensory tracts with a 56% reduction in axon number in the fasiculus gracilis compared to controls. The shorter fasiculus cuneatus showed reduction in axonal number to a lesser extent and this did not reach statistical significance (23%). These observations strongly support the hypothesis that HSP is a length dependent axonopathy in which degeneration begins in the terminal portions of the longest axons and proceeds in a retrograde manner toward the cell body. It had been hypothesized that the cell body was relatively unaffected by the degenerative process until late in the disease course and that isolated reports of a reduction in Betz cell number reflected an advanced stage in the disease process (Schwarz and Liu, 1956). However, pathological reports have begun to demonstrate cytopathology in HSP (White et al., 2000; Wharton et al., 2003). The cytopathology observed in HSP and the pathology of HSP caused by mutations in spastin are discussed in further detail in Chapter 5. However, in summary the cytopathology from the spastin post-mortem cases supports the concept that the pathogenesis of SPG4 involves cytoskeletal dysregulation. These observations correlate with the observations in cell models and molecular studies discussed later in this chapter. Whether similar cytopathology is present in other genetic subtypes of HSP will require further post-mortem studies.

17.4. Autosomal dominant HSP

To date 10 genetic loci for autosomal dominant HSP (ADHSP) have been identified. The genes at six of the ADHSP loci are known. Mutations in spastin (SPG4) and atlastin (SPG3) account for up to 40% and 10% of ADHSP, respectively. The frequency of HSP due to mutations in NIPA1 (SPG6), KIF5A (SPG10), Hsp60 (SPG13) and BSCL2 (SPG17) is unclear at present, as only one or two reports of each have been published. The genes at SPG8, SPG9, SPG12 and SPG19 are currently unidentified.

17.5. SPG3/atlastin

The frequency of HSP due to mutation in atlastin has been reported to be as low as 8% and as high as 38%

depending on the methodology used. The phenotype tends to be pure HSP with childhood onset with a mean age of onset of 4.6 years in the largest published study (Elliott, 2004). Exceptions have been reported with onset occurring in the fifth and sixth decades in one family (Sauter et al., 2004). Occasional reports of incomplete penetrance include a clinically unaffected 80 year old obligate carrier and asymptomatic carriers aged 62 and 25 years old (Tessa et al., 2002; D'Amico et al., 2004; Durr et al., 2004). Disease progression tends to be slow with only approximately one fifth of individuals requiring the use of a wheelchair later in life. Features of pure HSP observed in the large series by Durr et al. included mild pes cavus (15%), diminished vibration sense (13%) and sphincter disturbance (25%) (Durr et al., 2004). In comparison with SPG4, distal wasting was more common whereas dorsal column involvement was less common. Perhaps reflecting the relatively young onset of the phenotype compared to other forms of HSP, scoliosis was seen in a fifth of cases (Durr et al., 2004).

Initially it appeared that mutation in atlastin was limited to missense change in several hotspots. However, increasingly mutations are being identified scattered throughout the gene with missense changes now reported in exons 4, 7, 8, 9, 12 and 13 (Zhao et al., 2001b; Muglia et al., 2002; Tessa et al., 2002; Dalpozzo et al., 2003; Abel et al., 2004; D'Amico et al., 2004; Durr et al., 2004; Hedera et al., 2004; Sauter et al., 2004). Furthermore, a novel insertion has been identified which causes a frameshift and premature truncation of the protein (Tessa et al., 2002).

Atlastin is a 64 kDa protein that is located predominantly in the brain, being particularly enriched in pyramidal neurons and in the cerebral cortex and hippocampus (Zhao et al., 2001b; Zhu et al., 2003). Atlastin has a predicted protein structure indicating that it is a member of the dynamin/Mx/guanylate-binding superfamily of large GTPases. Evidence suggests it is a multimeric integral membrane protein which may be involved in Golgi membrane dynamics or vesicle trafficking (Zhu et al., 2003).

17.6. SPG4/spastin

The gene for SPG4 was identified as spastin in 1999 by Hazan et al. (1999). Spastin is a large gene on chromosome 2p21-p22 comprising 17 exons. The gene encodes a 616 amino acid protein which contains several conserved domains (Hazan et al., 1999). These include an AAA cassette (ATPase associated with diverse cellular activities) and MIT domain (microtubule interacting and trafficking) (Ciccarelli et al., 2003). Spastin is an AAA protein, a group of proteins whose functionality is imparted by an AAA cassette. This is an approximately 230 amino acid highly conserved sequence. The AAA cassette through ATP hydrolysis provides the energy for the interaction of the AAA protein with other proteins. Outside of the AAA little homology is seen between AAA proteins resulting in many diverse roles for this group of proteins including protein degradation, vesicular transport and mitochondrial function. Spastin shares closest homology with members of the meiotic subgroup of AAA proteins, particularly katanin, a microtubule severing protein and VPS4 an endosomal trafficking protein.

17.6.1. Spastin expression and subcellular localization

Spastin is ubiquitously expressed, although in mouse tissues higher expression was noted in the brain, particularly cortex and striatum (Charvin et al., 2003). Three alternatively spliced spastin transcripts have been identified at mRNA level including or excluding exon 4, exon 8 or exon 15 (Svenson et al., 2001a). The exon 4 splice variant would appear the most abundant of the alternatively spliced forms and the ratio of this splice variant and the full length transcript is seen to vary on a tissue specific basis (Charvin et al., 2003). The significance of this at present is unclear. Exon 4 of spastin contains several putative glycosylation and phosphorylation sites which may be important in post translational modifications of the protein.

Investigating the subcellular localization of spastin, Charvin et al. demonstrated purely nuclear staining in HeLa cells, mouse liver and kidney cells. In mouse neural tissues staining was confined to the nuclei of neurons, with glial cells not demonstrating staining for spastin. In comparison, work in HEK cells demonstrated a purely cytoplasmic and perinuclear localization for spastin (Errico et al., 2002; McDermott et al., 2003a). The discrepancy was argued to be due to differences in experimental approaches, however it appears that spastin has a complex sub-cellular localization. Wharton et al. (2003) examined spastin expression in normal human CNS tissue using immunohistochemical techniques. Spastin was observed to be a neuronal protein with no evidence of expression in glial cells. The protein was predominantly expressed in the cytoplasm, but a weaker diffuse nuclear staining was seen in specific neuronal groups including those within the basal ganglia, hippocampus and cerebellar molecular layer. Therefore, it appears spastin has a specific subcellular localization in different neuronal populations. Further work has demonstrated that in dividing HeLa cells the subcellular localization changes through different stages of the cell cycle. During interphase spastin was

mainly confined to the nucleus, but became associated with the centrosomes, the spindle microtubules, the midzone and finally the midbody during cell division (Errico et al., 2004). In post-mitotic neuronal cultures (NSC34) spastin was seen to have nuclear and cytoplasmic staining which was noted to be enriched at the growth cone of the neurites (Errico et al., 2004).

17.6.2. Spastin and the cytoskeleton

Work in both neurons and non-neuronal cells has demonstrated that spastin, like the homologous protein katanin, interacts transiently with microtubules resulting in microtubule severing (Errico et al., 2002; McDermott et al., 2003a). Furthermore, missense mutations in spastin, which disrupt ATP hydrolysis, caused an abnormal stable interaction between microtubules and spastin, with loss of severing action. These observations have been supported by observations that recombinant spastin makes internal breaks along the lengths of microtubules both with purified in vitro microtubules and when added to permeabilized cytosol extracted fibroblasts expressing GFP-tubulin (Evans et al., 2005). Missense mutations with predicted effects on ATP binding and hydrolysis, as well as other disease associated mutations were observed to impair this microtubule severing action.

The abnormal stable association of mutant spastin with the microtubule cytoskeleton was demonstrated to perturb kinesin mediated transport of organelles, such as mitochondria and peroxisomes, on the cytoskeleton (McDermott et al., 2003a). Evidence of an abnormal distribution of mitochondria was also seen in motor neurons in post-mortem tissue from HSP cases associated with spastin missense mutations (McDermott et al., 2003a; Wharton et al., 2003). These observations suggested that a gain of function or dominant negative mechanism rather than haploinsufficiency may lead to the SPG4 phenotype.

Further evidence for the role of spastin as a microtubule severing protein came from work that demonstrated that spastin interacts with NA14, a 119 amino acid protein of undetermined function known to localize to centrosomes. The interaction with spastin occurs at the N-terminus between amino acids 50 and 87 (Errico et al., 2004). The NA14 interacting domain of spastin is necessary for the association of spastin with microtubules as evidenced by the failure of the K388R mutant spastin to stably bind microtubules when this region is mutated. The authors argue that NA14 is a molecular adaptor involved in targeting spastin to the centrosomes, allowing spastin microtubule severing activity in a specific subcellular location. The observation of spastin staining at the midzone and midbody suggest a role for spastin microtubule severing activity in cytokinesis. Other interacting proteins or the N-terminus of spastin per se may target spastin to these and other cytoskeletal structures. In neurons spastin has been shown to be enriched at areas associated with dynamic microtubule activity and it seems likely that microtubule severing by spastin plays a role in regulating this activity (Errico et al., 2004).

Whether inefficient severing of microtubules or the disruption in axonal transport due to abnormal interaction of mutant spastin with microtubules results in axonal degeneration remains to be proven.

Studies in *Drosophila* have confirmed a role for spastin as a microtubule regulator (Sherwood et al., 2004; Trotta et al., 2004). *Drosophila spastin*, *Dspastin*, exhibits 48% identity and 60% similarity at the amino acid level with human spastin. *Dspastin* was present in cytoplasm but not in nuclei of neurons and muscle cells. *Dspastin* was expressed at a low level throughout neuromuscular structures but was clearly enriched at the neuromuscular junction (Trotta et al., 2004). RNAi-mediated knockdown of *Dspastin* resulted in pupal lethality suggesting spastin is an essential gene. Similarly overexpression of *Dspastin* was fatal in the embryonic stage. In adult tissues, except at low levels, overexpression caused severe neurodegeneration and cell death (Trotta et al., 2004). At low levels, *Dspastin* RNAi expression caused a reduction in the presynaptic terminal area, an increase in the amount of stable microtubules within the synapse and an increase in neurotransmission strength. Treatment with nocodazole, a destabilizer of microtubules, was seen to correct neurotransmission strength. Conversely, overexpression of *Dspastin* caused a reduction in the amount of stable microtubules in the presynaptic boutons and a decrease in neurotransmission strength. The reduction in synaptic function could be corrected with taxol, a stabilizer of microtubules. These data suggested that *Dspastin* regulates stability of microtubules at the neuromuscular junction and consequently plays a role in maintaining the synapse and in modulating the synaptic efficacy dictating neurotransmission strength (Trotta et al., 2004). However, Sherwood et al. (2004) demonstrated conflicting results in *Drosophila* studies. *Dspastin* null mutants could survive a number of days suggesting *Dspastin* is not an essential gene. Spastin-null mutants had an increase in synaptic boutons that were observed to have a reduction in the number of microtubule bundles and reduced synaptic transmission. It is not easy to explain the discrepancies between the work of the two groups, but it may stem from differences in experimental design. Despite the differences, both studies confirm a role for spastin as a regulator of microtubules, even though the exact nature of the interaction requires further investigation.

A recent study has suggested an alternative, but perhaps related function, for spastin. Using a yeast 2 hybrid approach, Reid et al. (2005) identified CHMP1B as an interacting protein with spastin. The function of CHMP1B has not been precisely defined, but it appears to either regulate the function of or be a structural component of ESCRT (endosomal sorting complexes required for transport)-III complex. This is a complex which targets cargoes to the multivesicular body. The multivesicular body is a late endosomal structure that fuses with lysosomes leading to degradation of its contents. Supporting the interaction was the observation that spastin co-localized with both endosomal proteins and with tagged CHMP1B in various cell lines. The sequence region of spastin required for the interaction of spastin with CHMP1B lies between amino acids 80 and 194, the area containing the MIT domain. Spastin shares homology with another protein in the endosomal pathway, VPS4. VPS4 like spastin contains a MIT domain and belongs to the same AAA subfamily. VPS4 regulates the membrane association of the ESCRT-III CHMPs including CHMP1B and, given the similarities, spastin may play a similar role.

Although the evidence to date has suggested that spastin acts as a regulator of microtubules, it is possible that spastin may also play a role in membrane trafficking events. If spastin has a role in the endocytic pathway there are several possible explanations as to why a disruption in this process may lead to the axonal degeneration seen in HSP. An abnormality in the endocytic pathway would be predicted to cause multiple abnormalities in plasma membrane receptor levels and consequent abnormalities in signaling, perhaps including activation of apoptotic pathways or repression of survival pathways. A further possibility is the disruption of endosomal traffic within axons. Neurotrophic factors are known to travel in early endosomes toward the nucleus by axonal transport and clearly a disruption in neurotrophic availability would be predicted to be deleterious to the axon (Delcroix et al., 2003).

17.6.3. Spastin genetics

Mutation in the spastin gene is the cause of HSP in up to 40% of cases (Heinzlef et al., 1998; Hazan et al., 1999; Burger et al., 2000; Fonknechten et al., 2000; Hentati et al., 2000; Lindsey et al., 2000; De Bantel et al., 2001; Higgins et al., 2001; Mead et al., 2001; Namekawa et al., 2001, 2002; Svenson et al., 2001a,b, 2004; Ki et al., 2002; Meijer et al., 2002; Morita et al., 2002; Patrono et al., 2002; Proukakis et al., 2002, 2003; Sauter et al., 2002; Yabe et al., 2002; Bonsch et al., 2003; Molon et al., 2003; Qin et al., 2003; Chinnery et al., 2004; Falco et al., 2004; Nicholas et al., 2004; Nielsen et al., 2004a,b; Orlacchio et al., 2004a,b; Tang et al., 2004). Most often there is a clear autosomal dominant family history, but spastin mutations have also been described in patients with an apparently sporadic spastic paraparesis (Lindsey et al., 2000; Sauter et al., 2002; Falco et al., 2004). Unfortunately, at least 132 different mutations in spastin have been described, the majority being private mutations. These mutations are spread throughout the whole gene without any identified hotspot, meaning that the whole gene must be directly sequenced in the search for spastin mutations. Stopping when a mutation is found is no longer a possible shortcut, as several families are now described with more than one mutation in spastin (Chinnery et al., 2004; Svenson et al., 2004). Some researchers reported a failure to identify a spastin mutation in pedigrees with tight linkage to the SPG4 locus (Burger et al., 2000; Lindsey et al., 2000; Meijer et al., 2002; Starling et al., 2002b). Recently a mutation in the 5′UTR was identified as causing HSP in one family and it seems likely that mutation in this and other non-coding regions may explain this observation (Iwanaga et al., 2005).

Of the 132 spastin mutations published to date, truncating mutations (41%) are the commonest and all would lead to a protein missing all or part of the highly conserved AAA cassette. Similarly splice site mutations are common (28%) and the majority of these affect splicing of exons within the AAA cassette. There is evidence that the truncated or mis-spliced transcripts are unstable, perhaps leading to reduced or absent translation of the mutated allele. Missense mutations account for 29% of mutations and the majority occur within the highly conserved AAA cassette. There are areas outside of this region near the N-terminus and at the C-terminus where missense changes have occurred, perhaps indicating a functional importance for these areas that is presently unknown.

The breadth of the described mutations in spastin suggested that haploinsufficiency was the most likely pathogenic mechanism. Furthermore, the presence of unstable truncated transcripts, undetectable truncated mutant protein and ‘leaky’ mutations gave support to the idea of a dynamic threshold for spastin levels which when breached would lead to the development of an HSP phenotype (Burger et al., 2000; Svenson et al., 2001a,b; Patrono et al., 2002). Contrary to this, the stability of missense mutant transcripts, the abnormal interaction of missense mutant spastin with microtubules and the work in *Drosophila* suggest that a dominant negative or gain of function may be the pathogenic mechanism (Errico et al., 2002; McDermott et al., 2003a; Molon et al., 2003; Sherwood et al., 2004; Trotta et al., 2004). This issue is likely to be resolved as better disease models are developed.

17.6.4. Clinical features in patients with mutations in spastin

Spastin mutation is most often associated with a pure HSP phenotype consisting of an early adult onset of a progressive spastic paraparesis (Heinzlef et al., 1998; Hazan et al., 1999; Burger et al., 2000; Fonknechten et al., 2000; Hentati et al., 2000; Lindsey et al., 2000; De Bantel et al., 2001; Higgins et al., 2001; Mead et al., 2001; Namekawa et al., 2001, 2002; Svenson et al., 2001a,b, 2004; Ki et al., 2002; Meijer et al., 2002; Morita et al., 2002; Patrono et al., 2002; Proukakis et al., 2002, 2003; Sauter et al., 2002; Yabe et al., 2002; Bonsch et al., 2003; Molon et al., 2003; Qin et al., 2003; Chinnery et al., 2004; Falco et al., 2004; Nicholas et al., 2004; Nielsen et al., 2004b; Orlacchio et al., 2004a,b; Tang et al., 2004). The age of onset reported for spastin related HSP varies greatly not only between families, but also within the same family, ranging from infancy to the eighth decade. Incomplete or age dependent penetrance has been observed in several families with individuals in their 70s, who are obligate carriers of spastin mutation, being free of signs (Fonknechten et al., 2000; Qin et al., 2003).

Similar variability in disease severity is seen. The majority of affected individuals retain the ability to walk independently or with minimal support. At the extremes patients are described as asymptomatic with little in the way of spasticity or as being wheelchair-bound or bedridden (Lindsey et al., 2000; Morita et al., 2002; Namekawa et al., 2002; Patrono et al., 2002; Qin et al., 2003; Chinnery et al., 2004; Nicholas et al., 2004; Svenson et al., 2004). Figure 17.2 illustrates a patient with spastin mutation severely affected with scoliosis and marked contractures affecting upper and lower limbs. The cause of this variation is for the most part unclear. However, recently in a number of pedigrees an explanation for such variation was identified (Chinnery et al., 2004; Svenson et al., 2004). Families have been reported in which two independently segregating mutations in the spastin gene were present. When one mutation was inherited the patients were observed to have a milder phenotype compared to patients in whom both mutations were present. In one pedigree the missense change S44L segregated independently with the missense change P361L (Chinnery et al., 2004). The proband had a severe pure HSP phenotype of childhood onset. The mother was clinically unaffected but

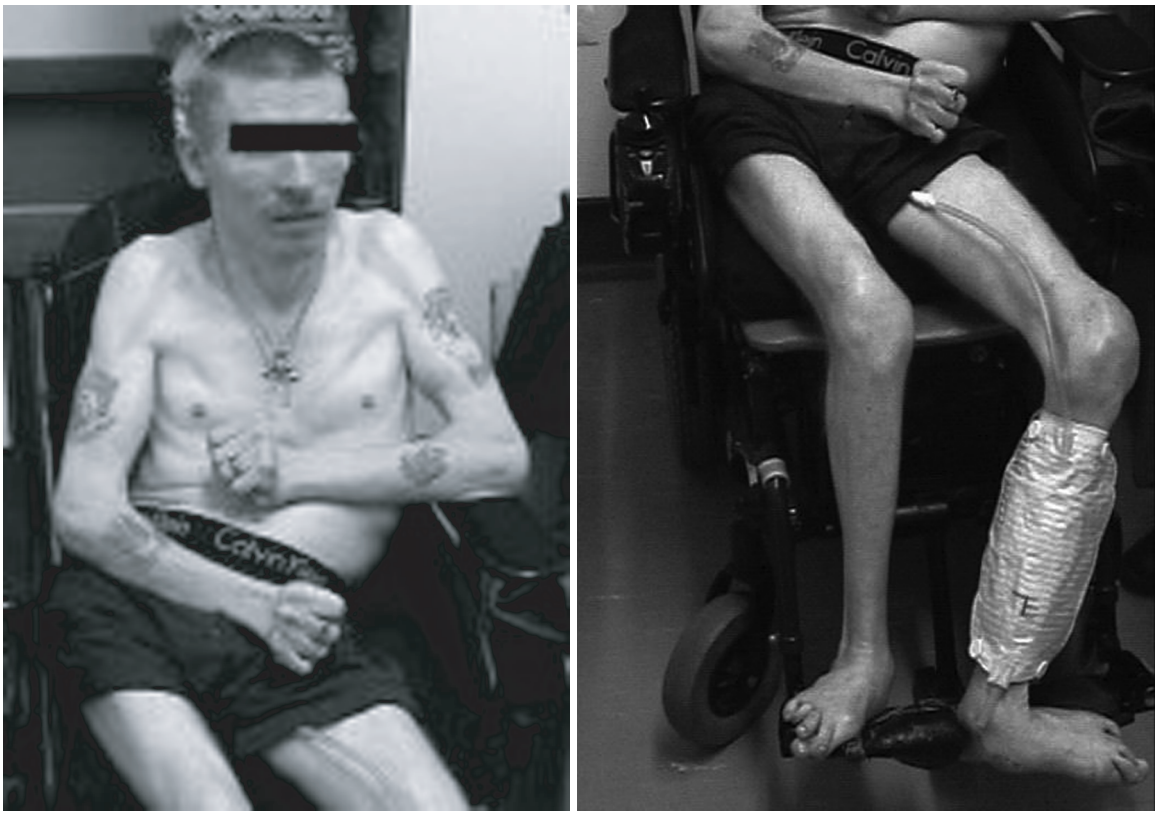

Fig. 17.2. A patient with mutation in the spastin gene with a particularly severe phenotype including scoliosis, severe tetraparesis, amyotrophy and secondary contractures.

carried the P361L change. The maternal grandfather of the proband was reported to be heterozygous for P361L and clinically had a pure HSP phenotype with severe spasticity. The father of the proband who harbored the S44L mutation did not report any walking difficulties but on examination was clearly affected. Further pedigrees have been described in which S44L and P45Q independently segregated with D470V and R526G, respectively (Svenson et al., 2004). Individuals with both mutations had younger age of onset compared to pedigree members with only one mutation. In the case of the S44L/D470V pedigree a more severe infancy onset phenotype was observed when both mutations occurred in an individual, with additional features in some including progressive cognitive decline and severe sensory loss. There is debate as to whether the S44L and P45Q missense changes are pathogenic per se or merely act as phenotype modifiers in the context of another spastin mutation. Further work is required to clarify this issue.

Unfortunately there is not one feature that stands out from the symptoms and signs observed in the pure phenotype associated with spastin mutation as compared with the other causes of pure HSP. Combining the observations of the more detailed clinical reports published, additional features consistent with pure HSP include: brisk deep tendon reflexes in arms (39%), increased tone in arms (25%), diminished vibration sense in the lower extremities (46%), pes cavus (43%), sphincter disturbance (45%) and amyotrophy (10%) (Fonknechten et al., 2000; Lindsey et al., 2000; Meijer et al., 2002; Namekawa et al., 2002; Patrono et al., 2002; Proukakis et al., 2002; Bonsch et al., 2003; Nicholas et al., 2004; Orlacchio et al., 2004b; Tang et al., 2004).

Although the common phenotype associated with spastin mutation is pure HSP, there are increasing reports of more complicated phenotypes. The most common complicating feature is cognitive impairment and even frank dementia. In the three families reported with dementia the clinical features were somewhat different (Heinzlef et al., 1998; Webb et al., 1998; White et al., 2000). In a French family reported by Heinzlef et al. (1998), a dementia with cortical features was seen in one family member, whereas cognitive impairment was described in other family members. In a family reported by Webb et al. (1998), one member died of a dementing illness at age 62 years, while other family members were found to have a subcortical dementia on neuropsychometric assessment. White et al. (2000) reported an index case with a rapidly progressive dementia with extrapyramidal changes in late life and unusual neuropathological features. Two family members had memory impairment in old age and one had borderline learning difficulties. In all three families the nature of the association of the dementia with the spastic paraparesis is unclear given the intrafamilial variation seen in each pedigree. The occurrence of dementia in these families may result directly from the expression of the mutant spastin protein or merely be a chance phenomenon. Supporting a role for spastin mutation in the development of dementia, McMonagle et al. (2000) found evidence of subclinical cognitive impairment in pure spastin HSP. They hypothesized that cognitive impairment is frequently present in spastin HSP, when specifically sought with neuropsychometric testing, and may even pre-date the onset of the spastic paraparesis (Byrne et al., 2000). Recently, the same group have shown a progressive cognitive decline in a group of SPG4 patients from three families, followed over a 3 year period, with seven out of 11 patients developing dementia of a mild or moderate severity (McMonagle et al., 2004). Other groups have reported SPG4 pedigrees with cognitive impairment in one or two family members, the significance of which is unclear (Lindsey et al., 2000; Orlacchio et al., 2004a; Svenson et al., 2004; Tang et al., 2004). No clear genotype phenotype correlation has been observed in pedigrees in which cognitive impairment is reported, with missense changes at different positions in the gene and a truncating mutation all being described.

There are reports of a variety of additional clinical symptoms and signs occurring in patients with spastin related HSP. These include; epilepsy (Heinzlef et al., 1998; Mead et al., 2001; Meijer et al., 2002; Orlacchio et al., 2004b), erectile dysfunction (De Bantel et al., 2001), amyotrophy (Lindsey et al., 2000; Falco et al., 2004; Tang et al., 2004), sensory disturbance (Svenson et al., 2004), constipation (Namekawa et al., 2001), multiple sclerosis (Mead et al., 2001), thin corpus callosum (Orlacchio et al., 2004b), foot drop (Meijer et al., 2002) and cerebellar signs (Meijer et al., 2002; Proukakis et al., 2002; Nielsen et al., 2004a; Tang et al., 2004). An interesting complicated family with the missense change T614I was reported in which spastic paraparesis segregated with congenital arachnoid cysts and pes cavus in all affected individuals (Orlacchio et al., 2004a). Cognitive impairment was also present in six of the 16 affected members.

17.7. SPG6/NIPA1

To date three families have been identified with NIPA1 mutations (Rainier et al., 2003; Reed et al., 2005). In one report a further 81 HSP families were screened for NIPA1 mutation with only one positive identified, suggesting SPG6 is not a frequent form of HSP (Rainier et al., 2003). The age at onset of SPG6 tended to be in the teenage years or early adulthood, ranging from

9 to 35 years of age. The phenotype of SPG6 is reported as pure ADHSP. However, in a recent large British pedigree additional features of epilepsy, cognitive impairment and postural tremor were present in a number of affected individuals (Reed et al., 2005).

The function of *n*on*i*mprinted in *P*rader-Willi/*A*ngelman loci 1 (NIPA1) is unknown. It is expressed ubiquitously at low levels but shows significantly higher expression in CNS tissues (Rainier et al., 2003). NIPA1 is predicted to have nine transmembrane domains and is likely to function as a membrane receptor or transporter (Reed et al., 2005). Missense mutations have been identified in exons 1 and 3 and are predicted to disrupt the transmembrane domains (Rainier et al., 2003; Reed et al., 2005). These mutations are likely to be pathogenic through a dominant negative, gain-of-function mechanism. Class I deletions in NIPA1 give rise to Prader-Willi or Angelman syndrome. Neither of these disorders cause a progressive spastic paraparesis, indicating that haploinsufficiency of NIPA1 is not the pathogenic mechanism in SPG6.

17.8. SPG10/KIF5A

The gene at the SPG10 locus has been identified as KIF5A (Reid et al., 2002). Two families have been identified with mutations in this gene (Reid et al., 2002; Fichera et al., 2004). Both families display a pure HSP phenotype with a young onset in infancy or teenage years. The mutations described are missense changes predicted to affect the microtubule binding domain of kinesin.

KIF5A is a member of the kinesin heavy chain group of proteins. *KIF5A* is expressed exclusively in neurons and forms part of the kinesin I complex, a microtubule motor responsible for anterograde travel from the neuronal cell body to the distal axon (Xia et al., 1998; Goldstein and Yang, 2000; Goldstein, 2001). The mutation (N256S) in one family identified to date affects an invariant aspargine residue (Reid et al., 2002). In homologs this residue has been demonstrated to be crucial in the activation of the ATPase activity upon binding to microtubules (Yun et al., 2001). In the *Saccharomyces cerevisiae* homolog, Kar3 mutation affecting the same residue decouples nucleotide and microtubule binding of the motor, resulting in a block in microtubule-dependent stimulation of motor ATPase activity (Song and Endow, 1998). A similar effect is seen with the same mutation in the Ncd kinesin motor of *Drosophila* (Song and Endow, 1998). The other missense change identified (R280C) affects a highly conserved arginine residue and is predicted to cause a structural change which alters microtubule binding (Fichera et al., 2004).

Interestingly, a further family had been linked to the SPG10 locus in which no mutation in the *KIF5A* gene has been identified (Reid et al., 2002). The phenotype in this family was distinct, having a much later adult onset with a mean age at onset of 30 years. Also, older members had additional moderate to severe amyotrophy in addition to the spastic paraparesis (Ashley-Koch et al., 2001). This would suggest a further SPG locus lies within the 40-cM interval defined by this family.

17.9. SPG13/Hsp60

The gene at the SPG13 locus has been identified as the mitochondrial chaperonin *Hsp60* (heat shock protein) (Hansen et al., 2002). Only one family has been identified with mutation in the Hsp60 gene and the phenotype described was of pure HSP, with a mean age at onset of 39 years old (range 17–68) (Fontaine et al., 2000). Comparison was made with a group of patients with SPG4 and it was observed that SPG13 affected individuals had a higher frequency of severe functional handicap. Screening of a further two families with suspected linkage to SPG13 and 20 autosomal dominant families without linkage data revealed one polymorphism but no other mutations in Hsp60.

Hsp60 is involved in ensuring correct protein folding in the mitochondria and it seems likely that a disruption in its function would lead to an impairment in mitochondrial biogenesis. The V72I mutant Hsp60, identified in the SPG13 pedigree, was unable to support growth in *E. coli* cells in which the homologous chromosomal *groESgroEL* chaperonin genes had been deleted (Hansen et al., 2002). Further work is needed to determine if this is via a haploinsufficiency or dominant negative mechanism.

17.10. SPG17/BSCL2/seipin

The gene at the SPG17 locus has been identified as BSCL2 (Berardinelli-Seip congenital lipodystrophy type 2) and mutations have been identified in four unrelated families (Windpassinger et al., 2004). The phenotype of SPG17 is complicated with distal amyotrophy co-segregating with the spastic paraparesis. This phenotype was first reported by Silver (1966) in two families with autosomal spastic paraparesis and distal amyotrophy of the hands. This complicated phenotype of HSP is referred to as Silver syndrome and several cases have been reported over the years (Patel et al., 2001; Windpassinger et al., 2003, 2004; Warner et al., 2004). Silver syndrome is genetically heterogeneous as demonstrated by reports of Silver syndrome without linkage to SPG17 or mutation in BSCL2 (Patel et al., 2001; Warner et al., 2004; Windpassinger et al., 2004).

In the three families reported with linkage to SPG17, the age at onset of symptoms ranges from 8–63 years old. Several asymptomatic individuals have been reported suggesting reduced penetrance. Variation was seen regarding whether amyotrophy occurred in the hands or peroneal muscles predominantly, although the former was more common. In the hands thenar and first dorsal interosseous muscles tend to be preferentially affected (see Fig. 17.1). Foot deformity, most commonly pes cavus, was frequently seen. Less frequent observations were reports of bladder disturbance and disturbance in vibration sense, most often seen in individuals with longer disease duration. Progression in these families was often slow over many decades. The neurophysiological assessments indicated the amyotrophy was likely to be secondary to anterior horn cell dysfunction or motor neuropathy.

The gene mutated in Silver syndrome, BSCL2, encodes the protein seipin, an integral membrane protein of the endoplasmic reticulum (Windpassinger et al., 2004). Northern-blot analysis revealed a brain specific ~1.8 kb BSCL2 transcript and a ubiquitously expressed 2.2 kb transcript. Both transcripts are translated into a predicted protein of 398 amino acids. BSCL2 has 11 exons and both mutations described (N88S and S90L) have been in exon 3 and destroy a predicted N-glycosylation of the protein. Transfection experiments in NSC34 cells demonstrated that the mutant seipin formed aggregates in the cytoplasm. This may be secondary to the loss of the glycosylation site in the mutant seipin which would be predicted to lead to the generation of misfolded and dysfunctional seipin, prone to aggregation. As null mutations in BSCL2 lead to Berardinelli-Seip congenital lipodystrophy type 2 it has been hypothesized that a gain of function mechanism underlies the motor neuron degeneration in SPG17. Heterozygous missense mutations in BSCL2 are also associated with distal hereditary motor neuropathy type V in which, as in Silver syndrome, predominant hand wasting and weakness is observed. The same missense change N88S identified in Silver syndrome individuals has also been associated with a dHMNV phenotype. This suggests that Silver syndrome and dHMNV are extreme phenotypes of the same disorder.

17.11. The remaining ADHSP loci

17.11.1. SPG8

The gene at SPG8 has not yet been identified. Three families have been linked to this locus and all have a pure HSP phenotype (Hedera et al., 1999; Reid et al., 1999; Rocco et al., 2000). Disease onset tended to be in adult life ranging from 18–60 years in the published families. Other than a hint of a more severe phenotype, there were no obvious differences between the pure HSP phenotype at this locus compared to other pure ADHSP loci.

17.11.2. SPG9

Two families have been reported with linkage to the SPG9 locus. The phenotype is an interesting subtype of complicated HSP. An Italian family has been reported in which the phenotype is complicated by cataracts, gastro-esophageal reflux with vomiting and amyotrophy (Seri et al., 1999). A further British family has been reported with cataracts, learning difficulties and skeletal abnormalities (Slavotinek et al., 1996; Lo Nigro et al., 2000). In both families a predominantly axonal motor neuropathy was identified as the cause for the amyotrophy. The age at onset ranged from the first year of life through to the fourth decade, with a suggestion of genetic anticipation in subsequent generations.

17.11.3. SPG12

Two large families have been linked to the SPG12 locus (Reid et al., 2000; Orlacchio et al., 2002). There is a suggestion of an earlier age at onset compared to HSP other ADHSP loci with the mean age at onset being 14.1 ± 4.4 years and 6.9 ± 6.2 years in the pure HSP families described. Disease progression was rapid with 75% of one of the families requiring constant use of a wheelchair within 4 years of disease onset.

17.11.4. SPG19

The gene at this pure HSP locus is unknown. Only one family has been linked to this locus in which symptom onset was relatively late (range 36–55 years), with a benign course and a high frequency of urinary disturbance (Valente et al., 2002).

17.12. Autosomal recessive HSP

Recessively inherited HSP is less common than autosomal dominant HSP. Both pure and complicated phenotypes have been described in families with autosomal recessive HSP (ARHSP). ARHSP pedigrees have been linked to 13 loci (Table 17.1) and three genes have so far been identified: SPG7/paraplegin, SPG20/spartin and SPG21/maspardin. Four loci have been identified for pure ARHSP: SPG5, SPG24, SPG27 and SPG28 (Hentati et al., 1994; Hodgkinson et al., 2002; Wilkinson et al., 2003; Meijer et al., 2004; Bouslam et al., 2005). An Arab family with spastic paraparesis complicated by skin and hair pigmentation abnormalities

and mild cognitive impairment has been linked to the SPG23 locus (Blumen et al., 2003). Other families have been described with a similar phenotype with additional axonal neuropathy which may also link to this locus (Abdallat et al., 1980; Lison et al., 1981; Stewart et al., 1981; Daras et al., 1983; Bamforth, 2003). In the family linked to SPG24, the index case has a severe spastic dysarthria, making the phenotypic classification as pure or complicated a little blurred. Interestingly the SPG27 locus overlaps that for complicated ADHSP at SPG9. Two further loci SPG7 and SPG11 have both pure and complicated phenotypes associated (Casari et al., 1998; Murillo et al., 1999). There is no apparent clinical difference between the phenotype of dominant or recessive pure HSP.

17.13. SPG7, mitochondria and neurodegeneration

Paraplegin is a nuclear encoded mitochondrial protein comprising 795 amino acids (Casari et al., 1998). The precise function of paraplegin is unknown, but insights have been gained by the study of the homologous yeast proteins, Afg3, Rca1 and Yme1. These yeast proteins, like paraplegin, are members of the AAA class of proteins. They belong to the metalloprotease sub-group, unlike spastin which belongs to the meiotic group of AAA proteins. In yeast, Afg3 and Rca1 form a high molecular weight complex localized to the inner mitochondrial membrane where they perform various roles including: participating in ATP synthase assembly, respiratory chain complex formation and the degradation of incompletely synthesized mitochondrial polypeptides (Tauer et al., 1994; Tzagoloff et al., 1994; Paul and Tzagoloff, 1995; Langer and Neupert, 1996; Rep and Grivell, 1996). Deletion or mutation of the conserved proteolytic site of either Afg3p or Rca1p leads to dysfunction of respiratory chain activity and an impaired ability to degrade incompletely synthesized mitochondrial polypeptides. These studies show the essential role the paraplegin like genes Afg3p and Rca1p play in normal mitochondrial biogenesis in yeast. A number of paraplegin-like genes, AFG3L2 and YME1L1, have now been identified in humans, which are highly homologous to the same group of yeast proteins (Banfi et al., 1999; Coppola et al., 2000). AFG3L2 is also localized to the inner mitochondrial membrane, where it has been demonstrated to form a 900 kD complex with paraplegin, which possesses proteolytic activity (Atorino et al., 2003). In cultured fibroblasts from SPG7 patients the AFG3L2-paraplegin complex was absent. The SPG7 fibroblasts were demonstrated to have an increased sensitivity to oxidative stress and a reduced respiratory chain complex I activity that could be reversed by the exogenous expression of wild type paraplegin. The reduction in complex I activity would appear to be due to the requirement of the AFG3L2-paraplegin complex for the correct assembly of complex I.

A mouse model for SPG7 has been developed. Paraplegin-deficient mice appeared developmentally normal at birth (Ferreirinha et al., 2004). Motor problems on the rotarod were detected as early as 4 months and continued to progress. They began to lose weight in comparison to control littermates at 12 months and by 17 months began to develop scoliosis and a gait disturbance. The first neuropathological changes occurred at 4 months in synaptic mitochondria. The abnormal mitochondria were seen to be hypertrophic, swollen and had abnormal cristae. By 8 months abnormal mitochondria were seen throughout the axon with signs of mitochondrial degeneration also present. In the older littermates mitochondrial dysfunction was evident with a reduction in ATP synthesis (age > 23 months) and a reduction of complex 1 activity (age > 26 months). Changes to the gross morphology of axons were not seen until 8 months, when axonal swellings were detected in the white matter of the spinal cord (Fig. 17.3). These progressed with age but clear axonal degeneration was not seen until 15 months. The swelling and degeneration was seen prominently in the longest axons of the fasciculus gracilis (cervical region) and in the lateral and anterior funiculi (lumbar region). Axonal swellings were also seen in the optic nerves. A progressive axonopathy was noted to affect the sciatic nerves in older littermates, first appearing at 19 months. The axonal swellings were comprised of accumulations of neurofilaments and organelles, including swollen mitochondria, suggesting an impairment of anterograde axonal transport. An impairment of retrograde transport was also demonstrated in paraplegin-deficient mice over 17 months in the long lumbar motor neurons.

The evidence from the paraplegin-deficient mouse model suggests that a deficiency in paraplegin causes a disruption in mitochondrial function (Fig. 17.3). The mechanisms by which a paraplegin deficiency may disrupt mitochondrial function include: an accumulation of abnormal or misfolded proteins in the inner mitochondrial membrane, incomplete respiratory chain formation and an impaired turnover of a mitochondrial regulatory protein. The work in SPG7 fibroblasts suggests that the failure to correctly assemble complex I, caused by the lack of AFG3L2-paraplegin complex, may be a major contributor to the mitochondrial dysfunction. Mitochondrial dysfunction may damage an axon in a number of ways and has been implicated as the pathogenic mechanism in a number of neurodegenerative disorders (discussed later). In SPG7 it appears that a major consequence of the mitochondrial dysfunction is a disturbance in axonal transport. Disruption in axonal transport is a recurring theme in the pathogenesis of HSP at several loci and is discussed in detail later in the chapter.

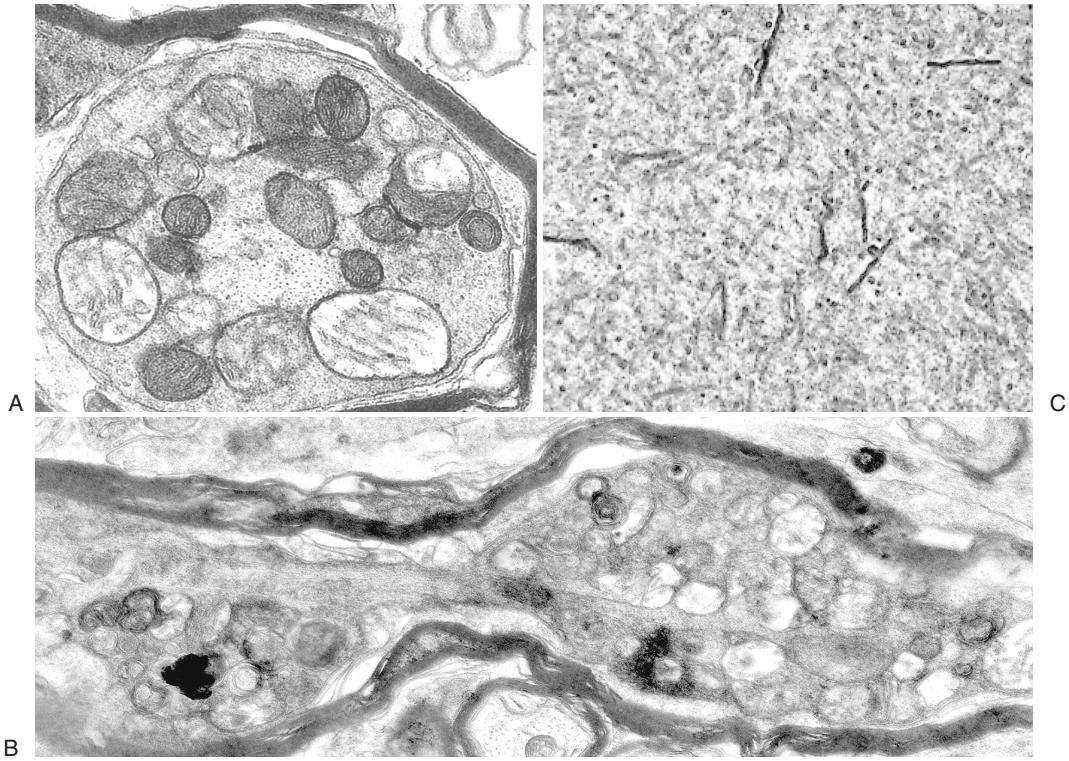

Fig. 17.3. (**A**) Spinal axon of a 12-month-old *Spg7–/–* mouse containing degenerating mitochondria. (**B**) Longitudinal section of a spinal axon of a 15-month-old paraplegin-deficient mouse, showing segmental swellings. Filamentous aggregates and degenerating mitochondria accumulate in the axoplasm. (**C**) Enlargement of the axoplasm of a spinal cord axon showing that neurofilaments have lost their normal orientation and form disorganized bundles. Reproduced from Ferreirinha et al. (2004) with permission from the American Society for Clinical Investigation. Reproduced with permission via Copyright Clearance Center.

17.13.1. Clinical features of SPG7

To date only seven pedigrees have been identified with HSP due to paraplegin mutation (Casari et al., 1998; McDermott et al., 2001; Wilkinson et al., 2004). In the majority of cases the mutations are private and are predicted to disrupt important functional domains. In all but one, affected individuals are either homozygous for paraplegin mutation or are compound heterozygotes with two different mutations on each allele. An exception was described in an English family in which the proband was a compound heterozygote (ERR484-6del/A510V), whilst the father of the proband only carried the deletion and yet was found to be clinically affected (McDermott et al., 2001). The authors suggested that SPG7 could therefore be inherited as an autosomal dominant trait. In all the other published families the inheritance is clearly autosomal recessive or sporadic.

The phenotype of SPG7 is varied, with both pure and complicated HSP described. There is no one specific complicated phenotype associated with SPG7. However, cerebellar signs or optic atrophy have been reported in more than one family and may provide a clue to the possibility of SPG7 when occurring with a spastic paraparesis. Other additional features described include distal amyotrophy, peripheral neuropathy, raised serum creatine kinase, cerebral atrophy and cerebellar atrophy. Mitochondrial dysfunction as indicated by the presence of muscle fibers that stain negative for cytochrome c oxidase has been reported in three families with paraplegin mutations (Casari et al., 1998; McDermott et al., 2001). Others have reported biochemical changes consisting of a reduction of complex 1 and complex II/III activities but no histochemical changes in muscle biopsies from individuals with SPG7 (Wilkinson et al., 2004).

17.13.2. Mitochondria and HSP

One of the key functions of mitochondria is the generation of ATP via the activity of the respiratory chain complexes. Intracellular energy deficits occur when the activities of the mitochondrial respiratory chain complexes are disturbed. Other detrimental intracellular consequences of mitochondrial dysfunction include: increased generation of reactive oxygen species, oxidative stress and impaired intracellular calcium homeostasis. The role of mitochondrial impairment and oxidative stress in other

neurodegenerative diseases such as Friedreich's ataxia, motor neuron disease and Huntington's disease is well recognized (Manfredi and Beal, 2000). The mechanism by which mitochondrial dysfunction causes the axonal degeneration in paraplegin-related HSP is unknown. The cell bodies of the long axons affected in HSP have to support very long axonal processes and this is a feature which may be predicted to place high metabolic demands on the cell. Therefore, it could be that these neurons may be sensitive to any disturbance in mitochondrial energy production, particularly as they are unable to store a large amount of energy.

The evidence from the paraplegin mouse model suggests two possible hypotheses for how mitochondrial dysfunction could lead to the dying back axonopathy seen in HSP. First, it may simply be the energy deficit in the distal axon, and in particular in the synapse, causing a failure of multiple processes required for healthy neuronal function. Over time synaptic function may deteriorate triggering gradual retrograde axonal degeneration. Second, although many intracellular processes may be predicted to be affected by an energy deficit, the effect on one specific process may be the main cause of the axonal degeneration. Axonal transport was demonstrated to be severely disrupted in the paraplegin deficient mouse. The long axons affected in HSP rely heavily on axonal transport, an energy dependent process, to transport molecules and organelles from the cell body to the distal axon up to a meter away. A failure in this vital transport system has been implicated in other distal axonopathies and is discussed elsewhere in this chapter.

The identification of a complex I deficiency in the paraplegin mouse may offer an explanation for the age related development and progression of the disease. Complex I has been observed to be especially susceptible to the age-related changes induced by oxidative stress (Wong et al., 2002). Therefore, the neuron may be initially able to compensate for the mitochondrial dysfunction caused by the paraplegin mutation. However, with the further additional burden of age related complex I deficiency, the compensatory mechanisms are no longer adequate and neurodegeneration occurs.

Whether mitochondrial dysfunction is a common feature in the pathogenesis of the HSP phenotype at the other loci remains to be seen. Mitochondrial dysfunction consisting of marked deficiencies in complex I and/or complex IV has been demonstrated in muscle biopsy tissue from groups of patients in whom mutations in spastin and paraplegin had been excluded (Piemonte et al., 2001; McDermott et al., 2003b). This suggests the possibility that at least two of the genes not yet identified that lead to HSP may be associated with mitochondrial dysfunction, one with complex I deficiency and the other with complex IV deficiency.

Mutation in a further nuclear encoded mitochondrial protein, heat shock protein 60 (Hsp 60), has recently been identified as the cause for pure ADHSP at the SPG13 locus and, given its function as a mitochondrial chaperone, it seems likely that HSP caused by mutation in this gene will be associated with mitochondrial dysfunction (Hansen et al., 2002). No evidence of mitochondrial dysfunction has been demonstrated in SPG4 patients. In the remaining dominant families at the SPG3, SPG6, SPG8 and SPG9 loci, only a small number of muscle biopsies have been performed for histochemical and biochemical analysis of mitochondrial function (Seri et al., 1999; Hedera et al., 2000). The results to date suggest there is no primary role for mitochondrial dysfunction at these loci. Further studies are now required to confirm these findings and also to investigate families linked to the more recently discovered dominant loci SPG10, SPG12, SPG17, SPG19 and particularly SPG13 caused by mutations in the mitochondrial chaperonin (*Hsp 60*) gene. No evidence of mitochondrial dysfunction was found in a large family linked to SPG5 and there have been no studies as yet investigating mitochondrial function in the other non SPG7 autosomal recessive families (Wilkinson et al., 2003).

17.14. SPG11

An increasing number of families are being described with HSP complicated by a thin corpus callosum (Fig. 17.4) (HSP-TCC) and progressive cognitive decline, suggesting this is a relatively common form of ARHSP (Murillo et al., 1999; Shibasaki et al., 2000; Casali et al., 2004; Winner et al., 2004). There had been a suggestion that this particular phenotype was more common in those of Japanese ancestry. However, relatively large numbers of European and North American families have now also been reported. A number of families with this phenotype have had linkage to the SPG11 excluded, confirming there is at least one further locus for HSP-TCC (Shibasaki et al., 2000; Casali et al., 2004). The MRI changes consist of a thin corpus callosum, particularly the rostral portion, often diffuse white matter signal change (Fig. 17.4) and in later stages frontoparietal atrophy. The clinical phenotype has been broadened to include individuals described with dysarthria, tremor, dysphagia, distal amyotrophy and not infrequently a peripheral neuropathy.

17.15. SPG14 and SPG15

In the one consanguineous Italian family linked to the SPG14 locus, the additional features co-segregating

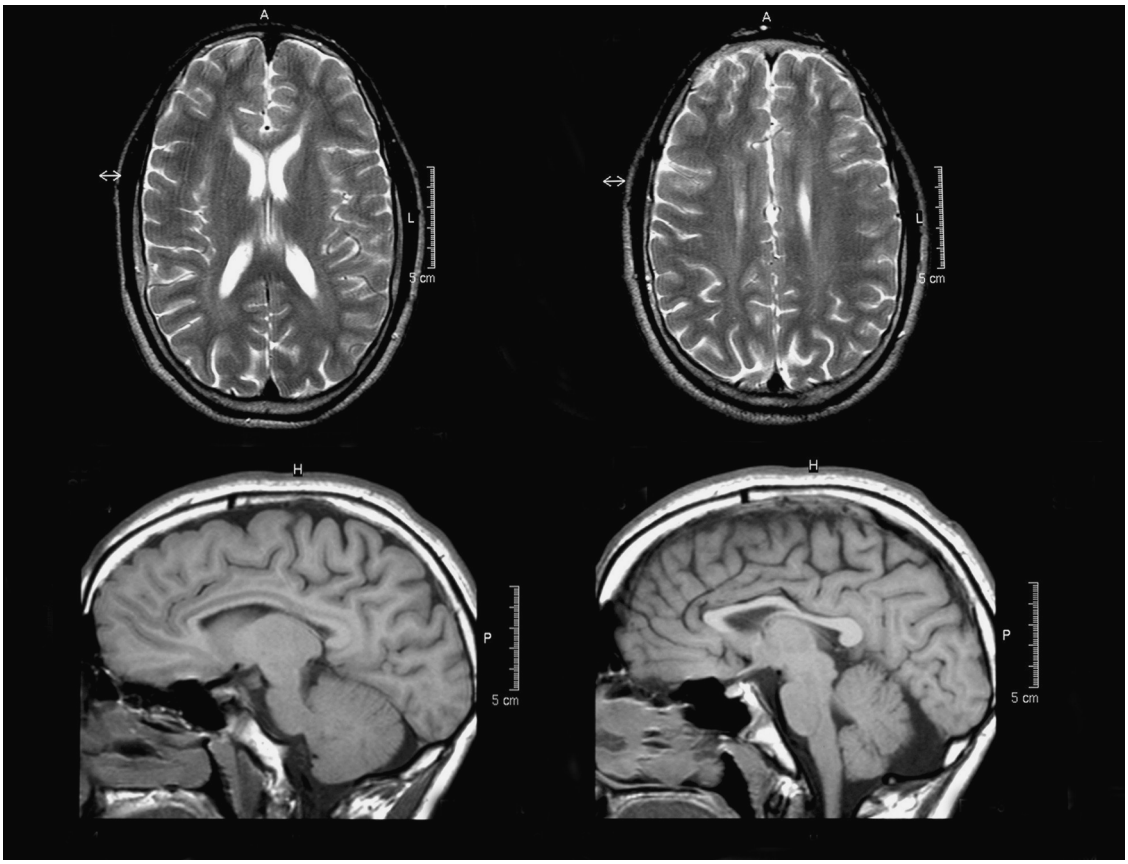

Fig. 17.4. MRI head scan from a patient with HSP showing thinning of the corpus callosum and subtle diffuse white matter changes within the cerebral hemispheres.

with the spastic paraparesis were a distal motor neuropathy and mild mental retardation (Vazza et al., 2000).

Two families have been linked to the SPG15 locus. Pigmentary macular degeneration, distal amyotrophy, fecal and urinary incontinence in late stage disease and mild cerebellar dysfunction were the cosegregating features. MRI imaging revealed atrophy of cerebral hemispheres, corpus callosum and brainstem (Hughes et al., 2001).

17.16. SPG20/Troyer syndrome

The Troyer syndrome, which occurs in high frequency in the old order Amish, has been linked to SPG20 (Patel et al., 2002). The Troyer syndrome is characterized by spastic paraparesis, distal amyotrophy, spastic dysarthria, developmental delay and short stature. A thorough reassessment of published cases failed to find evidence of Troyer syndrome outside the Amish population and added detail to the observed phenotype (Proukakis et al., 2004). Emotional lability was frequently observed, as was excessive drooling in the most advanced cases. Cerebellar signs including limb ataxia were observed, but not nystagmus. Dysphagia, dystonia and choreathetoid movements were also present in advanced cases. Skeletal abnormalities observed included kyphoscoliosis, pes cavus, hammer toes and hyperextensible joints in the hands. Pyramidal weakness and upper limb tone change were rarely observed. Deep white matter signal change was observed on T_2-weighted MRI images in all individuals scanned. The SPG20 gene comprises nine exons spanning a distance of 43.3 kb on chromosome 13q12.3. All patients have been found to be homozygous for the same mutation (1110delA) which causes a frameshift and a predicted truncated protein (Patel et al., 2002; Proukakis et al., 2004). The same SPG20 encodes a protein of 666 amino (72.7 kDa) named spartin (spastic paraplegia autosomal recessive Troyer syndrome) which is ubiquitously expressed in adult tissues. Spartin shares homology with SNX15, VPS4 and Skd1 human proteins involved in protein trafficking, suggesting a possible similar role for spartin. Spartin also shares homology with the N-terminal region of spastin, suggesting a similar function (Patel et al., 2002).

17.17. SPG21/mast syndrome

Mast syndrome is the complicated form of HSP mapped to SPG21 (Cross and McKusick, 1967). In one large Amish pedigree, disease onset was usually in childhood (Simpson et al., 2003). However, most patients functioned relatively normally into early adulthood before significant motor or cognitive difficulties developed. In advanced cases legs were described as rigid and patients became bed-ridden. Primitive reflexes and extrapyramidal movements were also observed. Imaging in three patients demonstrated a thin corpus callosum, generalized atrophy and white matter signal change in keeping with demyelination.

A mutation in the acid-cluster protein of 33 kDa gene (ACP33) has been reported (Simpson et al., 2003) in this family. ACP33 encodes the protein maspardin (*Mas*t syndrome, *s*pastic paraplegia, *a*utosomal *r*ecessive with *d*ementia). Maspardin has been shown to localize to vesicles of the endosomal/*trans*-Golgi network, suggesting a role in protein transport and sorting (Zeitlmann et al., 2001).

17.18. SPG25 and SPG26

An unusual phenotype, linked to SPG25, has been reported in an Italian family in whom the parents were first cousins (Zortea et al., 2002). In this family, four affected individuals developed spastic paraparesis in the fourth and fifth decades. The onset of spastic paraparesis was preceded or accompanied by radicular type back pain radiating into the limbs. Imaging revealed disk herniations often at more than one level. Three of the four affected family members had neurophysiological evidence of a mild peripheral neuropathy, although the feature differed in each individual. The gene that causes the susceptibility to disk herniation and spastic paraparesis has yet to be identified.

A single Kuwaiti family has been reported with linkage to SPG26 (Wilkinson et al., 2005). Affected individuals all had an onset of spastic paraparesis at 7 or 8 years. The additional complicating features described were dysarthria, distal amyotrophy in arms and legs, emotional lability and reduced IQ in three of five affected siblings.

17.19. X-linked HSP

In comparison to autosomally inherited HSP, X-linked HSP is quite rare. There are four X-linked HSP loci SPG1, SPG2, SPG16 and SPG22. Only the genes involved at the SPG1 and 2 loci have been identified and their molecular biology is relatively well understood and is discussed below. One SPG16 family with a pure phenotype has been identified having a NOR insertion into Xq11.2 (Tamagaki et al., 2000). Earlier, Steinmuller et al. (1997) reported a family in whom SPG1 and 2 was excluded and linkage suggested a locus within Xq11.2-q23. In this family the phenotype was severe with additional mental retardation, upper limb involvement, visual impairment and bladder and bowel dysfunction. Allan-Herndon-Dudley syndrome (AHDS) has recently been reclassified as a complicated form of HSP at the SPG22 locus (Bohan and Azizi, 2004; Fink, 2004). The original description of AHDS was in a multigenerational family in which affected males demonstrated severe mental retardation and hypotonia at birth. Few individuals ever walked and in adult life developed generalized muscular atrophy, joint contractures and hyporeflexia (Allan et al., 1944). Further descriptions over the years have broadened the phenotype to include spastic paraparesis as well as dysarthria, ataxia, athetoid movements, neck drop, muscle hypoplasia, scoliosis, pectus excavatum, long thin face, large simple ears and maxillary hypoplasia (Bundey and Griffiths, 1977; Stevenson et al., 1990; Bundey et al., 1991; Bialer et al., 1992; Claes et al., 2000). A Brazilian family with a pure X-linked HSP phenotype has also been linked to the SPG22 locus (Starling et al., 2002a).

17.20. SPG1 and CRASH syndrome

SPG1 is caused by mutations in the L1 cell adhesion molecule gene (*L1CAM*) (Jouet et al., 1994). The phenotype tends to be complicated, with mental retardation and congenital musculoskeletal abnormalities, most notably the absence of extensor hallucis longus (Kenwrick et al., 1986). Mutations in the same gene were identified in X-linked hydrocephalus, X-linked agenesis of the corpus callosum and the syndrome of MASA (mental retardation, aphasia, shuffling gait and adducted thumbs). There is marked interfamilial and intrafamilial in families with *L1CAM* gene mutations. In a number of families more than one of the possible *L1CAM* phenotypes has been observed. The diseases are now considered to be part of a clinical syndrome with the acronym CRASH, for corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia and hydrocephalus (Fransen et al., 1995).

L1CAM is a transmembrane glycoprotein, which is mainly expressed by neurons, and Schwann cells (Joosten and Gribnau, 1998). The protein has six domains homologous to members of the immunoglobulin superfamily and 5 homologous to fibronectin type III domains (Bateman et al., 1996). The mutations identified in L1CAM tend to cluster in regions throughout L1CAM and can be divided into those mutations that act mainly

by significantly altering protein domains and those predicted to alter the surface properties of the protein. Mutations affecting key residues in the domains were more likely to produce a more severe CRASH phenotype with death within 1 year, compared to those affecting surface residues. Mutations affecting the fibronectin domains were more likely to produce a severe hydrocephalus than those affecting the immunoglobulin domains (Michaelis et al., 1998). L1CAM plays an essential role in the development of the nervous system, being involved in neuron-neuron adhesion, axon outgrowth and pathfinding (Brummendorf and Rathjen, 1996). L1CAM plays an important role in corticospinal tract formation (Cohen et al., 1997; Dahme et al., 1997; Fransen et al., 1998; Demyanenko et al., 1999). In a transgenic mouse model, loss of L1CAM disrupts the normal guidance of corticospinal axons across the midline at the level of the pyramids. The normal decussation at the pyramids is stimulated by a chemorepellent molecule secreted by the ventral spinal cord known as Sema3A (Castellani et al., 2000). L1CAM is a component of the Sema3A receptor complex and mice deficient in L1CAM fail to respond to Sema3A in vitro.

17.21. SPG2 and Pelizaeus-Merzbacher disease

Both pure and complicated HSP pedigrees are seen at this locus (Johnson and McKusick, 1962; Keppen et al., 1987; Goldblatt et al., 1989; Bonneau et al., 1993; Cambi et al., 1996). Complicated HSP linked to SPG2 consists of a core phenotype of spastic paraparesis, cerebellar syndrome and mental retardation. Interfamilial variation is seen with the core features occurring to various degrees, with or without additional features such as optic atrophy. Similarly, intrafamilial variation occurs (Johnson and McKusick, 1962; Goldblatt et al., 1989; Bonneau et al., 1993).

SPG2 is caused by mutations in the proteolipid protein (*PLP*) gene (Saugier-Veber et al., 1994). Mutations in the *PLP* gene also cause Pelizaeus-Merzbacher disease (PMD). PMD is a dysmyelinating disease, the classical form having onset in infancy and death in late adolescence or young adulthood. It is characterized by nystagmus, ataxia, spasticity, abnormal movements, optic atrophy and microcephaly. There is a more severe subtype which shows a rapid progression and death in the first decade (Renier et al., 1981). The phenotype of disease caused by mutations in the PLP gene can be considered as a continuous spectrum with milder SPG2 at one end and the more severe PMD at the other (Inoue, 2005). Neuroimaging in PMD and to a lesser extent in SPG2 demonstrates diffuse hyperintensity of white matter on T_2-weighted images, resembling the appearance of newborn infants (Inoue et al., 2001).

PLP is the major myelin protein of the CNS, accounting for approximately 50% of total myelin protein in the adult brain. An alternatively spliced form of PLP missing 35 amino acid residues, called DM20, is also produced from the PLP gene. The function of PLP/DM20 is not confirmed. It seems likely that these proteins play a role in stabilizing and maintaining the myelin sheath (Boison and Stoffel, 1994; Kluggmann et al., 1997). A further role for PLP/DM20 in glial/axon communication has been suggested. This followed the observation that *Plp* null mutants developed normal myelin sheaths despite lack of PLP/DM20, but subsequently went on to develop a profound axonopathy (Griffiths et al., 1998). Some authors have suggested the two isoforms may have different functions, although this is yet to be confirmed. The DM20 isoform is present earlier in development and may play a specific role in glial cell development (Ikenaka et al., 1992; Yu et al., 1994; Timsit et al., 1995; Peyron et al., 1997).

A wide range of often private mutations have been described in PMD/SPG2 including duplications, missense, frameshift, nonsense and splicing mutations. Point mutations in the PLP gene within exon 3b, encoding the 35 amino acid segment specific to PLP and spliced out from DM20, tend to produce the milder SPG2 phenotype (Saugier-Veber et al., 1994; Sivakumar et al., 1999; Cailloux et al., 2000). Truncating mutations predicted to result in non-functional protein similarly result in a milder phenotype of PMD or SPG2 (Bond et al., 1997). This is in contrast to the majority point mutations in PLP, occurring outside of exon 3b, which lead to the development of the more severe dysmyelinating PMD phenotype (Raskind et al., 1991; Sistermans et al., 1996; Garbern et al., 1997, 2002; Inoue et al., 2002). Duplications, the commonest form of mutation reported in PLP, are also associated with a PMD phenotype (Sistermans et al., 1998; Woodward et al., 1998; Inoue et al., 1999; Mimault et al., 1999). These genotype phenotype correlations suggest that the PMD phenotype arises not from a reduction in functional PLP but rather from a toxic gain of function conferred by the missense change or, in the case of gene duplication, by overdosage of wild type PLP. There are three mutational mechanisms hypothesized to produce a PMD/SPG2 phenotype (Inoue, 2005). First, conformational changes of the PLP protein secondary to point mutations leads to accumulation of misfolded PLP in the endoplasmic reticulum. The accumulation of misfolded protein may trigger the unfolded protein response pathway which may lead to apoptosis of oligodendrocytes. With genomic duplications, excessive PLP accumulates in the late endosomes/lysosomes with cholesterol. This aggregation may disrupt the normal trafficking of the myelin raft necessary for

normal myelination. In the mutations where PLP is not translated, compact myelin is formed lacking PLP. This myelin is fragile and prone to subsequent demyelination changes. Furthermore, lack of PLP leads to the disruption of myelin-axon interactions, resulting in axonal degeneration. The evidence in the *plp* null mouse suggests that this degeneration may be secondary to a disruption in fast axonal transport caused by the absence of PLP/DM20 (Edgar et al., 2004).

17.22. Neurodegeneration and HSP

A number of different biological processes have now been implicated in the pathogenesis of HSP (see Fig. 17.5). In the developing nervous system, disruption of axon outgrowth and pathfinding and dysmyelination have been demonstrated to cause a HSP phenotype with mutations in L1CAM and PLP, respectively. In the developed nervous system neurodegeneration appears to occur because of a complex interaction between the selective vulnerabilities of the long axons affected in HSP and impairment of cellular processes including: axonal transport, endosome pathways, mitochondrial biogenesis and cytoskeleton regulation (Fig. 17.5). A tempting unifying hypothesis is that the dying back length dependent axonopathy of HSP is due to a perturbation of axonal transport. In SPG10 disruption in axonal transport would be expected secondary to mutation in the major motor protein KIF5A. In SPG4 it appears a disruption in the microtubule cytoskeleton due to spastin mutation may deleteriously affect axonal transport and, given the similarities of spartin, SPG20 may be due to a similar mechanism. The aggregations seen in cell models with mutant seipin may cause a disruption of axonal transport in SPG17. Atlastin and maspardin appear to play a role in the endosome pathway and vesicle trafficking, as may spastin and spartin. Disruption in the endocytic pathways may perturb axonal transport by packaging or targeting vital cargoes for the distal axon incorrectly or causing a blockage due to an accumulation of mishandled proteins. The process of axonal transport is dependent on efficient ATP production and a disruption in mitochondrial function due to paraplegin mutation has been demonstrated to cause abnormal axonal transport, Hsp60 would be predicted to have a similar effect. Recent evidence in the *plp* null mouse suggests a secondary disruption in fast axonal transport may contribute to the neurodegeneration in SPG2. These conjectures are supported by the mounting evidence for disruption in axonal transport in other length dependent axonopathies, with mutations in the motor proteins KIF1B and dynactin, causing Charcot-Marie-Tooth disease 2A and motor neuron disease, respectively (Zhao et al., 2001a; Puls et al., 2003). These hypotheses require further investigation with functional studies. It is hoped that, with the current speed of progress in the field, including the development of animal models, the uncertainties around selective vulnerability and neurodegeneration in HSP will soon be understood, allowing the development of targeted therapies for this group of disorders.

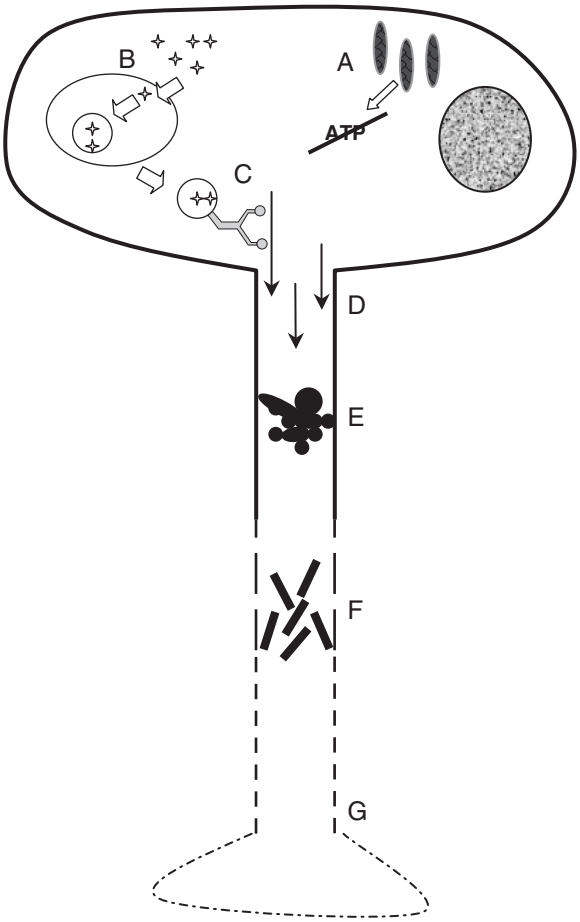

Fig. 17.5. Possible mechanisms of neurodegeneration in HSP. (**A**) Paraplegin and Hsp60 – Mitochondrial dysfunction. (**B**) Atlastin, spastin and maspardin – Impairment of endocytic pathways with consequent miss packaging and miss trafficking of intracellular cargoes. (**C**) KIF5A – Dysfunctional motor protein. (**D**) Spastin, KiF5A, spartin and PLP – Disrupted axonal transport. (**E**) Seipin – Intracellular aggregates, possibly interfering with axonal transport. (**F**) Spastin and spartin – Disrupted regulation of the microtubule cytoskeleton. (**G**) 'Dying back' axonal degeneration.

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Chapter 18

Toxic disorders of the upper motor neuron system

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18.1. Introduction

Dysfunction of the motor system has been associated with dietary dependence on food plants with neurotoxic potential, notably the grass pea (chickling pea) and cassava (manioc), in various and geographically distinct regions of the world (Fig. 18.1). Reliance on grass pea (*Lathyrus sativus* or related neurotoxic species) or on insufficiently processed bitter cassava (*Manihot esculenta* Crantz) as staples, the latter in association with malnutrition, has resulted in epidemics of (neuro)lathyrism and konzo (neurocassavism), respectively; these are clinically similar self-limiting neurodegenerative disorders confined to the upper motor neuron system. Studies of outbreaks of lathyrism and konzo have suggested individual susceptibility to the toxic effects of these plants varies with subject age, gender, nutritional status and motor activity, as well as the toxin content of grass pea (lathyrism) or cassava (konzo), methods of food preparation and duration of consumption. The common clinical picture is a spastic paraparesis or, in the potentially more severe cassava-associated disorder, a tetraparesis. Electrophysiological studies reveal prominent pyramidal dysfunction in both lathyrism and konzo; neuropathological data are sparse or lacking, respectively. Molecular mechanisms of these remarkably similar neurotoxic disorders have yet to be understood. Though there is no effective treatment for these persistently disabling conditions, lathyrism and konzo can both be prevented by modifying food preparation or changing diet.

18.2. Epidemiology

18.2.1. Historical background

Lathyrism has affected humans and animal populations since antiquity. The disease was known to ancient Hindus, to Hippocrates (460–377 BC), Pliny the Elder (AD 23–79), Pedanius Dioskurides (AD 50) and Galen (AD 130–210) (Desparanches, 1829; Hippocrates, 1846; Proust, 1883; Hubert, 1886; Schuchardt, 1887; Spirtoff, 1903). Lathyrism affects populations reliant on food products derived from neurotoxic species of the Lathyrus genus, member of the Fabacae family, most commonly *Lathyrus sativus* (grass pea) (Stockman, 1917; Selye, 1957; Dwivedi and Prasad, 1964; Rao et al., 1969; Kislev, 1985). Sale of flour prepared from *L. sativus* was banned by George, Duke of Württemberg (1671) because of its "paralysing effects on the legs" (Schuchardt, 1887). Years later (1873), the disease was referred to as *latirismo* ("lathyrismus") (Cantani, 1873). Since the 17th century, outbreaks of lathyrism have been reported on the Indian sub-continent (Bangladesh, India, Pakistan), in Europe (France, Italy, Germany, Romania, Spain, Ukraine), Afghanistan, China and North Africa (Algeria, Ethiopia and Eritrea) (Desparanches, 1829; Proust, 1883; Grandjean, 1885; Stockman, 1917, 1929; Selye, 1957; Barrow et al., 1974; Gebre-AB et al., 1978; Mannan, 1985; Kaul et al., 1989; Tekle-Haimanot et al., 1990; Spencer, 1994; Getahun et al., 1999) (Figs. 18.1 and 18.2(A)).

Konzo (Fig. 18.2(B)), a lathyrism-like handicapping disorder, was first documented by Trolli (1938) in the

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Fig. 18.1. World map depicting zones where lathyrism has been reported (dark gray) and those affected by konzo (light gray).

south-western region (Bandundu province) of Zaïre, the former "Belgian Congo," presently the Democratic Republic of Congo (DRC) (Trolli, 1938). Despite the existence of striking clinical similarities between lathyrism and konzo, outbreaks of these two diseases occur in two distinct, non-overlapping geographical areas of the globe (Fig. 18.1). Whereas lathyrism has occurred among populations that cultivate the grass pea, outbreaks of konzo have been reported mainly in sub-Saharan Africa among those who subsist on cassava as staple food (Rosling and Tylleskär, 2000). Cassava is a member of the Euphorbiacea family (DeBower, 1975). The plant was cultivated in tropical America for approximately 5000 years before it was exported into Africa around the 1600s by Portuguese traders to feed slaves (Jones, 1959; DeBower, 1975; Allem, 1994; Olsen and Schaal, 1999). Today, it is used worldwide and constitutes the prime source of dietary calories for over 500 million people in the tropics and subtropics (Cock, 1982). It is not known whether konzo has occurred during ancient times and/or among the Indians from the Amazonian forests who cultivate cassava (Lathrap, 1970).

Available literature indicates the disease was known to the local populations (Yaka) of the Bandundu province in the DRC in the late 1800s. The Yaka named the disease "*konzo*", which means 'tied legs' in kiyaka, the local spoken language (Trolli, 1938; van den Abeele and Vandenput, 1956; van der Beken, 1993).

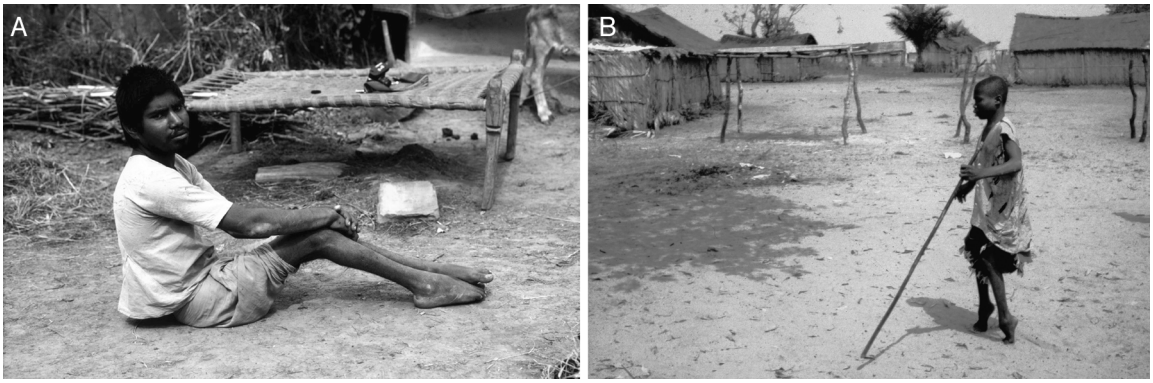


Fig. 18.2. (**A**) Young Indian male affected by lathyrism (courtesy of Third World Medical Research Foundation). (**B**) Young Congolese male affected by konzo (photograph by Thorkild Tylleskär, by permission).

This designation was a good description of the 'cross-legged gait' of affected subjects. Since the 19th century, outbreaks of konzo have occurred in many others countries of the sub-Saharan Africa including Mozambique (where it is called *mantakassa*), Tanzania, Central African Republic, Cameroon, Angola and Uganda (Fig. 18.1) (Ministry of Health Mozambique, 1984a,b; Cliff et al., 1985; Rosling, 1989; Tylleskär et al., 1991, 1994; Howlett et al., 1992; Banea et al., 1993; Davis and Howarth, 1993; Mlingi et al., 1993; Lantum, 1998; Bonmarin et al., 2002; Rosling and Tylleskär, 2000; Tshala-Katumbay et al., 2001a).

18.2.2. Role of environmental and contextual factors

Epidemics of lathyrism and konzo usually appear when adverse environmental conditions result in heavy dietary reliance on the grass pea (Figs. 18.3(A, B)) or on cassava (Figs. 18.3(C, D)), respectively (Spencer, 1999). Both the grass pea and cassava are annual high-yielding crops with relative resistance to harsh environmental conditions such as drought, insect attacks and/or pests. Grass pea has a high protein content (with most of the essential amino acids well represented) while cassava is a rich source of carbohydrate (Gopalan and Balasubramanian, 1963; Roy and Roa, 1978; Cock, 1982). Food products derived from these two plants usually make up a major component of the diet among populations in areas endemic for either lathyrism (grass pea) or konzo (cassava). However, adverse environmental conditions, such as extreme weather leading to flood or drought, pestilence or war, have forced populations to rely almost exclusively on food products made from these two drought-resistant crops; this situation has often led to outbreaks of characteristic neurologic disease (Spencer, 1994; Rosling and Tylleskär, 2000).

During World War II, outbreaks of lathyrism occurred among a group of Romanian Jews interred in a German hard-labor camp in the Ukraine and among Germans held in a French prisoner-of-war camp. The causative chain of lathyrism is best illustrated in the outbreak that occurred in the Ukrainian town of Wapniarka (Kessler, 1947; Cohn and Streifler, 1981a,b). On September 16, 1942, 1200 Romanian Jewish males were put on a diet of 400 g grass pea daily cooked in salt water and 200 g of bread. Three months later, there was a monophasic outbreak of lathyrism involving 800 inmates. Ukrainian and Russian inmates who had survived prior to the arrival of the Romanians on a diet of 200 g per day grass pea remained free of disease for 3–6 months, but developed lathyrism when their intake was doubled with the arrival of the latter. Those previously incarcerated were affected earlier and in a greater proportion than prisoners brought (in relatively good health) from their homes. The outbreak of lathyrism among Wapniarka inmates was documented by Kessler, a physician and prisoner who himself developed mild lathyrism. He identified the disease and its cause, and persuaded the guards to change the diet. Consumption of the grass pea was subsequently stopped at the end of 1942 and no new cases were thereafter reported (Kessler, 1947).

Other illustrative examples include outbreaks of lathyrism on the Indian sub-continent and in Ethiopia. In India, the disease historically has been mainly confined to Uttar Pradesh, Madhya Pradesh (where Acton identified the presence of 60,000 cases) and Bihar

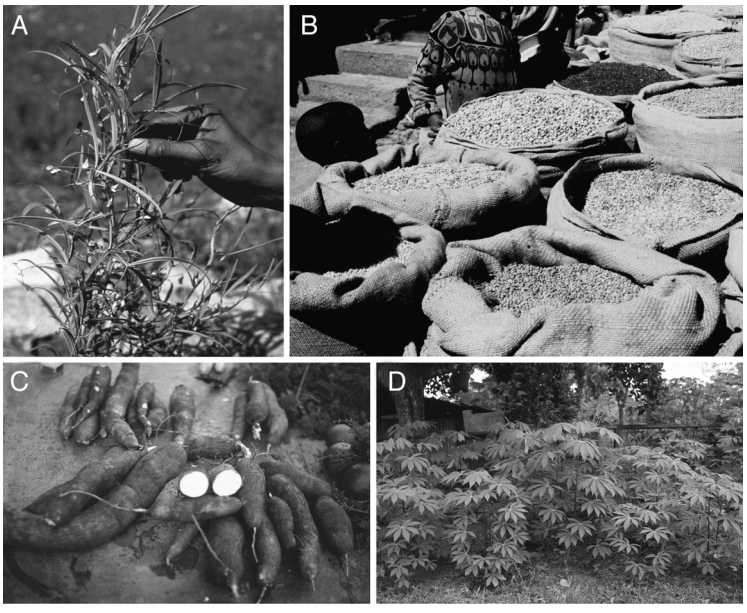


Fig. 18.3. Two drought-resistant plants associated with neurological disease. The grass pea plant (A) and seeds (B) that are grown in areas affected by lathyrism. (C) Cassava roots and plant (D) are cultivated in areas affected by konzo.

(Acton, 1922; Dwivedi, 1989). In these settings, lathyrism has occurred mostly among small land-holders and farmers who cultivate grass pea. In Bangladesh, a large producer and consumer of the grass pea, especially in the northwest of the country, the prevalence rates of lathyrism have fluctuated in proportion to the production and consumption of the grass pea. Low prevalence rates (0.48%) of lathyrism during the period 1945–1950 rose dramatically to reach the level of 66.45% during the famine period of 1971–1975 (Haque, 1989). A more recent outbreak of lathyrism has been reported in northeastern Ethiopia where drought has led to famine and excessive consumption of the grass pea (Getahun et al., 1999).

Clinical similarities between konzo and lathyrism have played an important role in identifying dietary factors in the causation of konzo. Literature from the 1930s indicates that konzo was thought to be caused by either infection or food toxins (Trolli, 1938). Decades later, epidemiological studies showed a consistent and reliable pattern of disease occurrence. Konzo occurs among populations that rely on cassava as a major crop for their subsistence. However, the disease affects mainly those whose diet is almost exclusively made up of food products that derive from insufficiently processed (toxic) bitter cassava. Shortcuts in processing have often occurred in times of drought, war and/or disruption of social conditions that have forced populations to rely on insufficiently processed (toxic) bitter cassava as a staple (Rosling and Tylleskär, 2000).

For example, because of favorable weather and road conditions in the province of Bandundu during the dry season, there is increased cassava trading which in turn creates opportunities for local vendors to reduce the processing (detoxification) time for the cash crop in order to sell it quickly and massively. As a consequence, the diet of the local population becomes increasingly dominated by insufficiently processed bitter cassava. By the end of the dry season and early in the rainy season (July–November) outbreaks of konzo are reported. Similar trends in cassava trading and subsequent outbreaks of konzo have been reported months after an epidemic of Ebola had affected the same province in 1995. Increase in cassava trading and shortcuts in cassava processing were noticed months after the military roadblock that was made to quarantine populations affected by Ebola (Tylleskär et al., 1995; Banea et al., 1997a,b; Tshala-Katumbay et al., 2001a). In Mozambique, particularly in the northern regions, outbreaks of konzo (*mantakassa*) have been associated with famine, severe drought (1981) and war (1992–1993), and consumption of insufficiently detoxified bitter cassava (Ministry of Health Mozambique, 1984a,b; Cliff, 1994; Cliff et al., 1997).

Because poverty and war appear to be linked to the occurrence of konzo, it is not surprising the disease persists in the DRC and neighboring countries, including Angola, Uganda and Central African Republic, all theaters of armed conflict.

18.2.3. Prevalence, age and gender distribution

While isolated cases of lathyrism and konzo may occur, they usually appear in epidemic form. In recent times, prevalence rates of lathyrism have been as high as 66.45%, particularly during the famine period (1971–1975) in northwest Bangladesh (Haque, 1989). While outbreaks are now rare, living cases of lathyrism are found in Bangladesh, China, Ethiopia, Eritrea, India and Spain, the latter occurring during the Spanish Civil War when food was scarce (Spencer, 1994).

As of 2004, the total number of cases of konzo has been estimated to be as high as 100,000, with most of the cases occurring in regions of sub-Saharan Africa (Cassava Cyanide Diseases Network, 2004). Accurate regional prevalence rates (perhaps as high as 5%) are difficult to obtain because of fluctuating and unreliable demographic data (Rosling and Tylleskär, 2000).

Both lathyrism and konzo affect individuals across the span of postnatal life except that neither disease has been reported in children under 2 years of age. These two diseases show differential patterns of gender and age susceptibility that are difficult to explain. In the case of lathyrism, young males are commonly and more severely affected than young females, and this differential gender sensitivity appears to be related to factors other than grass pea intake (Spencer et al., 1984). Women tend to develop lathyrism before puberty, during pregnancy or after menopause (Dwivedi, 1989). However, in the case of konzo, young males and females seem to be similarly affected, while women of childbearing age are more susceptible to the disease than men of the same age (Tylleskär et al., 1995). As a result, women tend to be more affected in konzo-affected areas, whereas male cases predominate in lathyrism areas. Whether this impression reflects the true population distribution of these diseases is unknown since the two conditions occur in distinctly separate regions that have not been subjected to a comparative epidemiology survey. Population and individual genetic susceptibilities have not been looked for and cannot be ruled out.

18.3. Clinical picture

The most prominent signs of both lathyrism (Fig. 18.2(A)) and konzo (Fig. 18.2(B)) are symmetrical postural abnormalities and spastic (cross-legged or

scissoring) gait during ambulation (Gopalan, 1950; Ludolph et al., 1987; Howlett et al., 1990; Tekle-Haimanot et al., 1990; Rosling and Tylleskär, 1995; Tylleskär et al., 1995; Tshala-Katumbay et al., 2001a,b). In cases mildly affected by either disease, spasticity of legs is revealed only when the subject is asked to run. Those severely affected by either disease are often bedridden (with stiff legs due to excessive weakness, marked increase in muscle tone and joint contractures and/or ankylosis), or may be capable of moving by crawling on the rump. The phenotype of these two diseases is so uniform that the two entities may be clinically indistinguishable as evidenced by the following independently developed epidemiological criteria for lathyrism or konzo:

(a) Lathyrism: (1) a history of heavy ingestion of *Lathyrus sativus* or other neurotoxic *Lathyrus sp.* for at least 2 weeks prior to and at the time of acute or subacute onset of walking difficulty, (2) a largely symmetrical and pyramidal distribution of leg weakness, with exaggerated thigh adductor, patellar and ankle reflexes, in the presence or absence of ankle clonus and extensor plantar reflexes bilaterally, (3) an essentially normal sensory examination, and (4) intact mentation, cerebellar and cranial nerve function (Gopalan, 1950).
(b) Konzo: (1) a heavy reliance on cassava as staple food, (2) abrupt onset (< 1 week) of leg weakness and a non-progressive course of the disease in a formerly healthy person, (3) a symmetric spastic abnormality when walking and/or running, and (4) bilaterally exaggerated knee and/or ankle jerks without signs of disease of the spine (WHO, 1996).

The main criteria for differential diagnosis of lathyrism and konzo are geographic and nutritional. Whereas konzo has been reported only in sub-Saharan Africa in association with dietary dependence on cassava, lathyrism has occurred on many continents with use of grass pea as a staple. In Africa, lathyrism seems to be restricted to the Horn, notably Ethiopia and Eritrea. The clinical manifestations of lathyrism and konzo are almost identical, although there is a tendency for konzo to be a more severe upper motor neuron disorder with clinical evidence of pyramidal deficits in both upper and lower extremities (Ludolph et al., 1987; Spencer, 1994; Tshala-Katumbay et al., 2001b). Both diseases affect the poorest of the poor because of their dependency on single inexpensive staples, and minimal nutrition or malnutrition is common and possibly necessary for florid disease to emerge. Excessive physical activity is often reported at onset and may be a predisposing factor that stresses the motor pathway and promotes cortical motor neuron susceptibility to the plant-derived toxins in cassava and grass pea.

18.3.1. Prodromal phase

During the prodromal phase, subjects affected by lathyrism or konzo experience mainly motor symptoms such as a sensation of leg weakness, heaviness or stiffness; muscle cramping usually confined to calf musculature and tremulousness. Acutely reversible sensory symptoms are often reported and may include paresthesia, numbness, muscle aching and a sensation of electrical discharge (Lhermitte's sign) in the back and legs. Urological involvement is not common. Blurred vision and difficulties in swallowing have been occasionally reported in konzo. Clinical deficits are usually greater at the onset of the disease and the subject may be bedridden. Once the course of the disease has stabilized, deficits are mainly confined to the motor system. The most noticeable feature is the cross-legged (scissoring) gait of affected subjects who are able to walk and/or run.

18.3.2. Neurological examination

On physical examination, the main clinical picture consists of an isolated symmetric spastic paraparesis. Deep tendon reflexes of the lower limbs are exaggerated and extensor plantar responses can be elicited in most cases when patients are tested in the recumbent position. Ankle clonus is frequently found. Upper extremities also show pathological reflexes in severely affected subjects, with palmomental and superficial abdominal reflexes often present in konzo and the former rarely in lathyrism (Ludolph et al., 1987; Spencer, 1994; Tshala-Katumbay et al., 2001a,b). Severely affected subjects may show a spastic tetraparesis associated with weakness of the trunk. In some cases, exaggerated tendon reflexes can occur as an isolated feature in the absence of functional deficit such as muscle weakness. Disuse muscle atrophy may be seen. Cognition, hearing, coordination and sensory function, as well as urinary, bowel and sexual functions, remain normal (Ludolph et al., 1987; Tshala-Katumbay et al., 2001a,b).

Subjects affected by konzo may also present with pseudobulbar signs – not reported in lathyrism – that mainly consist of speech and/or swallowing problems. These signs are often encountered in severe cases. The longest motor tracts are consistently involved before and to a greater extent than shorter ones. Thus, subjects with speech and/or swallowing problems always show pyramidal signs in the legs and arms. A subject with signs in the arms (weakness, increased deep tendon reflexes or Hoffman's sign) will always have pronounced

spasticity of the lower extremities (Tylleskär et al., 1995; Tshala-Katumbay et al., 2001a,b).

Degrees of severity of the disability, often inappropriately referred to as stages, vary from mild to severely affected subjects. Based on the ability to walk, the following classification has been proposed:

- Lathyrism: mild form (Acton's no stick-stage or stage I) represents subject with mild spastic gait with no need for a walking stick, complaints of increased leg stiffness, brisk deep tendon reflexes at knee or ankle and Babinski's sign equivocal or present. The moderate form (Acton's 1-stick stage or stage II) represents subject with spastic gait that requires the use of a stick for support during ambulation, mild rigidity and increased deep tendon reflexes and ankle clonus and Babinski sign present. The moderately severe form (Acton's 2-stick stage or stage III) presents with severe spastic gait that requires the use of two sticks for support during ambulation, crossed adductor gait, markedly increased deep-tendon reflexes with clonus at ankle and knee and Babinski's sign present. The severe form (Acton's crawler stage or stage IV) represents crawling, wheelchair-bound or bedridden state. Subject shows extreme muscle rigidity and has completely lost use of his legs (Acton, 1922; Spencer, 1994).
- Konzo: mild form represents subject able to walk without support, in the moderate form the subject has to use one or two sticks, and in the severe form the subject is unable to walk (WHO, 1996).

With cessation of exposure to cassava, spasticity remains largely stable across the lifespan, with painful calf muscle spasms representing an ongoing major symptom. Very few subjects with konzo may suffer a sudden second aggravating episode, which is in fact an episode identical to that experienced at the initial onset of the disease.

The spastic para/tetraparesis that characterizes konzo may be associated with other signs. Ophthalmologic studies have shown konzo patients with bilateral optic neuropathy in addition to their para/tetraparesis. This condition encompasses visual impairment, temporal pallor of the optic disks and defect of visual fields. A pendular nystagmus has also been reported in few cases. The presence of visual symptoms at disease onset and/or optic neuropathy on subsequent examination seems to not be correlated with the severity of konzo (Mwanza et al., 2003a,b).

There is evidence of another cassava-associated neurological disorder that develops in older subjects who have a heavy intake of incompletely detoxified cassava. The typical pattern is a slowly evolving ataxic neuropathy with or without evident pyramidal deficits, as well as occasional visual and sensorineural auditory deficits. The disorder was first reported in Nigeria (Osuntokun, 1973) and more recently in Kottayam District, Kerala, India, where patients are reported to improve with a nutritious diet (Madhusudan, personal communication, World Congress of Neurology, Sydney 2005). Other neuromuscular syndromes that have been reported in association with cassava dependency include a "motor neuron–cerebellar–Parkinson–dementia" syndrome among Nigerians (Osuntokun, 1981), proximal myopathy and a movement disorder resembling ballism among Indians (Madhusudan, personal communication, World Congress of Neurology, Sydney 2005). Extrapyramidal disorders of this type are seen in subjects with mildewed sugarcane poisoning, which raises the question of fungal contamination of food in cassava-associated cases with ballistic movement disorders (Ludolph et al., 1991). Other conditions that have been associated with cassava dependency include thyroid dysfunction and growth stunting, and a type 3 (tropical) diabetes mellitus (Ihedioha and Chineme, 1999; Rosling and Tylleskär, 2000; Mbanya et al., 2003). Indian cases with cassava-associated ataxic neuropathy reportedly do not present with diabetes.

18.4. Ancillary investigations

18.4.1. Clinical chemistry

The clinical chemistry profile of lathyrism has not been reported. Studies on konzo have focused on understanding underlying nutritional and metabolic factors and levels of exposure to the culpable cyanogenic compounds in cassava. Serum levels of albumin and prealbumin (indicators of protein nutritional status), and urinary sulfate – reflecting the dietary intake of sulfur-containing amino acids needed to convert cyanide (CN) to thiocyanate (SCN) – are usually below normal reference values in most of the subjects affected by konzo. Serum and urinary levels of SCN may reach values as high as 1000–1500 μmol L^{-1}. However, these biochemical values remain non-specific to konzo subjects and may also be found in apparently normal subjects living under the same conditions in zones endemic for the disease. SCN has been the most useful chemical biomarker for cassava cyanogenic exposure because (a) cheap, specific and sensitive determination methods are available, (b) it is a very stable metabolite and urinary samples do not need to be frozen and (c) it has a slow urinary excretion with a half-life in serum l of 3 days; thus, urinary levels of SCN reflects almost the mean daily SCN load during preceding days. Other analytical methods exist to measure levels of the

minor cyanide metabolite aminothiazoline-carboxylic acid in urine and/or cyanate (OCN) in plasma (Lundquist et al., 1979, 1983, 1993, 1995; Rosling, 1994; Spencer, 1999). These methods could potentially be used to study the relationship between cassava cyanogenic exposure and occurrence of neurological disease. There is no biological marker for lathyrism.

18.4.2. Electrophysiology

Comprehensive electrophysiological testing (Table 18.1) has been performed on subjects with lathyrism or konzo mainly to assess the functional integrity of motor pathways. Magnetic or electric stimulation of the motor cortex has been used to assess the motor pathways subserving the upper and lower extremities. Additional investigations have included peripheral nerve conduction studies (NCS) and conventional needle electromyography (EMG), evaluation of somatosensory evoked potentials (SEP) and visual evoked potentials (VEP) and electroencephalography (EEG). Because of the paucity of data using morphologic (neuropathologic) and imaging techniques, electrophysiological testing has provided a major contribution to the understanding of the lesion in lathyrism and konzo.

18.4.2.1. Studies of peripheral nerve conduction and electromyography

Most subjects with lathyrism or konzo have normal motor and/or sensory peripheral nerve conduction

Table 18.1

Comparative clinical electrophysiology in lathyrism vs. konzo

	Lathyrism	Konzo
Causal factors	Heavy dietary reliance on Grass pea (*Lathyrus sativus* sp.). Minimal nutrition possibly necessary for florid disease to emerge. Toxic candidate: (β-N-oxalylamino-L-alanine (see pathogenesis).	Heavy dietary reliance on insufficiently processed bitter cassava (*Manihot esculenta Crantz*) and low dietary intake of sulfur amino acids. Toxic candidates: cyanogenic glucosides (linamarin and/or lotaustralin) and metabolites (presumably thiocyanate and/or cyanate) (see pathogenesis).
Main clinical picture	Spastic para/tetraparesis.	Spastic para/tetraparesis. Pseudobulbar signs, optic neuropathy and goiter may be found.
MEP studies	Increased stimulation strength needed to trigger a volley.* Frequent inability to elicit MEP. When present, central motor conduction time is increased.**	Frequent inability to elicit MEP.* When present, central motor conduction time is sometimes increased.**
Peripheral nerve conduction studies	Normal motor and sensory nerve conduction. Evidence of deficits has been found in a minority of subjects with long-standing disease (probably unrelated disorders such as poliomyelitis, diabetes mellitus).	Normal motor and sensory nerve conduction. Increased F-wave amplitude in legs.
SEP studies	Cortical responses following tibial stimulation frequently absent. If present, the latency is prolonged. Median SEP normal.	Cortical responses following tibial stimulation frequently absent. If present, the latency is prolonged. Median SEP often normal.
VEP studies	Not available.	Frequent delay and decreased amplitude of P100.
EEG studies	Not available.	Generalized slowing of background activity, non-specific paroxysmal activities, eye-opening and hyperventilation with no effect. Normal EEG in ~ 40% patients.

* Consistent with reduction of the upper motor neuron pool. ** Consistent with loss of pyramidal conductivity from spinal tract (axonal) damage.

(Hugon et al., 1990, 1993; Tshala-Katumbay et al., 2002b). Electrophysiological deficits have been noticed in only a few subjects with lathyrism, and these are likely to be related to supervening factors such as malnutrition or diabetes. Approximately 7% of a large cohort of Romanian Jews with lathyrism showed evidence of sensorimotor neuropathy and signs of muscle denervation almost 30 years after the onset of the disease (Cohn et al., 1977; Cohn and Streifler, 1981a,b; Drory et al., 1992). Similar abnormalities were reported in six of 14 Bangladeshi subjects with 9–13 years of lathyrism. Two of 10 subjects who developed lathyrism 45 years earlier had peripheral neuropathy (Hugon et al., 1990, 1993; Gimenez Roldan et al., 1994). Etiologies unrelated to lathyrism (diabetes, malnutrition) often account for the presence of peripheral neuropathy.

In konzo, F-waves in legs often display high amplitude, presumably reflecting dysinhibition at the level of the anterior horn cell. Only limited needle EMG has been done in konzo. Spontaneous activity has not been recorded in the examined muscles. In several muscles the motor unit potentials were small in amplitude and/or duration without an increase of polyphasic potentials. These minor changes may be explained by disuse atrophy seen in the patients (Karin Edebol Eeg-Olofsson, personal communication).

18.4.2.2. Motor evoked potential (MEP) studies

The integrity of motor pathways was investigated in 14 Spanish subjects with long-standing lathyrism (> 40 years) using techniques of transcranial magnetic stimulation with separate spinal cathodic stimulation at the cervical level (C6) for upper limbs and thoracic level, (T12) for lower limbs (Hugon et al., 1993). Transcranial electrical stimulation was used to study the motor pathway of 14 Bangladeshi subjects with stable lathyrism (duration of disease: 9–13 years) (Hugon et al., 1990).

For konzo, investigations were performed on Congolese subjects using either electrical unifocal scalp stimulation in 21 subjects (duration of konzo: 2–11 years) or transcranial magnetic stimulation in 15 subjects (duration of disease: 1–18 years) and two Tanzanian subjects (duration of konzo: 6 years) (Tylleskär et al., 1993; Tshala-Katumbay et al., 2002b).

Magnetic and electrical stimulation methods show that most subjects with konzo or lathyrism have abnormal motor evoked potentials (MEP). Common abnormalities include either the absence of responses in lower limbs or a prolonged central conduction time (delayed responses). Another major electrophysiological finding consisted of MEP abnormalities in upper limbs of subjects with konzo. Although more clinically affected in lower limbs, MEP studies revealed an absence of responses, even in apparently clinically preserved upper limbs. A similar abnormality (delayed MEP) has been found in a subject with lathyrism during the aforementioned study, although other subjects showed brisk tendon reflexes and the Hoffman sign.

MEP deficits were markedly increased in Spanish patients with severe form (grade III or IV) of lathyrism whereas such a correlation has not been found among subjects with konzo.

The significance of MEP abnormalities, together with normal peripheral nerve conduction, demonstrates (a) a selective dysfunction of the upper motor neuron system both in konzo and lathyrism, and (b) the possibility of a presynaptic cortical failure especially because magnetic stimulation revealed abnormalities in apparently preserved upper limbs. These findings also raise the possibility of a common pathogenetic mechanisms for lathyrism and konzo.

18.4.2.3. Somatosensory evoked potential (SEP) studies

Objective sensory deficit is not observed in either lathyrism or konzo. However, sensory symptoms such as paresthesia, pain and a sensation of electrical discharge in the legs may be present at the onset of the disease; these symptoms usually dissipate within weeks of onset (Spencer, 1994; Tshala-Katumbay et al., 2001a,b). Electrophysiological testing has revealed frequent abnormalities of tibial SEP (in lathyrism and konzo) and much less frequent abnormalities of the median SEP (and only in konzo). The main abnormality consists of absence of cortical responses after stimulation of posterior tibial nerve. Most patients have normal absolute latencies to both cervical and lumbar levels. Increased central sensory conduction time through the spinal cord is common in patients with prolonged latencies of cortical evoked potentials (Hugon et al., 1990; Tshala-Katumbay et al., 2002a).

The frequent tibial SEP abnormalities and associated history of sensory symptoms in the legs at disease onset suggests that somatosensory pathways are subclinically involved in lathyrism and konzo. Mechanisms underlying these SEP abnormalities, also commonly found in other motor neuron diseases, are unknown. They may indicate a more widely distributed lesion in the nervous system, with the motor system being predominantly affected; reflect the impact of motor system dysfunction on the input and output pathways of the somatosensory system without any structural or functional alterations of these pathways; or they may be attributed to other causes such as nutritional deficiencies.

18.4.2.4. Visual evoked potential (VEP) studies

Visual deficits are peculiar to cassava-associated disease and are not reported in lathyrism. A study of VEP

in 23 Congolese konzo subjects (duration of disease: 2–25 years) was conducted to investigate the nature of ophthalmologic manifestations often reported in konzo. Latencies of the P100 VEP-component and peak-to-peak amplitude of N75-P100, for each eye, were compared with values recorded in 38 apparently healthy subjects who served as a reference group. About half (11) of 23 konzo subjects showed symmetrical abnormalities of VEP consisting of both prolonged latency and decreased amplitude. There was no correlation between VEP abnormalities and the severity of the disease. These abnormalities, together with the spastic paraparesis in konzo, may well result from intoxication by cassava cyanogens and/or their neurotoxic metabolites, including free cyanide (Tylleskär et al., 1993; Mwanza et al., 2003a,b).

18.4.2.5. Electroencephalography

Standard EEG recordings have been performed on 21 konzo patients aged 10–49 years (disease duration: 2–11 years). These cases had no history of seizures, skull trauma, loss of consciousness or cognitive problems. However, they showed significant EEG abnormalities of which the main abnormality was a generalized slowing (θ rhythm) of background activity. In severely affected subjects, non-specific paroxysmal activity and decreased frequency of the post-central background rhythm occurred in addition to generalized slowing. Some patients had focal slowing of the background activity with a dominant distribution in the frontal areas. Generalized EEG abnormalities were more frequent in severe cases. Whether these abnormalities are intrinsic to the disease process of konzo is unknown. Plausibly, malnutrition and exposure to cyanogenic compounds in konzo-affected areas may interfere with thyroid function and thus brain development and/or function with consequences seen at EEG (Tshala Katumbay et al., 2000). There are no reports on EEG recordings in subjects affected by lathyrism.

18.5. Imaging

A study using magnetic resonance imaging (MRI) on two Tanzanian konzo subjects has revealed no abnormalities (Tylleskär et al., 1993). MRI has not been performed on subjects with lathyrism.

18.6. Pathology

Neuropathological data on lathyrism and/or konzo are rare and incomplete. A study of the brain of a subject who developed lathyrism 31 years prior to his death showed loss and shrinkage of pyramidal neurons in the upper part of the precentral gyrus (Filiminoff, 1926). Most neuropathological studies, which have focused on the spinal cord, show predominantly distal symmetrical degeneration of lateral and ventral corticospinal tracts, sometimes with distal degeneration of spinocerebellar and gracile tracts (Buzzard and Greenfield, 1921; Filiminoff, 1926; Puis and Devinals, 1943; Sachdev et al., 1969; Hirano et al., 1976; Striefler et al., 1977). An autopsy report on a man who developed the disease 32 years prior to death showed anterior horn cells with Hirano bodies but no reduction of the lower motor neuron pool (Hirano et al., 1976).

The two reported autopsies of konzo cases are of little value: one limited to the brain revealed punctate hemorrhages and cerebral edema 3 hours post-mortem; the other showed no abnormalities in the spinal cord (Trolli, 1938).

It is difficult with these sparse reports to determine the primary site of the lesion in either lathyrism or konzo, but evidence from clinical and electrophysiological studies indicates these are diseases mainly confined to the upper motor neuron and thus comparable to primary lateral sclerosis (PLS). There is no evidence to support the suggestion that lathyrism progresses to involve the lower motor neuron, the final picture resembling amyotrophic lateral sclerosis (ALS).

Muscle biopsies in five konzo subjects with small motor unit potentials on EMG revealed minor non-specific fiber atrophy, presumably due to muscle disuse (Karin Edebol-Eeg Oloffson, personal communication).

18.7. Pathogenesis

Clinical and neurophysiological studies of human lathyrism and konzo show these two diseases to be remarkably similar. One of the striking similarities, apart from the clinical picture and the abnormalities of motor evoked potentials, is that physical exertion appears to be an important triggering factor for the disease. Many subjects affected by lathyrism or konzo report intense physical activity (e.g. prolonged walking or bicycling) prior to a sudden onset of walking difficulties that eventually progressed into stable disease states. Whether these similarities underlie a common pathogenetic mechanism for lathyrism and konzo remains enigmatic.

18.7.1. Lathyrism

The link between lathyrism and excessive consumption of the grass pea by humans or domestic animals (e.g. ducks, geese, hens, peacocks, pigs, oxen, sheep, cows, bullocks, elephants and horses) has been known for centuries (Sleeman, 1844; Stockman, 1917; Sugg et al., 1944; Selye, 1957; Gardner and Sakiewicz, 1963). Several experimental studies have attempted to reproduce

the disease in animals. A study in the horse, allegedly the most susceptible species, indicated that a diet made exclusively of *L. sativus* precipitated signs after 10 days. Horses fed only 1–2 quarts per day succumbed after 2–3 months, and neurological manifestations appeared a month or more after the diet is withdrawn (Stockman, 1929). There have been several attempts to model human lathyrism in primates. Stockman (1917) claimed success, but details of his methods and results are not available. Rao et al. (1967) reported the induction of either flaccid or spastic paraplegia in macaques after intrathecal administration of synthetic β-N-oxalyl-amino-L-alanine (BOAA), the major neurotoxic compound extracted from the grass pea.

Other studies have shown that monkeys fed grass pea, plus an extract thereof, for up to 15 months, developed a spastic paraparesis (Srinavassa Rao and Roy, 1981). Signs of pyramidal dysfunction resembling the early phase of human lathyrism were demonstrated in well-nourished cynomolgus monkeys fed a fortified diet of pure *L. sativus* plus daily oral gavage with an alcoholic extract of grass pea, for a total daily intake of 1.1–1.4 g of BOAA/kg body weight (Spencer et al., 1986, 1988). Additional animals on a control diet of chickpea (*Cicer arietinum*) that was matched for protein, carbohydrate, fat, mineral and vitamin content, were given either pure BOAA (300 mg kg^{-1} per day, increasing by 300 mg kg^{-1} every 15 days) or an alcoholic extract of BOAA plus pure synthetic BOAA. The animals developed comparable neurological signs after 2–4 weeks (300 mg kg^{-1} per day, increasing by 300 mg kg^{-1} every 15 days), 2–6 weeks (alcoholic extract of BOAA plus pure synthetic BOAA) and 3–10 months (alcoholic extract of BOAA plus grass pea diet). Affected monkeys showed a variable combination of neurological signs including fine tremor, periodic myoclonic-like jerks, mild-to-moderate increase in muscle tone of leg muscles, hind limb extensor posturing and a skater-like gait. The most severely affected monkey showed exaggerated patellar reflexes, crossed thigh adductor reflexes, bilateral extensor plantar reflexes and hind limb withdrawal after downward stroking of the tibia. Arm functions and skilled finger movements appeared to be intact. There was no sign of sensory dysfunction, cerebellar, cranial nerve or urological involvement. Neurological improvement occurred after cessation of grass pea administration. Both groups of BOAA-treated animals showed increase in central motor conduction time following transcranial (motor cortex) electrical stimulation. However, neuropathological studies showed no evidence of neuronal degeneration in motor cortex or spinal cord. These studies suggest (a) prolonged exposure to BOAA induces a pattern of motor neuron disease reminiscent of human lathyrism, (b) clinical signs consistent with neuroexcitation (myoclonus) occur early in primate lathyrism, as in the human disease, (c) early functional improvement is seen in both species and, by extrapolation, (d) BOAA is likely the key etiological agent in human lathyrism. Pharmacological effects of this potent excitatory amino acid may precede the onset of neuronal degeneration and account for reversible components of the disease. Studies with BOAA-dosed animals subject to malnourishment or excessive physical exercise have not been performed. It is possible that BOAA is a potent neurotoxin that may be largely excluded from the central nervous system in well-nourished subjects. Perhaps leakage of the blood – brain regulatory interface in association with poor nutrition or harsh exercise accounts for the sudden onset of disease in some cases.

The mechanism of action of BOAA appears to involve excessive neuronal stimulation (Watkins et al., 1966; Olney et al., 1976; Pearson and Nunn, 1981; MacDonald and Morris, 1984; Ross et al., 1987; Bridges et al., 1989; Riepe et al., 1995). Experimental studies show that BOAA (Fig. 18.4(A, B)) is both a potent glutamate agonist at alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors and an inhibitor of glutamate uptake in synaptosomal preparations (Spencer, 1999). Recent study indicates that BOAA neurotoxicity in vitro may also be partially mediated by activation of group I metabotropic glutamate receptors by an indirect mechanism (Kusama-Eguchi et al., 2004). Taken together, these observations suggest that BOAA induces neuronal degeneration as a

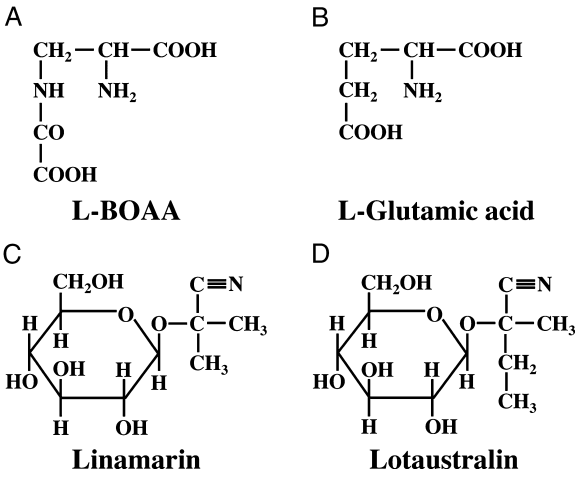


Fig. 18.4. Structural similarities between the AMPA agonist BOAA (**A**) and the principal CNS excitatory neurotransmitter glutamate (**B**). BOAA is also known as L-3-oxalyl amino 2-aminopropionic acid or β-N-oxalylamino-α, β-diaminopropionic acid or 3-N-oxalyl-2,3-diaminopropanoic acid (ODAP). (**C**) Linamarin and lotaustralin (**D**) are the main cyanogenic glucosides found in cassava.

result of excessive glutamatergic stimulation of nerve cells. The latter causes (a) an excessive influx of sodium ions (Na^+) and chloride ions (Cl^-), resulting in membrane depolarization, (b) increased activity of the Na^+/K^+ ATPase membrane pump, which diminishes ATP as the pumps attempts to restore the transmembrane ionic gradient, and (c) failure of the cell to re-establish ionic homeostasis. As a consequence, an osmotic pressure is created such that there is a massive entry of water into the cytoplasm, leading to a postsynaptic vacuolation of dendrites and perikarya that culminates in cell death. These pathophysiological events, which may develop within seconds following application of L-BOAA (10 μM) to mouse CNS explants in vitro (D-BOAA and the α-isomer of L-BOAA lack neurotoxic properties in rodent systems), constitute the excitotoxic theory of neuronal death as proposed by Olney (1980). A second delayed mechanism of neuronal degeneration is proposed to result from an increase in free intracytoplasmic calcium (Ca^{2+}) ions, which activates Ca^{2+}-dependent enzymes with subsequent dissolution of cytoplasmic structure (Choi, 1987).

Another line of enquiry proposes that L-BOAA exerts its neurotoxic effects via disruption of mitochondrial function. In vitro studies on mouse brain slices have yielded controversial results suggesting that L-BOAA inhibits the enzymatic activity of NADH, part of complex I of the mitochondrial electron transport chain (Pai and Ravindranath, 1993). Sabri et al. (1995) were unable to confirm these results.

Recent studies from the Ravindranath group suggest that L-BOAA triggers glutathione loss and protein thiol oxidation that disrupts mitochondrial complex I selectively in mouse motor cortex and lumbosacral cord, the regions affected in humans; and thioltransferase (glutaredoxin) mediates recovery of motor neurons from excitotoxic mitochondrial injury (Kenchappa et al., 2002; Ravindranath, 2002).

Clarification of the pathogenetic mechanisms of lathyrism awaits development of an animal (preferably primate) model that features cortical motor neuron degeneration. Based on previous experience, in which an early reversible (and presumably pharmacologic) model of primate lathyrism was induced in optimally nourished and largely sessile animals, persistent spastic paraparesis with cortical motor neuron and pyramidal tract degeneration is most likely to develop in poorly nourished animals subjected to vigorous exercise and a diet of grass pea plus grass pea extract or synthetic BOAA. A reliable primate model of human lathyrism would allow the testing of glutamate antagonists or other molecules as protective agents.

Nutritional surveys among human populations affected by lathyrism have shown that simple methods such as steeping and boiling leaches BOAA and detoxifies grass pea. Steeping dehusked seed in hot water for several hours and boiling the seeds in water removes 70–80% of the neurotoxin into the supernatant, which is discarded. Simulated kitchen-experiments show that steeping grass pea in a large volume of water for 3 min and decanting the excess water leaches about 30% of the neurotoxin. Steeping in a large volume of water (four parts water to one part seeds) for 1 hour may leach up to 90% of the toxic content. However, this method may result in considerable loss of water-soluble vitamins, especially thiamine and riboflavin. Parboiling is an improved method over steeping and the major portion of the toxic component is removed in the steam condensate. However, about 15% of the grain may be lost and therefore it impacts the quality of the final food products (Pushpamma, 1989; Spencer and Palmer, 2003).

18.7.2. Konzo

The molecular mechanisms of konzo have yet to be understood, and the absence of an animal model of konzo is a major drawback to studying this question. Epidemiological studies have consistently shown an association between the occurrence of the disease, a diet dominated by insufficiently processed bitter cassava, and a low protein intake (Rosling and Tylleskär, 2000).

Bitter (poisonous) varieties of cassava contain large amounts of cyanogenic glucosides, namely linamarin (~90%) and lotaustralin (~10%) (Fig. 18.4(C, D)). Levels of cyanogenic glucosides in cassava – the plant's chemical defense system against predators – depend on environmental conditions, including season, soil fertility and moisture (Sundaresan et al., 1987; Dixon et al., 1994; Mahungu, 1994). The glucosides are stored in cell vacuoles while a cyanogen-cleavage enzyme (β-glucosidase, syn. linamarase) exists in the cell wall. Once the physical integrity of the root tissue is disrupted, as in root processing for food preparation, the cyanogenic glucosides come into contact with linamarase and are hydrolysed, leading to the formation of glucose and cyanohydrins (Mkpong et al., 1990; Joachim and Panditteseker, 1991; Du et al., 1995). At pH > 5, the cyanohydrins spontaneously break down into ketones and hydrogen cyanide (HCN) gas escapes (O'Brien et al., 1992). Lower pH leads to persistence of cyanohydrins in the finished food product, with the result that HCN is released by bacterial enzymatic cleavage in the gut (higher pH) of the consumer (O'Brien et al., 1992; Rosling, 1994).

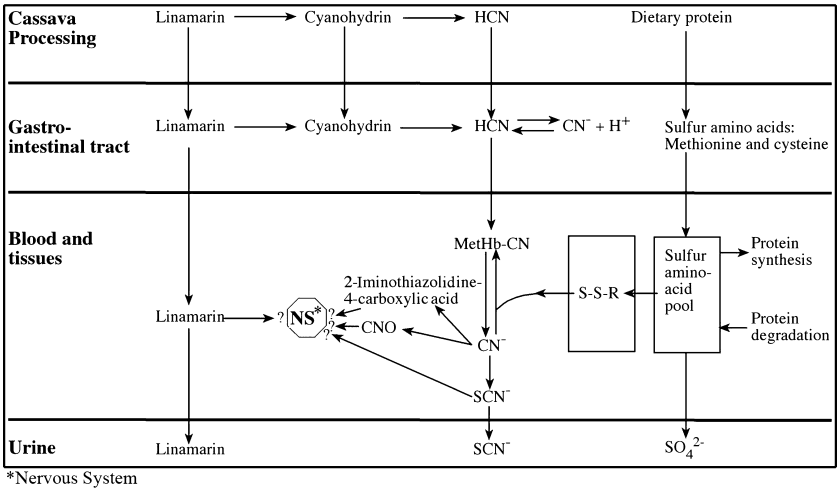
Many traditional processing methods have been developed to remove cyanogenic glucosides and their degradation products from cassava food products prior to consumption. These include methods such as soaking

or grating followed by drying or heating (Rosling, 1988; Nweke, 1994; Oke, 1994; Bokanga, 1995). Cyanogenic (toxic) exposure arises in populations where adherence to established effective processing techniques is no longer possible. The time necessary for proper cassava processing may not be available because of food shortages induced for example by armed conflict or drought. In some circumstances, a minor change in sequence or shortcut in one of the different processing steps can lead up to a 100-fold increase in the level of cyanogenic compounds in the final food product (Rosling, 1988). A single unprocessed fresh cassava root may yield enough hydrogen cyanide to kill a family within an hour of consumption.

Whereas linamarin (cyanogenic glucoside) is excreted intact in urine, a portion of ingested linamarin breaks down into cyanohydrin and thereafter into ketones and hydrogen cyanide. CN is normally sequestered by a saturable mechanism involving methemoglobin (MetHB). One major metabolic pathway of free CN (high acute toxicity) is conversion to thiocyanate (SCN, low acute toxicity), a rhodanese-regulated pathway that is reliant on the dietary intake of the sulfur amino acids methionine and cysteine. CN is also metabolized under normal conditions to OCN and trace amounts of iminothiazolidine carboxylic acid (Fig. 18.5). Under conditions of sulfur amino acid deficiency (low protein intake), protein catabolism appears to supply sulfane sulfur required for the SCN pathway. In these conditions, production of OCN is amplified several fold (Spencer, 1999; Tor-Agbidye et al., 1999; Rosling and Tylleskär, 2000).

Weeks or months of dependency on incompletely detoxified cassava combined with low intake of proteins – the source of sulfur amino acids providing the sulfur substrate for the conversion of CN to SCN – leads to outbreaks of konzo (Rosling and Tylleskär, 2000). Cyanide has been suggested to play a role in the genesis of neurological disorders including konzo because of its potential inhibitor effect on mitochondrial energy transformation that can secondarily lead to neuronal dysfunction and/or degeneration. This proposal is not supported by rodent studies of chronic cyanide intoxication (Spencer, 1999). Other neurotoxic candidates for the causation of konzo include: (1) the glucoside linamarin, which can enter pheochromocytoma 12 cells via glucose transporters and damage the cells by mechanisms not yet elucidated (Rosling, 1994; Sreeja et al., 2003), (2) the neurotoxic (seizing agent) agent 2-iminothiazolidine-4-carboxylic acid, (3) OCN, an established neurotoxic compound that induces axonopathy in humans and animals and (4) SCN, a chaotropic agent that preferentially increases glutamate binding to neuronal AMPA-binding sites resulting in an effect comparable to the AMPA receptor action of BOAA (Spencer, 1999). Conceivably, OCN may have similar chaotropic properties but the substance appears not to have been tested in pharmacologic assays able to demonstrate the effect.

Cyanate probably has a significant role in cassava-related neurodegeneration (especially ataxic myeloneuropathy) because of its ability to carbamoylate proteins and induce neurological disease. Cyanate (via isocyanic acid) carbamoylates proteins irreversibly on the N-terminal and ε-amino groups of internal lysines to form homocitrulline residues as well as reversibly on thiol groups (Stark, 1965; Glader and Conrad, 1972). Carbamoylation induces functional changes in targeted proteins (e.g. actin, tubulin, tau, Cu-Zn superoxidase dismutase) (Stark, 1965; Mellado et al., 1982; Farias and Vial, 1993). Protein carbamoylation has been associated



*Nervous System

Fig. 18.5. Pathways tentatively illustrating the metabolism of linamarin in humans. Sulfur amino acids are required for the detoxification of cyanogenic compounds.

with effects that both promote and compromise health. On the positive side, OCN attenuates erythrocyte sickling in sickle cell anemia by reacting with the terminal amino group of valine in the β chain of hemoglobin S. The negative side of the equation is drawn from human and animal experience with the development of sodium cyanate (NaOCN) as an anti-sickling drug (Cerami et al., 1973; Alter et al., 1974; Ohnishi et al., 1975; Peterson et al., 1975; Nicholson et al., 1976) and with systemic effects of chronic renal disease (uremia) mediated through cyanate as a metabolite of urea (Ohnishi et al., 1975; Kraus et al., 1994; Kraus and Krauss, 1998). All these conditions result in CNS and/or PNS (axonal) degeneration. Humans treated for sickle cell anemia with NaOCN develop peripheral neuropathy (Ohnishi et al., 1975; Schaumburg et al., 1983). Primates treated with NaOCN show neuropathological changes in peripheral nerves, spinal cord and brain, notably the basal ganglia and cerebral cortex (Tellez et al., 1979; Tedeschi et al., 1991). Rodents treated with high doses of NaOCN develop acute neurotoxicity (seizures and opisthotonos), while rats treated for prolonged periods with lower equivalent doses display chronic neurotoxicity (lethargy, weakness, spasticity, peripheral neuropathy) (Cerami et al., 1973; Kogure et al., 1975). Impaired tissue oxygenation and a marked decrease in overall learning capacity have also been observed in rats injected with OCN (Cassel et al., 1973). Though the literature suggests that OCN induces neuropathy by carbamoylating proteins, molecular mechanisms of OCN-associated neurotoxicity have yet to be understood. Studies are needed to identify key proteins (molecular targets) associated with the genesis of OCN-associated axonopathy.

SCN is an attractive candidate to induce konzo because an AMPA-mediated neuronal degeneration provides a conceptual model comparable to that of BOAA and lathyrism. The development of animal models for these two diseases will constitute a major step toward understanding of the pathophysiology of the motor system. OCN is an attractive candidate for the etiology of ataxic (myelo)neuropathy and perhaps other chronic disorders of the brain that have been linked to cassava food dependency.

18.8. Differential diagnosis

The diagnosis of lathyrism or konzo is relatively straightforward when the disease is epidemic, and several families within a community are affected within a common timeframe. The most important differential factor is the association with inadequate or malnutrition and either exclusive overconsumption of the grass pea or related neurotoxic *Lathyrus* spp. (e.g. *L. clymenum*; *L. saginata*) for lathyrism or insufficiently processed bitter cassava for konzo.

Lathyrism and konzo must be differentiated from Tropical Spastic Paraparesis (TSP), a neurological entity endemic to certain regions of tropical Africa, Seychelles, Japan and Latin America (Gessain and Gout, 1992; Proietti et al., 2005). In certain parts of the world, for example the Bandundu province of the DRC, TSP coexists with konzo on a large scale (Carton et al., 1986; Kayembe et al., 1990). Whereas konzo appears to be a toxiconutritional disease, TSP is etiologically linked to the human T-cell lymphotropic virus I (HTLV-I) (Gessain and Gout, 1992). Because of this association, TSP has been called also HTLV-I Associated Myelopathy (HAM). The differential diagnosis of konzo, lathyrism and TSP/HAM may be hampered because (a) either konzo or lathyrism may coexist with TSP/HAM and (b) a TSP/HAM patient may be seronegative to HTLV-I and resides in a konzo- or lathyrism-affected area. In these cases, the differential diagnosis is made by the history of the disease obtained after a carefully structured interview and physical examination. TSP/HAM is a slowly progressive spastic paraparesis whereas lathyrism and konzo are non-progressive conditions usually with an acute or subacute onset. In addition, there are objective signs of sensory and sphincter involvement in TSP/HAM, and lower motor neuron and/or sensory deficits may be evident in the extremities. Serological studies of subjects with lathyrism or konzo usually prove negative for HTLV-I (unless there is co-morbidity) (Gessain et al., 1985; Gessain and Gout, 1992; Tylleskär et al., 1996).

The process of identifying the cause of a spastic paraparesis may be challenging when the physician is faced with an isolated case and neither the history of the illness nor the dietary interview, nor the HTLV-I serological testing, provide essentials to confirm the diagnosis. In this situation, differential diagnosis should be made against other causes of non-compressive myelopathy (Table 18.2) including the subacute myelooptic neuropathy (SMON) syndrome due to clioquinol (5-chloro-7-iodo-8-quinolinol; iodochloroxyquin) intoxication (Konagaya et al., 2002, 2004; Benvenisti-Zarom et al., 2005), myelopathy associated with infectious agents or liver failure (Berger and Sabet, 2002; McArthur et al., 2005; Utku et al., 2005), hereditary spastic paraplegia (HSP), PLS and amyotrophic lateral sclerosis (ALS) (Strong and Gordon, 2005). In many cases, the history of the illness, the clinical examination that can reveal signs of systemic disease, the positive response to treatment (e.g. to antibiotics in disorders caused by infectious agents), genetics and laboratory tests in serum and/or cerebrospinal fluid, as well as the virological testing against other viruses such as

Table 18.2

Non-compressive causes of spastic paraparesis and their specific differential criteria

	Toxic-nutritional				Infectious			Neurodegenerative
	Lathyrism	Konzo	Combined degeneration of the spinal cord	SMON	TSP/HAM	HIV	Tuberculosis/ cysticercosis/ syphilis	ALS/PLS/HSP
Dietary/toxic factors	Grass pea	Cassava	B 12 deficiency	Clioquinol intoxication	No	No	No	Controversial in sporadic ALS cases
Onset	Acute/subacute	Acute	Subacute	Subacute	Subacute	Subacute	Subacute	Subacute
Upper motor neuron disorder	Yes	Yes	No	No	No	No	No	Yes (PLS, HSP) No (ALS)
Lower motor neuron involvement	No	No	Possible	No	Possible	Possible	Possible	Yes (ALS) No (PLS, HSP)
*Non-pure motor neuron disorder (sensory / sphincter signs)	No	No	Yes	Yes	Yes	Yes	Possible	No
Cranial nerve involvement	No	Optic neuropathy*	No	Optic neuropathy	Possible	Possible	Possible	Bulbar palsy but rare in HSP
Course	Non-progressive	Non-progressive	Progressive	Progressive	Progressive	Progressive	Progressive	Progressive
Virological tests	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Negative
Microbial link	No	No	No	No	No	No	Yes	No
Known genetic susceptibility	No	No	No	No	No	No	No	Yes

* More prominent in cassava-associated ataxic myeloneuropathy.

Human Immuno-Deficiency Viruses type I and II (HIV-I-II) and neuro-imaging (MRI) will help refine the diagnosis. An earlier detection of treatable causes of myelopathy such as tuberculosis, syphilis, cysticercosis or vitamin B12 deficiency is of paramount importance.

18.9. Treatment and prevention

There is no effective treatment for either lathyrism or konzo. Once the disease process has stabilized, the disability remains unchanged and irreversible. Attempts have been made to reduce muscle spasm and contractures. Little relief has been reported with use of centrally acting spasmolytics. Dorsal rhizotomy or partial surgical transaction of the thigh adductor muscles has been tried, the latter with some occasional success in subjects with lathyrism (Spencer, 1994; Rosling and Tylleskär, 2000). Intramuscular injection of botulinum toxin has been used with success to reduce adductor spasticity in patients with cerebral palsy (Mall et al., 2006) and should represent a valuable therapeutic tool in both konzo and lathyrism. Physical therapy may also be useful to reduce muscle spasm and prevent development of joint contractures and/or ankylosis.

During the last two decades, focus has been placed on preventing outbreaks of disease. In 1988, the Third World Medical Research Foundation brought together scientists from various disciplines and nations to form an International Network for the Improvement of *Lathyrus sativus* and the Eradication of Lathyrism (INILSEL) (Palmer et al., 1985). This initiative has stimulated scientists to perform carefully designed studies to understand epidemiological, socio-economic and agricultural factors underlying outbreaks of lathyrism. In parallel, similar efforts have been undertaken by several scientists under the auspices of the Cassava Safety Network and the Cassava Cyanide Diseases Network to understand causal factors of cassava-associated neurological disease and develop a strategy for its elimination (Rosling, 1988; Cassava Cyanide Diseases Network, 2003). Strategies for prevention include: (a) promotion of efficient detoxification methods for either cassava or the grass pea prior to its consumption, (b) introduction of other staples, cereals, vegetables and fruits to diversify the diet in lathyrism or konzo-affected areas, (c) development of genetically modified low-toxin or toxin-free strains of either grass pea or cassava in areas endemic for lathyrism or konzo, respectively, and (d) improvement of early warning systems to recognize the imminence of outbreaks through, for example, use of simple and effective methods such as picrate kits to monitor cyanide levels in cassava products and urinary SCN in populations at risk of developing konzo (Ernesto et al., 2002).

18.10. Summary

Lathyrism and konzo are two similar self-limiting cortical motor neuron disorders characterized by spastic disability of the lower extremities and, in severe cases, with disability in the upper extremities and even (in konzo only) pseudobulbar dysfunction. They are caused by prolonged and almost exclusive dietary dependence on grass pea (lathyrism) or insufficiently processed bitter cassava (konzo), with poor nutritional state a significant risk factor and excessive exercise an apparent precipitating factor. Only lathyrism has been partially modeled in laboratory primates. Experimental studies suggest these toxic neurodegenerative disorders are primary neuronopathies dominated by bilateral involvement of upper motor neurons and their axonal projections. Those cortical motor neurons with the longest axonal projections seem to be most vulnerable, possibly because they have the largest dendritic tree and available glutamate receptors that are likely targets of the culpable grass pea neurotoxin (BOAA) and the neurotoxic cassava metabolites (SCN, OCN). Onset of lathyrism and konzo is often abrupt, and some degree of clinical improvement is characteristic before the clinical signs stabilize into a pure form of spastic para/tetraparesis. Clinical progression may occur with continued intake of the offending foodstuff. Prolonged lower-level intake of cassava has been associated with distinct neurological syndromes in adults, notably an ataxic (myelo)neuropathy for which OCN is a likely etiological factor.

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Chapter 19

Therapies in amyotrophic lateral sclerosis: options for the near and far future

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The understanding of the pathobiology of amyotrophic lateral sclerosis (ALS) has increased substantially thanks to the generation of transgenic animal models, available since the discovery of mutations in the superoxide dismutase 1 (SOD1) gene in familial ALS (FALS) (Rosen et al., 1993). As the cause(s) of sporadic ALS (SALS) have not been identified, attempts to elucidate the pathogenesis of ALS have concentrated on the mechanisms of mutant SOD1-induced motor neuron degeneration. Insights into these mechanisms have allowed one to design treatment strategies for this form of motor neuron degeneration and hopefully for motor neuron degeneration in general. In the present chapter, we will attempt to highlight some of these options. For practical purposes, we artificially divided them into therapies that aim at the etiology of motor neuron degeneration directly, e.g. mutant SOD1 expression itself, those that interfere with the pathophysiological cascade leading to motor neuron degeneration and those that target the motor neuron or its environment in general.

19.1. Targeting the primary cause

The ideal therapy would be to selectively and completely shut off the expression of mutant SOD1 before damage to the cell occurs. Pessimists may think this is impossible. However, these three criteria (selectivity, completeness and timing) may not be that absolute, for the following reasons.

Time of toxicity onset is not all that clear. Electrophysiological studies such as motor unit number estimations in asymptomatic carriers of SOD1 mutations do not show convincing abnormalities until shortly before clinical disease onset (Aggarwal and Nicholson, 2001), although the reliability of this technique is uncertain. In mutant SOD1 mice, abnormalities of axonal transport can be found as early as 4 weeks postnatally (Borchelt et al., 1998), but obviously this overexpression model may be misleading when translating its results to the two-allelic expression in humans. It is obvious that the more sensitive parameter one uses, the earlier the first abnormalities will be seen. Hopefully, however, this is not that relevant. It may well be that damage to motor neurons induced by mutant SOD1 is reversible up to a certain stage. In a mouse model for Huntington's disease, it has been shown that striatal neurons can recover from mutant huntingtin-induced damage (Yamamoto et al., 2000). Whether this is true for motor neurons can now be studied experimentally using the siRNA approach mentioned below.

To switch off the expression of the mutated SOD1 entirely may not be necessary. At least in mice, there is a correlation between the level of expression of the mutated protein, the onset of disease and its rate of progression. This suggests that a partial reduction of the expression of the mutated SOD1 may already result in a substantial clinical effect: disease onset may be postponed (maybe till after the average life span) and the course may be more benign. Again, one should be careful extrapolating this to humans, for the same reasons mentioned above, but it is certainly encouraging. It should be noted that the same reasoning is likely to apply to other neurodegenerative diseases: it has been estimated that postponing the onset of Alzheimer's disease by 10 years may decrease its prevalence by 50%.

Similarly, it may not be necessary to downregulate mutant SOD1 expression selectively. A decreased

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SOD1 level may be an acceptable side effect. SOD1 knock-out mice do not develop major problems, although they are not entirely normal (Reaume et al., 1996). Obviously, we do not know whether this will hold for humans.

Several methods to downregulate SOD1 protein expression have been designed, and others will become available as our understanding of the biology of gene regulation, translation and protein synthesis and degradation increases. The recent development of siRNA technology demonstrates the feasibility of this concept (Miller et al., 2005; Ralph et al., 2005; Raoul et al., 2005). SOD1 shRNA can be delivered to motor neurons through the intramuscular administration of a viral vector such as the rabies-G-pseudotyped equine infectious anemia viral vector (Ralph et al., 2005) or an adenovirus-associated viral vector (Miller et al., 2005). These vectors apparently are retrogradely transported to the cell body. Using intramuscularly administered lentiviral vectors, the human SOD1 transgene has been effectively silenced in the $SOD1^{G93A}$ mouse, a treatment which delayed disease onset and prolonged survival by more than 100 days, which is a remarkable result. It should be noted that the animals were injected on day 7.

Mutant SOD1 expression can also be downregulated using shRNA delivered to the cell using a lentiviral vector injected into the spinal cord (Raoul et al., 2005). This approach has the theoretical advantage that not only SOD1 in motor neurons but also in glial and microglial cells will be downregulated.

These siRNA-based experiments will undoubtedly provide important information about the reversibility of mutant SOD1 toxicity, its mechanism of action, etc. However, the question arises whether this approach is feasible in humans, apart from vector-related problems which will be mentioned later. If selective downregulation of the mutated protein is necessary, molecular tools need to be developed that are suited for only one individual with a particular mutation, or a small group of patients carrying the same mutation. This represents quite a challenge. If selective downregulation is not necessary (see above), then at least a larger population can be targeted. Still, even then, only mutant SOD1 patients would benefit. Even if this treatment would result in a substantial and obvious effect, a clinical trial investigating this treatment and its toxicity will require a substantial effort. If other mutated genes are responsible for even a smaller portion of FALS than SOD1, the problem grows even bigger.

Maybe there are alternative methods to influence SOD1 expression. Some readily available small compounds that can be orally administrated can affect gene expression. Of interest to the present discussion are sodium valproate which upregulates SMN2 expression and ceftriaxone that has been reported to upregulate EAAT2 expression (see below). A small compound that downregulates SOD1 gene expression may well be found. That such molecules are unlikely to discriminate between mutant and wild type protein may be less relevant, as discussed above.

The question arises whether downregulating a protein may also be applicable in SALS. The answer depends on the pathogenic mechanism of SALS. If this form of the disease is caused by the (posttranslationally induced) toxic effect of a protein, then this protein can be targeted as well. Familial and sporadic prion disease (Creutzfeld-Jakob disease) is an appealing example of this. If wild type SOD1 ($SOD1^{C}$) converts into a disease-causing SOD1 ($SOD1^{ALS}$) in one individual out of every 100,000 each year, then downregulating SOD1 may be a strategy. There currently is no evidence that this occurs.

19.2. Targeting pathogenic pathways

A large series of studies have reported the involvement of a variety of cellular pathways in the mechanism of motor neuron death induced by mutant SOD1 (Bruijn et al., 2004). An equally large series of studies has reported the positive effect of interfering with these pathways on disease onset or survival of mutant SOD1 animals. Table 19.1 lists the drugs studied and the effects observed when treatment is started before onset of clinical abnormalities. Survival studies using double transgenic mice obtained by crossbreeding mutant SOD1 mice were not included. It is likely that there exists an even longer list of drugs that were found to have no effect, as these negative results almost never find their way to publication.

Some of the therapies have resulted in a substantial benefit, but none has stopped the disease from progressing. This suggests that multiple pathways are involved in parallel. Very little is known about the effect of a combination of these drugs, in a cocktail, just like is done with chemotherapy. The few reports on the use of such combinations certainly are encouraging (Kriz et al., 2003; Zhang et al., 2003).

In this section we will discuss different therapeutic interventions aimed at suspected pathophysiological pathways in mutant SOD1-induced cell death. Some of these strategies are likely to be applicable to non-SOD1-associated motor neuron degeneration and maybe to non-familial ALS.

19.2.1. Aggregate formation

The observation that many neurodegenerative diseases are characterized by neuronal inclusions, and that many

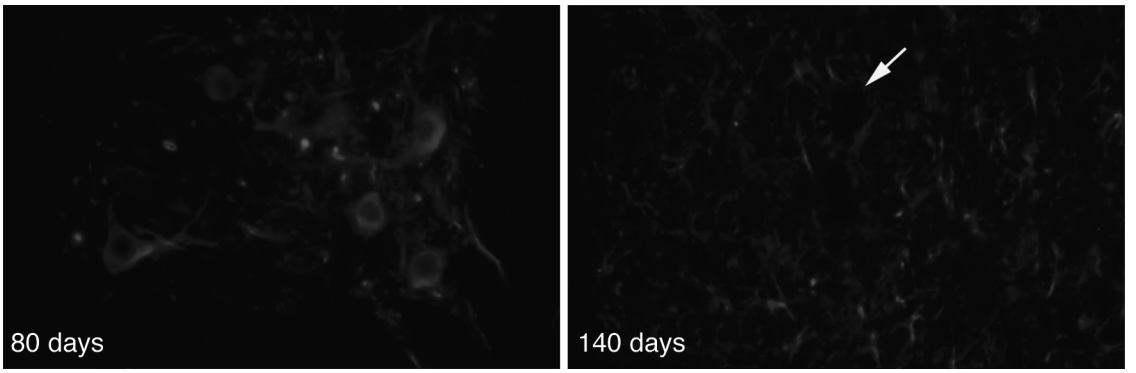

Fig. 19.1. For full color figure, see plate section. HSP27 immunoreactivity in spinal cord from presymptomatic (80 days of age; left panel) SOD1^{G93A} mice and symptomatic (140 days of age; right panel) mice. The protein is present in the motor neuron's cytosol in the early stages, but disappears from it later in the disease (arrow), while it is abundantly present in glial cells.

of the pathogenic mutated proteins have the tendency to form high molecular weight complexes, fibrils and aggregates, has generated the hypothesis that the toxic gain of function of these proteins resides in these (pre)aggregate complexes (Bruijn et al., 2004). The misfolding of the protein is a pivotal initiator for this, hence the name foldopathy. This is a highly interesting but hitherto unproven hypothesis that opens a window of opportunities. If one understands the physics, chemistry and biochemistry of this process, it may be interfered with. Understanding the reaction of the cell to such aberrant multimerization may identify therapeutic targets. One such example is already available. The cell reacts to the presence of misfolded proteins with a stress response which involves the upregulation of heat shock proteins, apparently as an effort to protect the cell. Motor neurons appear to be poor inducers of this defence line, which may (partially) explain the vulnerability of motor neurons in ALS (Batulan et al., 2003). Some heat shock proteins, although upregulated in glial cells, disappear from the cytosol of motor neurons (Vleminckx et al., 2002; Maatkamp et al., 2004) (Fig. 19.1). Providing motor neurons with HSP70 in vitro (Bruening et al., 1999) and treating mice with arimoclomol, a non-specific inducer of the heat shock response, has been reported to protect from mutant SOD1-induced motor neuron degeneration (Kieran et al., 2004).

Direct interference with the physical process of complex formation is an alternative option. In prion disease, considered to be a prion protein foldopathy, this has been considered (Brown, 2002). In ALS, this remains to be explored.

19.2.2. Mitochondrial dysfunction

Of special interest are those strategies that aim at what may be a common pathway in motor neuron degeneration. Mitochondrial dysfunction is one of them, as recent evidence suggests that mutant SOD1 aberrantly interacts with the mitochondrial membrane (Liu et al., 2004; Pasinelli et al., 2004; Vijayvergiya et al., 2005). Energy failure certainly is an interesting hypothesis to explain neurodegeneration, but interfering with it has proven not to be easy. The effect of creatine in mutant SOD1 mice has been suggested to be based upon restoring energy balance (Klivenyi et al., 1999; Zhang et al., 2003); in humans, no effect of creatine was found (Groeneveld et al., 2003; Shefner et al., 2004).

19.2.3. Cell death pathways

Another common pathway is the activation of the machinery of the cell to initiate death. Whether to call the latter apoptotic or not is not relevant to this discussion: suffice it to say that all evidence suggests that some kind of biochemically organized process leads to the death of the motor neuron in ALS. Inhibiting (part of) this cascade has been shown to affect the process of motor neuron degeneration, albeit temporarily so (for a review see Vila and Przedborski, 2003) and treatment of the mutant SOD1 mice with pharmacological agents that interfere with apoptosis has been shown to prolong survival (Table 19.1). It should be noted however that keeping the motor neuron from going through this final stage, the execution of death, may be insufficient to effectively influence a disease course determined by the dysfunction of motor neurons and their diseased axons.

19.2.4. Microglial activation

ALS spinal cord, both from humans and mutant SOD1 animals, is characterized by a marked proliferation of astroglial cells and the proliferation and/or infiltration of microglial cells (Henkel et al., 2004) (Fig. 19.2). Although neither the precise significance nor its

Table 19.1

Effect of drugs when administered before disease onset in an animal model. The therapeutic effect of gain in mean survival is represented in days and percentage compared to control

	Animal	Drug	Effect on onset: control (days) and the therapeutic gain (in days and %)*	Effect on survival: control (days) and the therapeutic gain (in days and %)	Reference
Anti-apoptosis	Mouse (G93A)	WHI-P131 (JAK3 inh)		134 + 66 (49.3%)	Trieu et al., 2000
	Mouse (G93A)	zVAD	103.5 + 20.2 (19.5%)	126.1 + 27.2 (21.6%)	Andreassen et al., 2000
	Mouse (G93A)	Rasagiline		211 + 29 (13.7%)	Waibel et al., 2004
Antioxidants	Mouse (G93A)	N-acetyl-L-cysteine		126.3 + 18.7 (14.8%)	Andreassen et al., 2000
	Mouse (G93A)	EUK-134		221 + 23 (10.4%)	Jung et al., 2001
	Mouse (G93A)	synthetic SOD catalase mimetic: EUK-8		221 + 17 (7.7%)	Jung et al., 2001
	Mouse (G93A)	Lipoic acid		128.2 + 7.7 (6.0%)	Andreassen et al., 2001
	Mouse (G93A)	Red wine		144 + 8 (5.6%)	Esposito et al., 2000
	Mouse (G93A)	Iron porphyrin		128.6 + 7.0 (5.4%)	Wu et al., 2003
	Mouse (G93A)	Ginseng	94 + 22 (23.4%)	132 + 7 (5.3%)	Jiang et al., 2000
	Mouse (G93A)	Co-enzyme Q10		135 + 6 (4.4%)	Matthews et al., 1998
	Mouse (G93A)	Carboxyfullerene		+ 9	Dugan et al., 1997
Cu^{2+} chelator	Mouse (G93A)	Trientine		126.7 + 10.2 (8.1%)	Andreassen et al., 2001
	Mouse (G93A)	d-penicillamine	124.6 + 10.1 (8.1%)	141.5+ 10.9 (7.7%)	Hottinger et al., 1997
	Mouse (G93A)	Trientine		123.6 + 6.5 (5.3%)	Nagano et al., 2003
Chemotherapy	Mouse (G93A)	Vincristine		117.8 + 14.2 (12.1%)	Bruce et al., 2004
	Mouse (G93A)	Cyclosporine A		130.7 + 10.3 (7.9%)	Kirkinezos et al., 2004
Anti-excitoxicity	Mouse (G93A)	Glutamate carboxypeptidase II inhibitor: 2-MPPA		190 + 29 (15.3%)	Ghadge et al., 2003
	Mouse (G93A)	AMPA rec antagonist: RPR 119990		158 + 20 (12.3%)	Canton et al., 2001
	Mouse (G93A)	Riluzole		127 + 12 (10.7%)	Gurney et al., 1998
	Mouse (G93A)	AMPA rec antagonist: NBQX	111.3 + 7 (6.4%)	129.9 + 13.4 (10.3%)	Van Damme et al., 2003b
	Mouse (G93A)	Antisense PNA to GluR3	118 + 11 (9.3%)	123 + 11 (9.4%)	Rembach et al., 2004
Growth factor	Mouse (G93A)	LV-VEGF	95 + 28 (29.5%)	125 + 38 (30.4%)	Azzouz et al., 2004
	Mouse (G93A)	AAV-IGF	91 + 31 (34.1%)	123 + 37 (30.1%)	Kaspar et al., 2003
	Mouse (G93A)	AAV-GDNF	101.3 + 12.7 (12.5%)	122.3 + 16.6 (13.6%)	Acsadi et al., 2002
	Rat (G93A)	VEGF	109 + 17 (15.6%)	121 + 22 (18.2%)	Storkebaum et al., 2005
	Mouse (G93A)	AVR-GDNF	108.2 + 7.9 (7.3%)	119.2 + 14.6 (12.2%)	Acsadi et al., 2002
	Mouse (G93A)	AAV-GDNF	91 + 16 (17.6%)	123 + 11 (8.9%)	Kaspar et al., 2003
	Mouse (G93A)	VEGF		132 + 11 (8.3%)	Zheng et al., 2004
	Mouse (G93A)	AAV-CT1	126 + 27 (21.4%)	172.6 + 13.3 (7.7%)	Bordet et al., 2001

Anti-inflammation	Mouse (G93A)	Celecoxib (COX2 inh)	+ 16	+ 28 (25%)	Drachman et al., 2002
	Mouse (G93A)	Rofecoxib (COX2 inh)		126 + 26 (20.6%)	Klivenyi et al., 2004
	Mouse (G93A)	Celecoxib (COX2 inh)		126 + 24 (19.0%)	Klivenyi et al., 2004
	Mouse (G93A)	Minocycline	107 + 18 (16.8%)	130.3 + 20.6 (15.8%)	Van Den Bosch et al., 2002
	Mouse (G93A)	Minocycline	94 + 9 (9.6%)	126 + 16 (12.7%)	Zhang et al., 2003
	Mouse (G93A)	Sulindac (COX2 inh)		123 + 12 (9.7%)	Kiaei et al., 2005a
	Mouse (G93A)	Minocycline	90.3 + 18.7 (20.7%)	125.6 + 11.2 (8.9%)	Zhu et al., 2002
	Mouse (G93A)	NDGA	115.9 + 5.1 (4.4%)	127.4 + 10.1 (7.9%)	Maatkamp et al., 2004
	Mouse (G37R)	Minocycline		343.6 + 21.8 (6.3%)	Kriz et al., 2002
Cell-based therapy	Mouse (G93A)	Umbilical cord blood cells	?	123.5 + 38.5 (31.1%)	Chen and Ende, 2000
	Mouse (G93A)	BM transplant		129 + 11 (8.5%)	Corti et al., 2004
	Mouse (G93A)	Umbilical cord blood cells		138 + 10 (7.2%)	Chen and Ende, 2000
Others	Mouse (G93A)	Lentiviral RNAi to SOD1	94 + 108.1 (115.0%)	129.3 + 98.5 (76.2%)	Ralph et al., 2005
	Mouse (G93A)	Arimoclomol		125 + 28 (22.4%)	Kieran et al., 2004
	Mouse (G93A)	Creatine		126 + 25 (19.8%)	Klivenyi et al., 2004
	Mouse (G93A)	Creatine		143.7 + 25.6 (17.8%)	Klivenyi et al., 1999
	Mouse (G93A)	PPAR agonist		123.8 + 11.1 (13.0%)	Kiaei et al., 2005a
	Mouse (G93A)	Creatine	94 + 7 (7.4%)	126 + 15 (11.9%)	Zhang et al., 2003
	Mouse (G93A)	β-Lactam antibiotic		122 + 13 (10.7%)	Rothstein et al., 2005
	Mouse (G93A)	5-hydroxytryptophan		142.5 + 13.8 (10.7%)	Turner et al., 2003
	Mouse (G93A)	Antisense PNA to p75NTR	117 + 11.2 (9.6%)	132.3 + 11 (8.3%)	Turner et al., 2003
	Mouse (G93A)	Valproic acid		270.5 + 20.5 (7.6%)	Sugai et al., 2004
	Mouse (G93A)	Cannabinoid		125.9 + 6.4 (5.1%)	Liu et al., 2004
Combination therapy	Mouse (G93A)	Rofecoxib and creatine		126 + 39 (31.0%)	Klivenyi et al., 2004
	Mouse (G93A)	Celecoxib and creatine		126 + 36 (28.6%)	Klivenyi et al., 2004
	Mouse (G93A)	Minocycline and creatine	94 + 18 (19.1%)	126 + 31 (24.6%)	Zhang et al., 2003
	Mouse (G93A)	Rasagiline and riluzole		211 + 41 (19.4%)	Waibel et al., 2004
	Mouse (G37R)	Minocycline, riluzole and nimodipine	317.8 + 29.4 (9.3%)	336.0 + 42.7 (12.7%)	Kriz et al., 2003
	Mouse (G93A)	Trientine and ascorbate		123.6 + 8.3 (6.7%)	Nagano et al., 2003

LV: Lentivirus; AAV: Adeno-associated-virus; AVR: Adenovirus; BM: Bone marrow.

* Only publications in which effect on disease onset was specifically mentioned are included. Excluded were those publications where motor onset or different parameters for disease onset were pointed out.

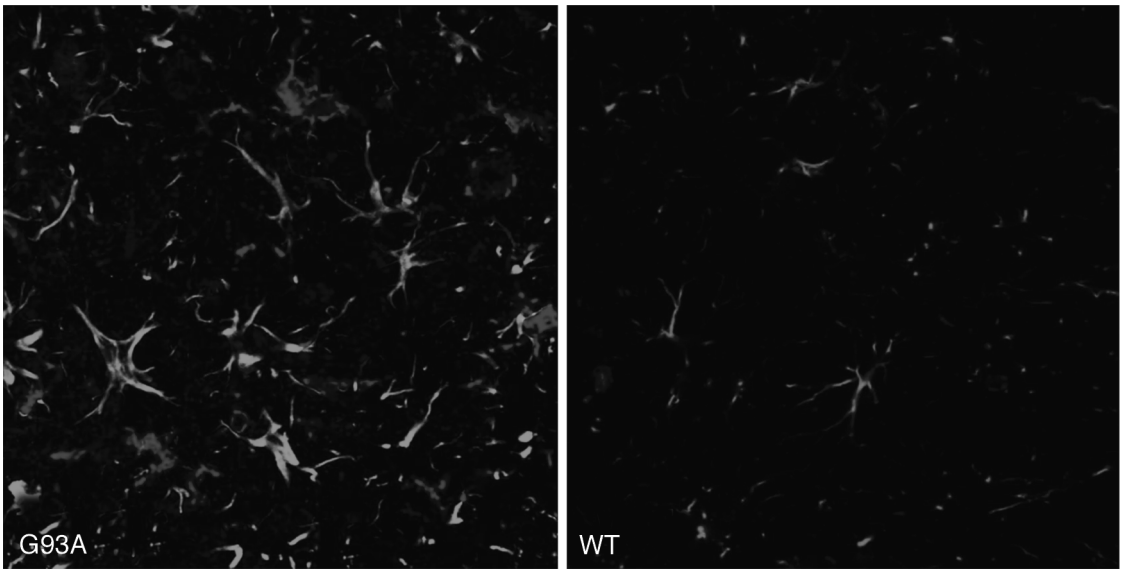

Fig. 19.2. For full color figure, see plate section. The spinal cord from early symptomatic SOD1^{G93A} mice (left panel) is characterized by microglial (blue; stained for activated p38 MAP kinase) and astroglial (green; stained for GFAP) proliferation. Spinal cord tissue from age-matched SOD1^{WT} mice is shown on the right.

mechanism have been elucidated, evidence suggests that at least the microglial cells contribute to the motor neuron degeneration (Zhao et al., 2004). Recent evidence even suggests that the pathogenic effect may come from microglia that express mutant SOD1, rather than from microglia that do not (Weydt et al., 2004).

It therefore seems to be a reasonable strategy to counteract this response. Minocycline has been shown by three independent groups to positively affect the disease course of mutant SOD1 mice (Table 19.1, Kriz et al., 2002; Van Den Bosch et al., 2002; Zhu et al., 2002) and is thought to exert this beneficial effect through inhibition of microglial activation (Kriz et al., 2002). It is unlikely to work through its inhibitory effect on gelatinase B/metalloprotease-9 (Dewil et al., 2005), although in vitro studies have suggested the latter to be a candidate to mediate the deleterious effect of microglia. Molecular tools to abort microglial proliferation/infiltration become available and will need to be tested in an ALS model.

If it is the mutant SOD1 expressing microglial cells that play a pathogenic role, then replacing them by 'normal' microglial cells may influence the disease. Evidence from experiments using bone marrow transplantation suggests it does, and will be discussed below.

This microglial proliferation is accompanied by the upregulation of a variety of other pro-inflammatory molecular cascades, of which one is the prostaglandin pathway. A marked upregulation of cycloxygenase-2 (COX-2) has been observed in neuronal and glial cells of SOD1^{G93A} mice, and was confirmed to be present in human ALS spinal cord (Almer et al., 2001; Yasojima et al., 2001; McGeer and McGeer, 2002; Maihofner et al., 2003; Okuno et al., 2004; Kiaei et al., 2005b). In an in vitro model, COX-2 inhibition was found to protect motor neurons (Drachman and Rothstein, 2000; Bilak et al., 2004). Furthermore, the treatment of SOD1^{G93A} mice with COX-2 inhibitors affected onset and progression of motor neuron degeneration in this animal model (Pompl et al., 2003; Klivenyi et al., 2004; Azari et al., 2005). There are not that many pathophysiological elements which received as much support from various research groups as the one on COX-2 upregulation. Still, and disappointingly so, a trial of celecoxib, a COX-2 inhibitor, in human SALS was announced to be negative.

19.2.5. Excitotoxicity

There is overwhelming evidence that *excitotoxicity* contributes to motor neuron death in ALS (reviewed in Van Damme et al., 2003a). The beneficial albeit limited effect of AMPA antagonists in mutant SOD1 mice is one of the stronger arguments (Table 19.1) (Canton et al., 2001; Van Damme et al., 2003b). Excitotoxicity to motor neurons is mediated by Ca^{2+} entering the motor neuron's cytosol through Ca^{2+}-permeable AMPA receptors. Motor neurons express a large number of these because of the limited expression of the GluR2 subunit in these cells (Van Damme et al., 2002). This GluR2 subunit determines the Ca^{2+} permeability of the

receptor complex. Interestingly, glial cells appear to be important regulators of the motor neuron's GluR2 expression, and mutant SOD1 appears to negatively affect this phenomenon (Van Damme, unpublished results). The crucial role of GluR2 in excitotoxicity to motor neurons is demonstrated by the fact that upregulating GluR2 attenuates (Tateno et al., 2004) and downregulating GluR2 enhances (Van Damme et al., 2005) motor neuron degeneration in mutant SOD1 animals. Understanding the molecular and cellular regulatory mechanisms of GluR2 expression may open therapeutic opportunities.

The loss of the glutamate scavenging transporter protein EAAT2 from glial cells may be the direct reason for glutamate to become toxic in ALS (Rothstein et al., 1992, 1995). Increasing EAAT2 levels by crossing mutant SOD1 mice with EAAT2 overexpressing mice has yielded rather disappointing results (Guo et al., 2003). Most interestingly, however, a recent report suggests that upregulating EAAT2 expression by treatment of mice with ceftriaxone positively affects the motor neuron degeneration in mutant SOD1 mice (Rothstein et al., 2005). Ceftriaxone was identified in a large scale high-throughput screening system, and reported to be able to upregulate EAAT2 expression. Treatment of mice with this antibiotic delayed onset and increased their survival. A trial in human ALS has been initiated.

19.2.6. Influencing modifying genes: SMN2

A low copy number of the SMN2 gene has been suggested to play a role in ALS (Veldink et al., 2001), although the findings have been somewhat contradictory (Gamez et al., 2002). Increasing SMN2 expression may thus be a therapeutic option. Sodium valproate (depakine) is able to increase the expression of SMN2, most probably through its inhibitory effect on histone deacetylase activity (Sumner et al., 2003). Treatment of mutant SOD1 mice with depakine indeed delays disease onset and increases the life span of these animals (Sugai et al., 2004). Obviously, as the effect of depakine on gene expression is likely to be rather non-specific, it is uncertain whether this beneficial effect is mediated through SMN2. A trial in human ALS has started.

19.3. Targeting the diseased neuron in general

A different set of strategies aims to provide neurotrophic support for diseased neurons or to populate the spinal cord with normal cells. The latter may be intended to replace the dying neurons or to provide the sick neurons with a normal and supportive environment. These approaches have the advantage of being independent of the etiology and pathophysiology of the motor neuron degeneration and thus may be applicable to all forms of ALS.

19.3.1. Neurotrophic factors

The use of neurotrophic factors has turned out to be disappointing. Clinical trials found IGF-1, CNTF, BDNF and others to be ineffective in human ALS. A variety of reasons for these negative results can be brought up. Lack of a biological rationale or the lack of knowledge of the bioavailability of growth factors, their pharmacokinetics and pharmacodynamics are some of the obvious ones. Recent studies, however, have indicated that growth factors continue to be an interesting therapeutic strategy, on condition they are efficiently targeted to the motor neuron. Delivery of the IGF-1 or GDNF gene using an adenovirus associated viral (AAV) vector attenuated motor neuron degeneration in mutant SOD1 mice (Acsadi et al., 2002; Manabe et al., 2002; Batulan et al., 2003; Kaspar et al., 2003; Guillot et al., 2004). It is thought that the vectors found their way to the motor neuron cell body through retrograde transport after intramuscular delivery. In one study, the beneficial effect in the mutant SOD1 mouse was among the largest seen (Kaspar et al., 2003). A viral vector strategy was also used by Azzouz et al. (2004), who delivered VEGF using a lentiviral vector, again intramuscularly injected. The effect on mutant SOD1 mouse survival was equally large (Table 19.1). The use of VEGF in ALS has a strong biological rationale. Mice in which the hypoxia response element (HRE) of the VEGF promotor has been deleted develop progressive motor neuron degeneration (Oosthuyse et al., 2001). In addition, polymorphisms in the VEGF promotor that are associated with low levels of VEGF are found more often in ALS patients than in controls (Lambrechts et al., 2003) and VEGF in the serum and CSF of ALS patients has been reported to be lower than in controls (Devos et al., 2004). The use of viral vectors has several disadvantages as we will discuss later. To avoid these, Storkebaum et al. (2005) studied the intracerebroventricular administration of recombinant VEGF. This was done in mutant SOD1 rats because of the mechanical advantage of this larger model. In the rat, mutant SOD1 induces a very aggressive form of motor neuron disease that can start in the cervical or lumbosacral region (in mice it always starts in the hindlimbs), and the former form is particularly severe. VEGF prolonged survival of the rats and modified the disease expression, in that almost no cervical onset was observed in the treated animals. How VEGF affects motor neuron degeneration is not clear: it has direct effects on motor neurons in vivo (Lambrechts et al., 2003) and in vitro

(Van Den Bosch et al., 2004), but a vascular component in its therapeutic action cannot yet be excluded.

The use of virally delivered and intracerebroventricularly administrated IGF-1, GDNF and VEGF is being studied for use in human patients.

19.3.2. (Stem) cell therapy

The use of stem cells to treat neurodegenerative diseases certainly has attracted major attention. In ALS, as in other neurological conditions, this approach is only in the first stages of development. Cells can be used for a variety of purposes. The most attractive, but maybe least realistic aim is to replace the neurons lost with cells that have the potency to differentiate into motor neurons. Alternatively, normal cells may provide a better environment for the diseased ventral horn. Finally, transfected cells can be used as carriers to bring gene products close to motor neurons in the spinal cord.

Embryonic stem cells can be differentiated into motor neuron-like cells in vitro (Wichterle et al., 2002; Miles et al., 2004). They acquire molecular, morphological and electrophysiological properties of motor neurons by treating them with substances such as retinoic acid and activators of the sonic hedgehog pathway. In vitro, these cells extend long axons and induce contractions of cocultured myoblasts through innervation (Harper et al., 2004). When transplanted into the chick spinal cord, such cells extend axons into the ventral root and form neuromuscular junctions (Wichterle et al., 2002). In an acute motor neuron death model (Sindbis virus infection) in the adult rat, embryonic stem cell-derived motor neurons survive and some of these cells form axons down into the ventral root on condition the inhibitory effects of myelin are overcome (Harper et al., 2004). It is unclear whether these cells also find their target tissue, the muscle. If they do, they would also need to be integrated into the spinal neuronal circuitry and acquire connections from descending tracts. These connections also need to be wired correctly, so that the cerebral command results in the contraction of the proper muscles. All this seems far fetched, but may not be impossible. The newly developed motor neurons may attract these descending axons just like they do during development, and there may be sufficient plasticity in the neural system for new connections to become functionally useful.

For the near future, it is maybe more realistic to consider stem cell therapy for a different purpose. Several experimental data suggest that motor neuron death in ALS is not cell autonomous. Cells other than motor neurons may contribute to it, in both ways: a bad environment contributes to motor neuron death, while a good environment may attenuate it. Experiments using chimeric mice which have partly wild type cells, and partly mutant SOD1 cells, and especially mice in which these cell types were mainly found on one side of the ventral spinal cord, have shown that wild type cells can keep the mutant SOD1 expressing motor neurons healthy and alive, while the mutant SOD1 motor neurons can induce degenerative changes in neighboring wild type cells (Clement et al., 2003). This of course suggests that 'normalizing' the ventral horn environment in ALS may be of benefit, and that the cells used for that purpose do not need to really replace the motor neurons. Maybe it is even better to use a non-differentiated cell, to enhance settlement and survival in the ventral horn. In some way there already is experimental evidence for this. Bone marrow transplantation has been reported to prolong survival of mutant SOD1 mice, and infiltration of the transplanted cells in the host spinal cord has been described (Corti et al., 2004). These cells had microglial characteristics, which is understandable given their hematogenous origin. Whether the clinical benefit observed is due to the effect of these (few) cells remains to be seen.

Stem cells can be generated from a variety of sources. Embryonic stem cells have been studied the best, but a variety of practical, biological and ethical factors make them maybe less likely to be used for treatment purposes. One of the drawbacks is their tendency to proliferate and form tumors. Multipotent progenitor cells can also be found in adult tissues, the most interesting one being the bone marrow. This cell type can be differentiated into neural cells and does not have the tendency to form tumors. Research on the use of these so called multipotent adult progenitor cells has only just started (Jiang et al., 2002; Zhao et al., 2002).

Research on the use of (stem) cells in neurodegenerative disorders in general and in ALS in particular is still in its infancy. Many more data on efficacy and safety, route or site of administration and quantity, need for immunosuppression, etc., should be obtained before even considering this an option for ALS patients.

19.4. Concluding remarks: Basic research and clinical relevance

When considering these treatment options for the near and far future, a number of issues need to be looked at critically. We will focus on the importance and limitations of the pharmacological studies in mutant SOD1 mice and of the potential use of viral vectors.

It is often mentioned that the use of the mutant SOD1 mouse has not been very reliable in terms of its predictive value for human studies. Creatine, celecoxib and others had an effect in mice, while they did not affect the disease in humans. Some researchers therefore do

not advocate their use. Several elements have to be considered.

Mouse trials are not always done using the same rigorous criteria as used in human trials, in terms of statistical methods used, number of mice studied and endpoint definition. These rigorous criteria should be adopted in order to avoid false positive (and false negative) results. In addition, a positive mouse trial should be replicated in another, independent study, as is required in medicine in general. There certainly are mouse trials not confirming previous results, which never make it into peer-reviewed journals, for obvious reasons. This could be overcome by creating a peer-reviewed website reporting these data in a short but clear format.

For very few if any of the compounds used in mouse trials, adequate information is available on dosage, kinetics, pharmacodynamics, etc. The extrapolations made for the human trials may be inadequate.

Most importantly, however, it should be noticed that in most mouse trials treatment is begun long before disease onset: for some of them mice were only 7 days old when the treatment was initiated. Patients with ALS enter trials 6 months to even 5 years after disease onset. In some studies, a cohort of mice is also treated at 'disease onset.' The effect of the compound of interest in these cases has always been clearly smaller, as can be seen in Table 19.2. Even in these studies "disease onset" often has a statistical meaning and certainly does not appear to mean the onset of clinical weakness. These compounds are therefore unlikely to affect the disease in mice if started after onset of weakness. Most of the trials in Tables 19.1 and 19.2 are therefore to be considered as proof of principle studies, rather than to provide preclinical information.

The use of viral vectors raises various issues. First, the safety of chronic use of these vectors needs more study. It is unknown whether humans develop an immune response to them. Second, the intramuscular administration needed to reach the motor neuron is poorly studied. It needs to be investigated how many muscles need to be injected to reach a critical portion of the motor neuron pool. Third, expression of the gene contained in the viral vector is currently difficult to control and is to be regarded an all or nothing phenomenon. That means that, if side effects occur, there is currently no way of shutting the expression of the transgene off. If the gene product has a narrow therapeutic window, this could present a major obstacle. Fourth, there also is the issue of making viral vector technology available to a large group of patients. The preparation of large amounts of these vectors will represent safety issues, among which there is the risk of mutation into a pathogenic vehicle, and will undoubtedly generate cost-benefit discussions for both the private sector and for health authorities.

The attractiveness of some of the ideas, such as the use of stem cells, has generated a lot of interest from both clinicians and lay people and, sad to say, has also induced a lot of unrealistic hope. In spite of the pressure of these desperately ill patients, scientists and clinicians should continue to maintain the principle that the best way to help ALS patients is to use rigorous scientific methods to provide answers to rigorous scientific questions.

Table 19.2

Effect of drugs when administered at disease onset in an animal model. The therapeutic effect of gain in mean survival is represented in days and percentage compared to control

Animal	Drug	Effect on mean survival (unless indicated otherwise): control (days) and the therapeutic gain (in days and %)	Reference
Mouse (G93A)	Arimoclomol	125 + 23 (18.4%)	Kieran et al., 2004
Mouse (G93A)	LV-VEGF	127 + 19 (15.0%)	Azzouz et al., 2004
Rat (G93A)	VEGF	118 + 10 (8.5%)	Storkebaum et al., 2005
Mouse (G93A)	β-Lactam antibiotic	122 + 10 (8.2%)	Rothstein et al., 2005
Mouse (G93A)	Cyclosporine A	11.8 + 12.4 *	Keep et al., 2001
Mouse (G93A)	Iron porphyrin	16.7 + 9.1 *	Wu et al., 2003

* Effect in mean survival interval starting from disease onset to endpoint.

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Chapter 20

Symptomatic therapy and palliative aspects of clinical care

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20.1. Introduction

While amyotrophic lateral sclerosis (ALS) is a progressive and terminal illness in most cases, aggressive symptomatic therapies can have a positive impact on the illness, prolong life and help maintain quality of life. In this chapter we will outline our philosophy for presenting the diagnosis to patients, treating various symptoms and discussing the timing and institution of palliative care.

20.1.1. Effects of the disease on physical and psychosocial domains

ALS is characterized by progressive upper motor neuron (UMN) and lower motor neuron (LMN) degeneration that affect the brainstem, cervical, thoracic and lumbosacral myotomes. The symptoms depend on the distribution of the involved motor neurons. UMN disease, which is the initial symptom in only a minority of patients (1.3 to 6%), leads to spasticity, slowed movements and mild weakness (Caroscio et al., 1987; Li et al., 1990; Rowland, 1998). Spasticity can be disabling; balance may be impaired due to stiffness and diminished reaction to postural shifts (Nardone et al., 2001) and the gait may be labored. Slowed hand movements can reduce dexterity. Emotional lability or pseudobulbar affect due to loss of normal inhibition of laughter and crying, complex acts that are thought to depend on neural pathways involved in emotion, respiration, vocalization and facial movements, may also be associated with UMN degeneration (Haymaker and Hartwif, 1988).

Symptoms due to LMN degeneration, including weakness, atrophy, cramps and fasciculation, often overshadow those associated with UMN disease. Weakness is the initial symptom in 58 to 63% of patients (Jokelainen, 1977; Gubbay et al., 1985), beginning asymmetrically in the limbs in approximately 2/3 of patients, and in the bulbar region in about 1/3 (Jokelainen, 1977; Gubbay et al., 1985; Li et al., 1990; Traynor et al., 2000). With onset in the arms, the weakness begins in a few myotomes, leading to difficulty performing tasks such as dressing, hygiene and eating. Weakness in the hand causes loss of finger dexterity and trouble picking up small objects, writing, turning keys and fastening buttons. Weakness of proximal arm muscles may lead to poor function due to the dependence on elbow and shoulder flexion for lifting, carrying and reaching. Initially, patients may have difficulty carrying heavy items, such as shopping bags or pots and pans. Eventually, function is lost all together, leading to dependence on caregivers. Joint immobility may result in painful contractures, most often in the fingers and the shoulders.

Weakness and atrophy in the legs usually begins in the distal muscles, especially the dorsiflexors of the ankle; tripping over the toes and foot-drop are frequent symptoms. While weakness in the legs may start unilaterally, it occurs in both legs simultaneously 13–20% of the time (Gubbay et al., 1985; Caroscio et al., 1987). As the weakness ascends, difficulty going up stairs, stepping over curbs or arising from chairs ensues. Falls and dependent edema are common. Eventually, walking, standing and bearing weight for transfers become impossible. Weakness of the axial musculature leads to head drop and kyphosis, which may cause pain, imbalance due to change in the center of gravity and problems with activities such as eating and driving that require an upright posture.

ALS is usually described as causing painless weakness, but pain can be a surprisingly common symptom. Pain can result from loss of mobility and the inability to

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turn in bed, joint contractures or bedsores. Occasionally pain appears without another explanation and may be a feature of the disease itself. Cramps can also be painful and may interfere with sleep or physical activity and limb fasciculation is annoying to some patients.

In bulbar onset ALS, dysarthria generally develops prior to dysphagia, with both UMN and LMN degeneration contributing to symptoms. UMN speech tends to be slow and effortful, with poor enunciation and a harsh, strained quality to the voice. LMN loss in the brainstem may cause slow and effortful speech, with breathiness of the voice and slurring of consonants (Hillel and Miller, 1989). In some, velopharyngeal incompetence causes air leakage through the nose and lends a hypernasal quality to the voice. With time, some patients become anarthric. Swallowing problems may be an early bulbar symptom and worsen with disease progression. Drooling, dehydration, malnutrition with weight loss and aspiration are all associated with dysphagia.

Shortness of breath or other respiratory symptoms usually occur later in the disease course. Symptoms of respiratory insufficiency include orthopnea, morning headaches and weakened cough. Patients develop dyspnea on exertion, with breathlessness during strenuous and then minor tasks such as eating or talking. Eventually shortness of breath occurs during periods of quiescence. Stridor, or paroxysmal laryngeal spasm, especially with coughing, is due to paradoxical adduction of the vocal cords leading to glottal narrowing. Respiratory failure and pulmonary complications of bulbar paralysis (i.e. aspiration pneumonia) are the most common causes of death in ALS.

Traditionally, cognitive function was considered to be normal in ALS. While overt dementia remains uncommon, recent studies using formal neuropsychological testing have revealed deficits in frontal executive function in as many as 50% of ALS patients (Massman et al., 1996; Lomen-Hoerth et al., 2003). The cognitive abnormalities may lead to changes in personality, language, judgment, decision-making and affect. The associated abulia and reduced judgment may render patients less able to participate in decisions about their medical treatments.

Depression and anxiety can be prominent features at all stages of the disease, from the time of diagnoses, to periods of concern over loss of independence, to the final stages as death approaches. Anxiety may accompany symptoms of respiratory insufficiency and depression may lead to reduced appetite, poor sleep, hopelessness and impaired decision-making ability.

While ALS is an incurable disease, many symptoms are amenable to supportive and adjunctive therapies, some of which may even improve the disease course. Unfortunately, there are few controlled trials of symptom management. As a result, the selection of therapies is still based largely upon physician experience and anecdotal reports. While neurologists are increasingly turning to published practice parameter guidelines, the lack of scientific data to guide use of many therapies has led to a wide variety of management practice (Forshew and Bromberg, 2003), and more controlled trials are needed.

20.1.2. The principles of treatment and care

20.1.2.1. Philosophy

The care of patients with ALS is especially challenging and differs from that of other disorders because ALS is terminal, its course is short and there are as yet no truly effective treatments (Mitsumoto et al., 2005a). The philosophy of care is therefore based on a holistic approach that respects patients' wishes, attempts to bring meaning and hope to circumstances that may make no sense, provides symptomatic support in the face of relentless physical disintegration and gives patients a sense of independence and dignity to the greatest extent possible (Thompson, 1990). This daunting task requires special skills and experience (Norris et al., 1985; Leigh, 1999; Mitsumoto and Del Bene, 2000; Mitsumoto et al., 2005b); a clinical interest in ALS and a commitment to treating patients with ALS are especially helpful.

Many ALS centers use a multidisciplinary approach to care. Patients are evaluated frequently so that impending problems are detected and treated aggressively by specialists. The overall care plan is centered on the patient's decisions, with the focus being respect, support and education for patients and their families. The neurologist and allied health team provide information for the patient and family to help ensure they understand the issues involved in treatment decisions; discussions include advanced directives related to nutritional and respiratory care (Mitsumoto et al., 2005b). With the advent of information technology and easy access to the Internet, the neurologist acts to contextualize and explain clinical and scientific advances in the field. The ALS team also directs patients and their families to services and support structures beyond the traditional confines of medical practice (Mitsumoto et al., 1998), ensuring that home care is used effectively and that palliative and hospice care are provided during the final stages. Until stronger neuroprotective agents are identified, the goal of ALS care is to help patients to reach the fullest physical, psychological and social potential possible, aiming to achieve the optimal quality of life throughout the course of the disease.

20.1.2.2. Practice parameters

Evidence-based medicine has begun to impact the field of neurology and neuromuscular diseases. In 1999, an American Academy of Neurology (AAN)-appointed task force published evidence-based practice parameters for the management of ALS (Miller et al., 1999a). The multidisciplinary task force included consultants in patient advocacy, ethics, practice parameter development and library research. Together, they reviewed the medical literature for human studies on ALS and ranked each paper based on its strength of evidence: randomized, controlled clinical trials (class I, resulting in standard of care recommendations), case-control and cohort studies (class II, resulting in guideline recommendations) and expert opinion, case series, case reports and studies with historical controls (class III, resulting in optional recommendations). Management recommendations were developed as either guidelines or options for methods to inform patients of the diagnosis, treat symptoms, manage nutritional and respiratory problems and institute palliative care. Two guidelines that have received particular attention address percutaneous endoscopic gastrostomy (PEG) and non-invasive positive pressure ventilation (NIPPV). In order to reduce the risk of undergoing a procedure, the parameter recommends that patients who develop dysphagia should receive PEG soon after symptom onset and before the forced vital capacity (FVC) falls below 50% of predicted. The parameter also recommends NIPPV for patients who have symptoms of respiratory insufficiency and whose FVC is less than 50% of predicted (Aboussouan et al., 1997; Kleopa et al., 1999; Miller et al., 1999a). Analyses of the impact of the parameters on patient care have been published (Bradley et al., 2001); under-utilization of PEG, NIPPV and symptomatic therapies were identified.

20.1.2.3. ALS care database

The ALS CARE database, funded by a pharmaceutical company manufacturing riluzole and the ALS Association, was developed to track outcomes and help standardize treatment for patients with ALS (Miller et al., 2001). Neurologists practicing in North America are eligible to contribute to the database and are encouraged to enroll all of their ALS patients. Data is supplied by neurologists, patients and caregivers, and includes information on diagnosis, prognostic factors, natural history and therapeutic interventions. Data management is based at the Center for Outcomes Research at the University of Massachusetts Medical School in Worcester, MA. The Center manages the data, performs analyses and protects the confidentiality of participants, including patients, neurologists and clinical sites.

Participating clinicians receive reports about their patients and how their outcomes compare with the aggregate of all patients in the database. They receive feedback about the overall level of satisfaction of their patients, about the education and information given to their patients and about the socio-economic and psychosocial impact of the disease on both patients and caregivers. The comparison of individual practice patterns to all neurologists in North America may help to standardize care.

Since its inception in 1996, more than 5,600 patients have been enrolled in the database by 387 neurologists at 107 clinical sites (Miller et al., 2005). Approximately two-thirds of the sites are affiliated with academic medical centers and over 90% of patients have been enrolled at academic centers. Eighty-nine percent of patients are either satisfied or extremely satisfied with their overall level of care. Ninety-six percent of patients were given the diagnosis in person, matching the recommendations of the AAN practice parameter, and, in 78% of interviews, the people who were considered most important to the patient were present when the diagnosis was given. Seventy percent of patients reported that the ALS clinic is their most valuable source of information compared with 23% for the ALS Association and 8% for Muscular Dystrophy Association (MDA).

Based on patient self-reports, symptomatic therapies for constipation, cramps, depression, disturbed sleep, spasticity and sialorrhea are generally effective, but a minority of patients received therapy for symptoms, suggesting that additional patients might benefit from symptomatic pharmacotherapy. ALS-specific treatments included riluzole (52%), high-dose vitamins or antioxidants (48%), non-traditional medications (15%) or other unspecified medications (42%). Over the past 7 years, utilization of riluzole has increased from 45% in 1998 to 61% in 2002. Just 26% of patients were enrolled in a formal ALS clinical trial.

The proportion of patients who received PEG when the FVC was less than 50% of predicted rose from 12 to 22% following publication of the practice parameter. Approximately 43% of patients for whom a gastrostomy was recommended had it inserted. Of those who did not accept the recommendation, 31% thought their swallowing was adequate, 16% reported not liking the concept of PEG, 4% received insufficient information to make a decision and 20% recorded a series of other reasons. In a study utilizing the same data, there was a strong correlation between PEG use and declining ALSFRS scores ($p < 0.0001$) (Mitsumoto et al., 2003). A positive impact of PEG was documented in 79% of patients overall, but in only 37% of patients who received PEG later in their disease course, suggesting that PEG may still be performed too late in many instances.

The proportion of patients with FVC less than 40% of predicted who received NIPPV rose from 9 to 21% following practice parameter publication (Bradley et al., 2001). Nearly 50% of patients not using NIPPV had tried it but could not adapt to its use. The data, albeit retrospective, show a positive impact of NIPPV on 5 year survival.

Sixty-four percent of patients died at home and 63% of these had used home hospice services. Eighty-nine percent of patients died peacefully, although 38% were said to have had some breathing difficulties at the end of life. Pain medications were administered to 80% of patients and oxygen was given to 25% during the terminal phase.

The data also document the stress and emotional burden of the disease upon the caregiver, who appears to experience increasing distress as the disease progresses. Nevertheless, at enrollment, 93% of caregivers reported that the opportunity to participate in the care of a loved one provided a measure of satisfaction and 92% of caregivers thought they were doing something important by caring for the patient. Since ALS is more common in men, wives are more likely to serve as caregivers, and they found care giving to be a greater burden (Coker et al., 2002).

There are some limitations to interpreting data contained in the ALS CARE database. These limitations, inherent to uncontrolled submission of data, include dissimilar demographics across treatment groups, meaning that comparison of outcomes may lead to inappropriate conclusions; selection bias because some clinics may not enroll all of their patients into the database; uncertainty as to whether enrolled patients are representative of the ALS patient population at large, and whether the conclusions are therefore generalizable; lack of means to confirm the diagnoses except in a small percentage of cases; and cross-sectional as opposed to longitudinal data acquisition.

Even with these limitations, however, analyses from large numbers of patients in the database suggest that the diagnosis of ALS is for the most part delivered in accord with the evidence-based recommendations and that treatment of important symptoms such as sialorrhea and pseudobulbar affect are effective. The data illustrate the need for increased utilization of effective symptomatic therapies, including PEG and NIPPV. A patient-driven web-based version of ALS CARE is planned that could provide more information about the standard of care outside academic centers than is currently the case.

20.2. Breaking the news

20.2.1. Presenting the diagnosis and how to convey hope

Giving a patient the diagnosis of ALS is a daunting task for the physician (Miller et al., 1999a) and of vivid importance for the patient and family (Silani and Borasio, 1999). Honesty and understanding from the neurologist help patients as they confront this initial hurdle and help cultivate the patient–doctor trust that will be necessary throughout the course of the illness. A tone of candor and hope should be set from the moment the neurologist meets with the patient and family to break the news. If not done appropriately, the effect can be devastating, leaving the patient with a sense of abandonment and destroying the patient–physician relationship.

Once the diagnosis is established, ten precepts serve as a foundation from which to present the diagnosis (Chad, 2005). First, allow adequate time for the task (McCluskey et al., 2004). The process of breaking the news may require 45 minutes or longer and should be followed by a near-term revisit for further discussion. Second, because the information is profound and life altering in its implications, the patient should not hear the news alone. If at all possible, a relative, friend or some representative of the patient's support network should be in attendance (Miller et al., 1999a). Third, the meeting room should be comfortable and quiet, and the neurologist should sit at a desk next to the patient, making eye contact with the patient and companion (Miller et al., 1999a). These simple gestures give the patient a sense of dignity and knowledge that the physician takes the problem seriously. Fourth, the neurologist should ascertain what patients know before informing them of the diagnosis (Chad, 2005). Many patients will already have the sense that their problem is a serious one; some will already know that ALS is a diagnostic possibility, or they may recognize that their problem is serious but have not yet become familiar with ALS, believing they may have some other neurological condition for which there are effective ameliorating treatments. Fifth, the neurologist should provide a preliminary warning that bad news is coming with words to the effect: "I have reviewed all the findings and I believe the problem is a serious one" (Chad, 2005). Sixth, it is best to give the diagnosis in a direct, empathetic and caring way, using simple language free of medical jargon, calling the disease by its widely known name (ALS), noting that it is a progressive disorder, with a variable prognosis (Borasio et al., 1998). Seventh, attend to the patient's verbal and nonverbal responses and remain sensitive to how much information patients want to learn about the disease, giving the news at their pace and allowing them to dictate what they are told (Miller et al., 1999a). Eighth, the neurologist should provide reassurance that he and the ALS team are an integral part of the patient's support system and will do whatever is possible to help maintain muscle function and to manage complications that arise

during the course of the illness (Mitsumoto and Del Bene, 2000). The neurologist might indicate that the treatment team "is here" to ensure the patient's well being, that the patient's wishes will be respected at all times and that the patient will never be abandoned (Miller et al., 1999a). Ninth, the neurologist should consider summarizing the main points of the discussion verbally or in writing and propose at least a preliminary plan of care before the patient leaves the office. The neurologist should let the patient know his plans for a revisit both in the near term and longer term for reassessment. It is also important to inform patients about ALS resources, such as the MDA and the ALS Association, and to let patients know of available therapeutic trials. The plan of care fosters the physician–patient bond while permitting patients and their caregivers to cope and to plan appropriately for the future (McCluskey et al., 2004). Last, the neurologist should support the patients' desire to seek a second opinion, which can help patients and family members know that the physician's mind is not closed to other possibilities (Bradley, 1994).

20.2.2. Research

Part of conveying hope to patients includes a discussion of ongoing research into better treatments. Discussions about research may begin during the time of initial diagnosis as part of the treatment plan. The neurologist may explain that although there is no cure at present, research is very active around the world with scientists making progress in understanding the disease better. Telling the patient that one day soon, a very bright researcher is going to unravel the mysterious biology of ALS, and that more effective treatments will follow, can provide hope (Rowland, 1997).

20.3. Pharmacotherapy

20.3.1. Specific therapy

Excess glutamate, an excitatory neurotransmitter, may be associated with neuronal degeneration in ALS. Riluzole, first developed as an anti-epileptic agent, inhibits the presymptomatic release of glutamate, although its exact mechanism in ALS is not yet known. In two placebo-controlled, double blind 12 month clinical trials, riluzole was shown to modestly prolong survival from 3 to 3.25 years (Bensimon et al., 1994; Lacomblez et al., 1996). The first, but not the second, trial showed mild slowing in deterioration of muscle strength compared to the placebo group. Neither study showed improvements in quality of life (Rowland, 1994; Sojk et al., 1997). Post-hoc analyses showed slight prolongation in the time it took patients on riluzole to move from milder to more severe disability (Lacomblez et al., 1996), but the effect was not apparent to patients, family members or physicians (Anonymous, 1997).

A total of four randomized controlled trials have examined the efficacy and safety of riluzole in patients with ALS. The Cochrane Library conducted a meta-analysis of the results (Class I evidence) (Miller et al., 2002). One study conducted in Japan used different outcome measures and was not included in the meta-analysis. The other three published trials (Bensimon et al., 1994; Lacomblez et al., 1996; Meininger et al., 1997) examined tracheostomy-free survival and included a total of 876 riluzole treated and 406 placebo treated patients. The results of the meta-analysis indicate that riluzole 100 mg per day prolongs survival by approximately 2 months.

The long-term safety of riluzole therapy has also been established (Lacomblez et al., 2002), including the elderly and those in advanced stages of the disease (Bensimon et al., 2002). The most common side effects of riluzole are nausea, fatigue, vertigo and somnolence. Serum transaminase elevation may occur, but rarely to levels that are clinically meaningful. There may be as much as a 70% inter-individual variability in serum concentration of riluzole, probably resulting from different rates of metabolism (Miller et al., 1996). Side effects such as diarrhea tend to be more frequent in those with high serum levels. Dose adjustment based on blood levels may be one approach to optimizing treatment (Groeneveld et al., 2003a).

A survey of 559 ALS patients from 10 medical centers was conducted in 1996 following approval of riluzole by the Food and Drug Administration (FDA) (Bryan et al., 1997). Only 43% of ALS patients had taken the drug, even though 90% knew about it. Reasons for not taking riluzole included perceived lack of benefit (45%), expense (31%), opinion of ALS physician (23%), risk of side effects (14%) and opinions of family and friends (10%). Sixty-three percent of patients who took riluzole paid less than $25/month while 12% paid over $600/month. Thirty-four percent had discontinued the drug due to lack of benefit, expense or adverse effects. The probability of taking riluzole varied from 18–75% between centers. Independent risk factors that significantly influenced the probability of taking riluzole included encouragement (OR = 6.8) or discouragement (OR = 0.1) by the ALS physician, as well as Medicare coverage (no pharmaceutical coverage; OR = 0.6). This study points out that the personal cost to the patient and encouragement from the physician are major factors determining who takes riluzole, with the decision to use riluzole determined in large part by whether or not riluzole is

included on drug formularies (Bryan et al., 1997; Ringel and Woolley, 1999). A recent report from the ALS care database suggests that 60% of US patients with ALS now take riluzole (Bradley et al., 2004). Use of riluzole is higher in Europe, approaching 100%, where it is approved for use in ALS and where the health systems cover the cost (Walley, 2004). The Canadian health system does not pay for riluzole, but many patients there are able to take riluzole based on compassionate use compensation.

20.3.2. Symptomatic treatment

20.3.2.1. Emotional lability

The characteristics of pseudobulbar affect, first described by Charles Darwin over 130 years ago, include uncontrolled laughter or crying, often with minimal or no provocation. Episodes are usually sudden, involuntary outbursts of emotion inappropriate to the context of the situation, with uncontrolled crying being more common than laughter. The symptoms can limit social interactions and quality of life.

Two theories have been proposed to explain pseudobulbar affect. Wilson (1924) suggested that interruption of pyramidal pathways from brain to brainstem yields a 'faciorespiratory response' and loss of inhibition that results in emotions that are dissociated from appropriate affect. Parvizi et al. (2001) reported that reciprocal pathways between motor cortex, brainstem and cerebellum comprise a circuit which controls emotional responses appropriate for specific cognitive and social context. Disruption of the circuit disconnects centers that regulate perceived emotion from those that involve displayed emotion. Both theories implicate a disconnection of neuronal circuitry that coordinate perceived and displayed emotion.

While there is no currently available FDA-approved therapy for pseudobulbar affect, selective serotonin reuptake inhibitors, tricyclic antidepressants and some dompaminergic agents have been beneficial in case reports (Table 20.1). Most clinical trials have been conducted in small numbers of patients with stroke or multiple sclerosis and have yielded variable results (Jackson and Rosenfeld, 2005).

Recently a combination of dextromethorphan hydrobromide (30 mg) and quinidine sulfate (30 mg) (DM/Q) was shown to be effective and fast acting in a phase III randomized controlled trial (Brooks et al., 2004). One hundred and forty patients received DM/Q or placebo twice daily for 4 weeks. DM/Q significantly reduced emotional lability and improved quality of life and quality of relationship scores. Based on these and similar results from a trial in multiple sclerosis, DM/Q is currently under consideration for FDA approval.

20.3.2.2. Sialorrhea and thick phlegm

In ALS, sialorrhea is caused by the loss of the ability to swallow rather than by increased saliva production. It is estimated that 50% of ALS patients suffer from sialorrhea (Sufit et al., 1999). Patients may wipe their mouths with tissues and even insert washcloths into their mouths to prevent drooling. In addition to being socially embarrassing, excess saliva can lead to aspiration pneumonia.

The AAN practice parameter recommends treatment with both pharmacologic and nonpharmacologic interventions (Miller et al., 1999a). Non-pharmacologic approaches include the use of suction machines and in-exsufflator or cough-assist. There have been no randomized trials comparing the efficacy of different agents in ALS. Treatment with anticholinergic medication (Table 20.1) is considered "first line" pharmacologic therapy, but the benefits can be self-limited, requiring additional medications after an initial improvement. Common side effects associated with anticholinergic therapy include constipation, fatigue and impotence. Urinary retention, blurred vision, tachycardia, orthostatic hypotension, confusion and dizziness occur less frequently. Anticholinergic medications are relatively contraindicated in patients with glaucoma, prostatic hypertrophy and cardiac conduction disorders (especially bifascicular block, left bundle-branch block and a prolonged QT interval). Side effects may be less common with sublingual hyoscyamine sulfate than with the oral anticholinergic medications.

Medication selection often depends upon the severity and frequency of the drooling. Sialorrhea associated with mealtimes or a particular time of day may be treated with as needed administration of hyoscyamine because of its transient benefit. Transdermal scopolamine, oral glycopyrolate or tricyclic antidepressant medications provide a more continuous effect (Jackson and Rosenfeld, 2005).

Analyses from the ALS CARE database indicate that over 70% of ALS patients treated with atropine, glycopyrolate or amitriptyline report benefit (Bradley et al., 2001). Patients who have difficulty swallowing medications may prefer sublingual, transdermal or liquid forms that can be administered through a PEG tube. Combinations of agents or more aggressive approaches, discussed below, can be considered if the response to standard medications is inadequate. In general, all of these medications may cause or aggravate constipation and so concomitant prescription of a stool softener may be helpful.

Several open label trials indicate that injections of botulinum toxin (BTX) into the salivary glands may help reduce sialorrhea (Giess et al., 2000; Portis et al., 2001). BTX acts by blocking acetylcholine release from presynaptic cholinergic nerve terminals and reduces

Table 20.1

Symptomatic medications for ALS

Symptom	Medication	Dosage
Sialorrhea	Tricyclic antidepressants	20–100 mg qhs
	Atropine sulfate	0.4 mg q 4–6 hours
		1–2 ophthalmic drops SL q 4–6 hours
	Glycopyrrolate	1–2 mg TID
	Hyoscyamine sulfate	0.125–0.25 mg q 4 hours
	Diphenhydramine	25–50 mg TID
	Scopolamine transdermal patch	0.5 mg applied behind ear q 72 hours
Emotional lability	SSRI antidepressants	20–100 mg qd
	Tricyclic antidpressants	20–100 mg qhs
	Mirtazapine	15–30 mg qhs
	Venlafaxine	37.5–75 mg BID-TID
	Dextromethorphan/quinidine	30 mg/30 mg BID
	Lithium carbonate	300 mg qd-TID
Fatigue	Amantadine	100 mg qAM, qnoon
	Modafinil	100–200 mg qAM
	Pemoline	18.75–93.75 mg qd
	Bupropion SR	150–450 mg qd
	Fluoxetine	20–80 mg qd
	Venlafaxine	75–225 mg qd
	Pyridostigmine	60 mg TID
Depression	Mirtazapine	15–30 mg qhs
	SSRI	20–100 mg qd
	Tricyclic antidepressants	20–100 mg qd
	Venlafaxine	37.5–75 mg BID-TID
	Bupropion	100 mg TID
Anxiety	Diazepam	5 mg TID
	Lorazepam	0.5–1 mg BID
	Buspirone	10 mg TID
	SSRI	0–100 mg qd
	Mirtazapine	5–30 mg qhs
Spasticity	Baclofen	10–60 mg TID
	Dantrolene	25–100 mg TID
	Tizanidine	2–8 mg QID
	Benzodiazepines	2–10 mg TID
Cramps	Quinine sulfate	260–325 mg qd
	Vitamin E	400 IU TID
	Phenytoin	300 mg qhs
	Diazepam	2–10 mg TID
Fasciculation	Gabapentin	300–600 mg TID
	Phenytoin	300 mg qhs
Urinary urgency	Oxybutynin	2.5–5 mg BID
	Amitriptyline	25–75 mg qhs
	Tolterodine	1–2 mg BID
	Oxytrol patches	3.9 mg qd

stimulation of the salivary glands. In one study, four of five patients had improved quality of life following injections of a mean of 46 mouse units (MU) into the parotid glands. A reduction of sialorrhea was first noticed 3 to 5 days after injection, with marked reduction as measured by the number of paper handkerchiefs used each day occurring 4 weeks later. Scintigraphy of the parotid glands 2 weeks after injection showed a reduction of radiotracer uptake.

In the second open-label pilot trial, five patients who received 15 MU injections into each parotid gland (Rowe and Erjavec, 2003) had a 52% reduction in mean saliva production. The duration of benefit ranged from 56–72 days. While there were no adverse events in these small studies, worsening of dysphagia, chewing difficulties, recurrent temporomandibular joint dislocation and swelling of the base of tongue have been reported (Bhatia et al., 1999; Tan et al., 2001; Winterholler et al., 2001). A multi-center controlled trial using BTX for sialorrhea is underway.

Radiotherapy may also reduce sialorrhea in ALS patients. In an open label trial of 12 Gy in two fractions given once a week, 14 of 19 patients (74%) reported a satisfactory response (Stalpers and Moser, 2002). Four patients had a relapse and were re-irradiated. Six patients reported pain in the parotid area and four noted dryness of the mouth. In a separate open label trial, two doses of radiotherapy of the submandibular, sublingual glands and the tail of the parotid gland were given to nine patients (Harriman et al., 2001). Effective control of drooling was achieved equally at either dose. Side effects included erythema and burning of skin in four patients, sore throat in two patients and nausea in one patient. One patient noted thicker saliva post-treatment. Controlled studies are needed to determine actual benefit and optimal dosing regimens.

Thick mucous secretions may occur independently or may co-exist with sialorrhea. Occasionally, treatment of sialorrhea can change the viscosity of saliva and produce thick phlegm. This problem can be exacerbated by inadequate water intake, a common problem in those with dysphagia. Patients with thick phlegm and weakened cough often report a sensation of something caught in the back of the throat. Pharmacologic treatments that may be helpful include high dose guaifenesin, nebulized acetylcysteine, nebulized saline or betablockers such as propranolol. An uncontrolled survey of alternative measures reported that dark grape juice, papaya tablets, sugar free citrus lozenges and grape seed oil can be helpful (Foulsum, 1999). Dietary modifications, including reduction of alcohol, caffeine and dairy products along with increased fluid intake may also help. Some find a cool mist humidifier to be helpful.

Anecdotal experience indicates that mechanical insufflation–exsufflation may be an effective method for improving clearance of upper airway secretions (Bach, 2002). A randomized trial of high frequency chest wall oscillation therapy (The Vest™), which has been reported to improve the clearance of thick phlegm in patients with cystic fibrosis (Arens et al., 1994), is also underway.

20.3.2.3. Laryngospasm

Laryngospasm, due to adduction of the vocal cords, is usually caused by aspirated liquids or saliva or acid reflux. It usually resolves spontaneously within several seconds, and repeated swallowing while breathing through the nose can reduce symptoms. Laryngospasm is almost never life threatening, but if the episodes occur frequently, pharmacologic treatment is warranted since the attacks can be anxiety provoking (Jackson and Rosenfeld, 2005). A few drops of concentrated liquid lorazepam applied sub-lingually will generally provide relief. Antacids and proton pump inhibitors can also be considered, particularly if there are other symptoms of gastroesophageal reflux disease (GERD) such as heartburn, acid taste, throat irritation or hoarseness. Since diaphragmatic weakness and overeating may worsen GERD, patients with reduced vital capacity or who use a PEG tube for feedings may benefit from peristaltic agents, such as metochlorpropamide, and either antacids or proton pump inhibitors.

20.3.2.4. Spasticity

Spasticity in ALS is caused by loss of the normal inhibition of LMN in the brainstem and spinal cord by degenerating UMNs. Baclofen, a gamma-amino-butyric acid (GABA) analog, facilitates motor neuron inhibition (Jackson and Rosenfeld, 2005). Progressive dose titration begins at 10 mg once to three times per day and increases by 10 mg every 3–5 days depending upon tolerance. Maximum tolerated effective doses can range from 30–180 mg per day and responses vary. Side effects include weakness, fatigue and sedation. Reducing spasticity can give patients a sense of looseness or weakness, which can be minimized by slow dose titration.

Dantrolene sodium reduces both rigidity and spasticity. Dantrolene acts by blocking calcium release at the level of the sarcoplasmic reticulum and has a theoretical benefit in reducing excess neuronal excitation. Dantrolene can be used in conjunction with baclofen and there may be a synergistic effect of the two drugs (Jackson and Rosenfeld, 2005). Dosing is initiated at 25 mg three times per day and is usually well tolerated and effective at higher doses. The maximum suggested dose is 100 mg four times daily. Liver function studies should be checked regularly, especially at higher doses.

Tizanidine, an alpha-two agonist, inhibits excitatory interneurons in the spinal cord and also reduces rigidity and spasticity. Tizanidine can be used in conjunction with other anti-spasticity medications. Side effects are similar to baclofen and optimal tolerance depends upon slow dose titration. Initial doses of 2–4 mg per day can be increased to as much as 36 mg per day in divided doses.

Benzodiazepines may be effective in treating painful spasms or cramps that can accompany spasticity. The use of these drugs must be weighed against the potential for sedation and respiratory suppression. Stretching exercises may also reduce spasms and cramping associated with spasticity (Ashworth et al., 2004). Tone reduction ankle orthotics (posterior leaf spring) can help stabilize the gait by counteracting the effects of spasticity.

If the maximum tolerated dose of oral medications is not effective, intrathecal baclofen is one alternative (Marquardt and Seifert, 2002). Direct administration of sterile baclofen into the CSF can minimize the side effects of oral administration. The magnitude of the intrathecal dose is approximately 1/1000th the oral dose. One advantage of intrathecal administration is the availability of variable dose delivery tailored to daily variation in symptoms. Additional bolus doses in the early morning or later in the evening can be programmed routinely. Prior to consideration of intrathecal dosing a test bolus dose of 50 μg is infused via lumbar puncture to assess the clinical response and the possibility of drug hypersensitivity.

Botulinum toxin injections have been reported to reduce spasticity due to other conditions but have not yet been formally studied in ALS. The risk of muscle paralysis may limit the use of BTX in large muscle groups in ALS.

20.3.2.5. Urinary urgency

Some patients, especially those with leg spasticity, may have urinary urgency or incontinence. Patients may need to urinate as frequently as every 1–2 hours, voiding only small volumes each time. Urinary frequency and urgency are confounded by impaired mobility and the fear of not being able to reach the commode in time. The presumed etiology of these bladder symptoms is spasm of the urinary sphincter or detrussor muscle.

The possibility of urinary tract infection or prostatism should be considered in those with symptoms of urgency and frequency. If no other cause can be identified, an empiric trial of a spasmolytic agent is indicated. Oxybutynin (Ditropan) is the least expensive and can be crushed and put through a PEG tube. An extended release form of Oxybutynin is also available that can be administered once a day, although it cannot be crushed. Tolterodine tartrate (Detrol) is longer acting than oxybutynin and can be prescribed twice daily. Oxytrol patches may be used in addition to the oral tablets for patients with refractory symptoms. Patients can avoid accidents by using a voiding schedule in which they attempt to urinate every 2–3 hours regardless of whether they have an urge or not.

Nighttime incontinence can usually be avoided by reducing fluid intake in the evenings, but men may need a condom catheter at night. Absorbent undergarments can also be used, but skin should be monitored closely for sores and protected with moisture repelling agents. For those with severe incontinence, an indwelling Foley or suprapubic catheter can be tried when other options have failed.

20.3.2.6. Jaw quivering and clenching

Some ALS patients develop jaw quivering or clenching due to UMN degeneration. These symptoms are often precipitated by pain, anxiety or cold. Jaw clenching can make suctioning or oral hygiene difficult to perform. Treatment with benzodiazepines such as clonazepam, diazepam or lorazepam may be helpful. Botulinum toxin injected at two sites within the masseter muscles may also be effective.

20.3.2.7. Edema

Dependent edema is a common consequence of immobility. Loss of skeletal muscle tone and mass results in reduced vascular tone that leads to limb edema. Chronic edema and static blood flow may cause pain, venous thrombosis, sensory nerve damage and impaired range of motion. Elevation of the legs is the simplest and most effective early intervention, but diligent compliance is essential for optimal benefit. Placement of pillows under the calves can facilitate drainage of distal edema. Proper planning is essential prior to ordering wheelchairs to ensure that placement of elevating leg rests are an available option. Motorized scooters, for example, do not offer such adaptability.

When periodic elevation alone is not effective in reducing edema, specialized elastic stockings can be effective and custom fit prescription elastic support stockings are most helpful. Stockings are prescribed based on the length and the degree of desired compression. Proper fitting requires accurate measurement of the calf and foot. Personnel specially trained for such fitting should be sought. Custom support stockings can be made for patients with unusual foot or leg sizes.

20.3.2.8. Sleep disturbances

Disordered sleep patterns in patients with ALS are often multi-factorial in etiology. Respiratory muscle weakness, difficulty repositioning in bed, anxiety, depression and pain can all interfere with normal sleep patterns.

Respiratory insufficiency, increased arousal and decreased total sleep time have all been recognized in patients with ALS (Arnulf et al., 2000). The consequences of impaired sleep include daytime fatigue, exacerbation of respiratory compromise, weakness and depression. Overall, impaired sleep can markedly affect quality of life and may worsen prognosis.

Solutions to address poor sleep are as varied as the diverse problems causing it. Simple physical adaptations such as a power hospital bed can enhance mobility, positioning and comfort. An inexpensive alternating pressure air pad or gel overlay mattress can also lessen the discomfort from limited nighttime mobility. NIPPV can improve respiration and sleep quality. Antidepressant medications may relieve anxiety and depression and promote sleep. Mirtazapine and the tricyclic antidepressants can be especially helpful.

Anxiolytic medications such as benzodiazepines, used specifically to induce sleep, can be helpful when used selectively. Pharmacological tolerance and withdrawal symptoms can become evident with chronic use. Treating the underlying cause of anxiety (respiratory difficulty, depression, fear or pain) is most effective. Zolpidem tartrate, a non-benzodiazempine sleep aid, is often effective and carries a low risk of respiratory depression. Antihistamine medications or chloral hydrate are also sedating.

Alternative pharmacologic agents such as melatonin, passionflower, lavender and hops have been effective for individual patients; however, their benefits are quite variable and untested (Jackson and Rosenfeld, 2005).

20.3.2.9. Fatigue

Fatigue and exercise intolerance are common symptoms in patients with ALS. Although fatigue is not a primary consequence of motor neuron degeneration it is often mistakenly interpreted as advancing weakness. Limitations in activities due to fatigue can reduce quality of life. The initial challenge in treating fatigue is in the identification of the primary etiology. Fatigue can be caused by physical fatigue with over-expenditure of energy; mental tiredness related to fear, stress or depression; anhedonia caused by depression; poor sleep from respiratory insufficiency, pain or depression; and medication side effects (Krupp, 2004).

Energy conservation is perhaps the first line and simplest method of treatment for fatigue. Patients with weakness may try to establish that their weakness is mild by attempting activities more appropriate for their premorbid state. Instructions that exercise, daily activity and travel are acceptable within the guidelines of energy conservation can be very therapeutic. It may be reassuring for patients that normal activities are possible and encouraged, but that a certain amount of rest is necessary too. Fear that fatigue is synonymous with weakness and is due to disease progression can be dispelled. Deconditioning from the lack of exercise can be more disabling and fatigue-inducing than controlled activity and exercise.

Nocturnal respiratory insufficiency may be an under recognized source of daytime fatigue. Standard pulmonary function tests are helpful but not required for a patient to benefit from NIPPV. Occasionally, when daytime fatigue contributes to a low pulmonary function test (justifying the onset of NIPPV therapy, see below), both the fatigue and pulmonary function may improve as a result of using NIPPV.

Pharmacologic treatments for fatigue are also often effective (Table 20.1). Response to medications can, however, be as idiosyncratic as the etiology of the fatigue. Medications taken for other reasons can contribute to fatigue and should be considered prior to addition of new drugs to manage side effects resulting from existing drugs.

Off label use of pyridostigmine (Mestinon) can reduce symptoms of weakness in some by enhancing neuromuscular junction transmission. Methylphenidate can also provide benefit in selected patients. Caution must be taken to monitor side effects of anorexia, restlessness, anxiety or palpitations. Modafinil (Provigil) can also be tried.

20.3.2.10. Constipation

Constipation is common in ALS, particularly when patients become less mobile. Constipation can be aggravated by anticholinergic medications used for sialorrhea or by narcotic medications used for pain or air hunger. Inadequate fluid intake due to dysphagia, arm weakness or a desire to minimize trips to the bathroom may worsen the problem. In later stages of the disease, abdominal wall muscles may weaken to the point that patients are unable to push the stool out, even if it is soft.

Initial management of constipation includes the use of stool softeners such as Sufack, Pericolase or Senekot. Simply increasing fluid intake and substituting medications with fewer anticholinergic effects can be effective. Increasing dietary fiber can help; a creative recipe for constipation is “power pudding,” which consists of equal parts of prunes, prune juice, apple sauce and bran (Gelinas, 2001). Two tablespoons with each meal and at bedtime, along with adequate fluid intake and fruits and vegetables in the diet may be helpful. If not, Milk of Magnesia or Dulcolax tablets can be added to the regimen. In patients with a PEG tube, Lactulose can be administered as long as the patient is not impacted. Enemas or magnesium citrate can be used in urgent situations.

20.3.2.11. Pain

Pain is often under recognized in ALS. Immobility, emotional distress, muscle spasms, edema or the illness itself may all cause pain. Early recognition of the common precipitants leading to pain is the first line of treatment. Often pain can be treated without medication by directly treating the cause, including use of stretching and range of motion exercises, massage, physical therapy, limb elevation and support hose to reduce edema.

Medical managements may include nonsteroidal anti-inflammatory drugs or benzodiazepines and opioids. The latter are generally safe but may lead to constipation, respiratory depression in high doses and tolerance. Liberal use of narcotics and anxiolytics is often necessary at the end stages of the illness to prevent suffering.

20.3.2.12. Cramps and fasciculation

Cramps and fasciculation are common symptoms in ALS and can be bothersome to patients. Cramps can often be reduced by stretching exercises, which patients can learn from a physical therapist. If physical measures are not adequate, quinine (Connolly et al., 1992), vitamins E and C (Khajehdehi et al., 2001) and anti-spasticity agents such as baclofen (Forshew and Bromberg, 2003) are often helpful. The treatment of fasciculation is less clear cut. Most patients are relieved by the reassurance that fasciculation do not mean that the disease is accelerating. For those who continue to be bothered, gabapentin (Romano, 1996) or anti-spasticity agents can be tried (Forshew and Bromberg, 2003).

20.4. Multidisciplinary clinic

The multidisciplinary care (MDC) clinic, first established by the MDA for children with muscular dystrophy, has over the past two decades become the way most large centers care for patients with ALS (Norris et al., 1985). The MDA and ALS Association now both certify ALS clinics or centers that provide expertise in diagnosis, management and research of ALS (Mitsumoto et al., 2005a). The MDC clinic is conducive to a holistic approach to treatment. Allied health care professionals in the MDC clinic develop expertise in ALS because of dedicated experience in caring for numerous patients. The patient and family benefit from having questions and concerns addressed by professionals from different disciplines, which conserves energy and time. Some data suggest that patients cared for at MDC clinics may survive longer (Traynor et al., 2003).

The MDC is not a perfect solution to ALS care, however. Undergoing evaluations by different therapists in succession over hours may be tiring for patients, and the time allocated with each therapist may be insufficient when the problems are complex. In the US, the greatest barrier to the MDC approach is the high cost to the clinic, which may not be reimbursed by insurance payers.

While MDC services are ideally available at the clinic (Mitsumoto et al., 1998), the format of each clinic varies depending on the characteristics of the clinic and the director's philosophy. Most clinics are directed by neurologists who have special expertise in ALS, but occasionally they are run by physiotherapists or other specialists. Some clinics see only follow-up patients with an established diagnosis, whereas other clinics see a mixture of new patients and established patients. Regardless of these differences, access to neurological care, diagnostic laboratories and clinical research are keys to a successful MDC.

The neurologist is responsible for patient care (Mitsumoto et al., 2005a), directs the ALS MDC clinic and oversees the care provided by the other health care professionals. Neurologists make orders based on recommendations given by the team members and prescribe appropriate equipment and braces (Figs. 20.1–20.4). At regular follow-up visits, treatment options, such as enteral feeding and NIPPV, are discussed. The topics that the neurologist discusses with patients and their families may overlap with those of the nurse and other therapists, but repetition often helps patients and families to better understand complex issues, not only because they hear the information repeatedly but because each team member educates from a unique perspective. Neurologists, along with the ALS nurse or social workers, also discuss preparation of a health-related living will and durable power of attorney for health care.

The ALS nurse has special expertise and interest in ALS and provides nursing care for patients. The nurse, often an advanced nurse practitioner or a registered

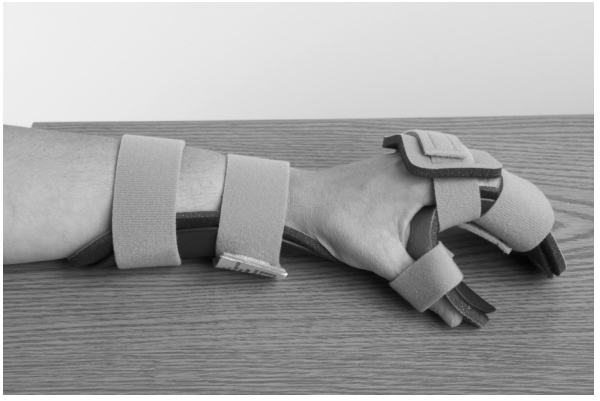

Fig. 20.1. Resting hand splint or wrist-hand-finger orthosis. Used to support wrist and finger extensor muscles and to prevent contractures due to weakened muscles.

Fig. 20.2. Posterior leaf spring orthosis. Off-the-shelf stock ankle foot orthosis used to support foot drop.

nurse specializing in ALS, has a key role in the MDC clinic, including coordinating the activities of the other health professionals, providing nursing care, coordinating hospice or home care and being an educator and advocate for the patients. Because the neurologist may be perceived as an authority figure, making decisions

Fig. 20.3. A portable key-board-activated communication device with voice output. The device is lightweight and is powered by a rechargeable battery.

Fig. 20.4. In-exsufflator or cough assist device, used to clear secretions from the throat.

difficult for some patients, the nurse is in a unique position to explain the MDC path to the patient and family. The nurse is responsible for ensuring that communication between patients, families and team members is sufficient for patient compliance and satisfaction. The nurse can also explain information provided by voluntary disease organizations, such as the MDA, ALS Association and the National Institutes of Health. At the end of the clinic, the nurse coordinator moderates a team conference, in which a final plan of care is discussed and implemented by all the team members (Mitsumoto et al., 2005a) and the ALS nurse schedules the next appointment.

At the MDC clinic, physical therapists evaluate limb strength, trunk strength and motor skills. They develop exercise programs for each patient to help maintain existing motor function and they evaluate the need for orthoses and assistive devices to maintain independence. Physical therapists also work with occupational therapists to recommend home equipment for patient safety and mobility.

Occupational therapists address the skilled motor functions that enable patients to engage in activities of daily living. They work with physical therapists to evaluate hand and arm function, they give recommendations for splinting and adaptive devices and they discuss activity modification, such as energy conservation and work simplification.

A dietitian or nutritionist assesses nutritional status by evaluating appetite and weight and determining whether the patient is at risk for poor nutrition. The dietitian often works with a speech pathologist to evaluate the degree of dysphagia and can recommend strategies to modify swallowing or prepare food that help maintain calorie balance. The dietitian also helps to determine whether enteral feeding is required. For those patients who undergo PEG, the dietitian selects a nutritional formula based on the patient's daily caloric, protein and fluid requirements.

Speech pathologists are responsible for evaluating bulbar function. When evaluation at the MDC clinic is not sufficient to determine the degree of swallowing impairment, the speech pathologist may request a modified barium-swallow test or videofluoroscopy, which can identify subtle dysphagia and silent aspiration. These procedures precisely identify swallowing impairments, and are a good opportunity to educate patients about dysphagia and how to swallow food of various textures. Speech pathologists work with dietitians to teach patients how to compensate for progressive dysphagia. They also counsel patients in regard to augmentative communication devices.

A social worker assesses health insurance coverage and assists the patient in applying for disability payments. Social workers provide referrals for financial resources, community resources, ALS advocacy group chapters and support groups. When the disease progresses to a stage at which patients require home or hospice care, the social worker makes the necessary arrangements and applications for patients. The social worker discusses advance directives, durable power of attorney for health care and preparation of a living will. They also provide emotional and psychological support.

Voluntary disease organizations often send a representative to the ALS clinic to provide educational literature and information about the many services they offer, including equipment loans and patient transportation services. They also often organize patient and caregiver support groups, which can be important to patients and their families because they provide the opportunity to meet and talk with others who are going through the same experience.

A pulmonologist, who may not be a regular on-site member of the MDC team, is usually available to see patients who have respiratory problems. Because respiratory muscle weakness may occur rapidly, a pulmonary consultation is sometimes required urgently. Thus, the pulmonologist develops a close consulting relationship with the ALS center and should be readily available for consultation. When respiratory support is required during gastrostomy, the pulmonologist works with the gastroenterologist or radiologist performing the gastrostomy. The pulmonologist also helps to regulate NIPPV administration and to oversee those patients who elect permanent ventilatory support.

The gastroenterologist is consulted when enteral feeding is necessary and the patient agrees to PEG. Gastroenterologists evaluate the patient's gastrointestinal status and perform the procedure, usually in an outpatient endoscopy suite. Patients whose respiratory status is unstable before the procedure may stay overnight for observation. For those patients with FVC below 50%, an interventional radiologist may be consulted to perform a radiologically guided gastrostomy (RIG), which requires only a local anesthetic and is somewhat less invasive. When a patient's FVC is below 50%, a pulmonologist also is usually consulted. Both the gastroenterologist and dietitian follow the patient during the immediate post-procedure period.

An orthotist, either in or outside of the hospital setting, fits ankle-foot orthoses and neck braces for those who develop head-droop. Orthotists and biomechanical engineers may collaborate to create customized braces that are unavailable commercially. An oral surgeon may be consulted to create a palatal lift for patients with marked dysarthria due to bulbar palsy and velopharyngeal incompetence (Esposito et al., 2000).

Depression is common in ALS, but, if properly managed, psychiatric consultation usually is unnecessary. In general, all members of the ALS clinic attempt to provide psychosocial support to patients and their families. Occasionally, an overwhelming catastrophic reaction to the disease, stress-induced adjustment problems or marital problems stemming from stress may become serious issues. In these instances, a psychiatry or clinical psychology referral may be necessary. A chaplain can provide pastoral or spiritual care for patients, families and even for health care professionals in the ALS clinic.

At the end of each MDC clinic, the team members discuss the patients seen that day. Usually the ALS nurse facilitates the discussion and the team reviews the issues and recommendations. The problems and concerns of each patient and family are addressed and solutions and suggestions are presented. The team discusses specific treatment plans, including symptomatic medical treatment, clinical trial participation, consultations, home care and hospice referral. The nurse records the key points and the team decides on a treatment plan for each patient, which the nurse then prepares. The nurse or individual team members arrange to discuss the recommendations with the patient. Because of the meeting's collaborative nature, innovative ideas and suggestions may be raised. Psychologically, the meeting is also important for team members because it can foster camaraderie, provide hope and boost morale after working with patients afflicted by a devastating disease.

Ideally, the MDC clinic develops a care path individualized for each patient and his or her caregiver. At the MDC clinic, the health care providers determine whether the care path is working for the patient, how well the patient is following the care path and whether the plan requires modification. MDC team members and patients may use e-mail to communicate between clinic visits; however, in the US, the Health Insurance Portability and Accountability Act (HIPAA) now requires secured encryption technology and a legally acceptable confidentiality statement.

20.4.1. Difficulties facing MDC clinics

The cost of operating an MDC clinic is high because multiple health care professionals need to be on site (Mitsumoto et al., 2005a). Medicare, the principle government insurance for the elderly or disabled in the US, does not pay for therapy given by allied health care professionals at the doctor's office on the same day. Reimbursement is only provided when therapy is done at a separate formally designated facility. Because commercial medical insurance generally follows Medicare guidelines, the physician can charge for only a regular follow-up fee even after the patients have been evaluated by multiple health care professionals at the MDC clinic. Essentially, none of the allied health care services is reimbursed by the insurance payor. This lack of reimbursement imposes an enormous financial burden on most MDC clinics and, as a consequence, the very existence of the ALS MDC clinic is in jeopardy.

The MDA provides financial support to their designated ALS clinics, of which there are more than 30 throughout the US. However, this financial support is contingent upon treating more than 40 other neuromuscular diseases. The MDC arrangement is a prerequisite for ALS Association certification, but, unfortunately, the ALS Association can only provide modest financial support to their certified clinics. A number of prominent MDC clinics are affiliated with ALS foundations and local ALS chapters that provide some financial support. The MDC concept and structure seem to be widely accepted and promoted, but operating the clinics is costly and additional financial support is necessary for most.

Not all ALS MDC clinics in the US are run identically. The structure and service may vary between clinics, depending not only on the level of financial support that the clinic receives, but also on each clinic's philosophy and development of innovative ideas. The key to effective treatment is to structure and operate an MDC clinic in the way that allows us to care for patients with ALS and their families in the ways that they need most. Generating new ideas to not only sustain MDC clinics in the US but also to allow them to thrive is absolutely essential.

The MDC concept has also been adopted in Canada, Europe and Japan (Mitsumoto et al., 2005a). Different countries face different financial and personnel hurdles, but the clinic structure is similar to that in the US. The Japanese have developed a particularly effective home-care system. The national governments of France and Belgium have established and funded regional multidisciplinary clinics, and the ALS Care Center first established in the UK has become a leader of comprehensive care worldwide. In Switzerland, one clinic has begun to influence ALS care throughout the country. In contrast, in Ireland, a single comprehensive MDC clinic must work within the structure of the Irish health care system. In Montreal, pet therapy has been found to enhance patient and caregiver comfort during the clinic, adding a unique aspect to their comprehensive MDC clinic. The clinics have been adapted to each country's health care system, societal customs and the needs of patients and families.

20.5. Physical rehabilitation

Rehabilitation helps patients to reach their fullest potential within their physical limitations, desires and life plans (Krivickas et al., 2005), as determined by the patient and those concerned with his care. The goal is to reach optimal function despite disability, even if the disability cannot be reversed (Haas, 1993). Rehabilitation in ALS, therefore, is a fluid process that attempts to anticipate changes and stay ahead of accumulating symptoms in order to maintain quality of life.

Rehabilitation can help during all stages of ALS, but the strategies change as the disease progresses. For example, patients with early ALS need help designing an aerobic exercise and strengthening program. As increasing spasticity develops, patients may return to physical therapy to learn stretching and range of motion exercises. Orthoses are prescribed once more marked weakness develops and, in later stages, a therapist may teach the caregiver to transfer the patient. Continuous therapy is not covered by Medicare or most commercial insurance agencies, so that intermittent referrals and visits are often needed. The physical therapist in the MDC clinic can help oversee the timing of referrals.

20.5.1. Exercise

Patients are usually given an exercise program early in the course of ALS to help delay disability in strong muscles. Exercise entails stretching, strengthening and aerobic exercises. There is a paucity of literature on all types of exercise in ALS, but experience and common sense suggest benefits. Stretching and range of motion exercises help prevent contractures, decrease spasticity and reduce muscle spasms.

Case reports support strengthening (Dal Bello-Haas et al., 1998) and lack of exercise can lead to deconditioning that may compound weakness produced by ALS. Studies of other motor neuron diseases report that muscles that are only mildly affected can be strengthened by a moderate resistance strengthening program (Aitkens et al., 1993), but a study in a heterogeneous group of neuromuscular patients suggested that overuse weakness can develop in muscles exercised with high

resistance (Kilmer et al., 1994). Overuse weakness and muscle damage do not appear to occur in muscles with at least 3/5 strength on the MRC scale. In general, the improvement in strength is proportional to the initial muscle strength, suggesting that a strengthening program should be initiated as early as possible in the course of the disease. To date, no studies have demonstrated translation of the modest strength gains into improved function or slowing of disease progression. Well-controlled randomized trials are needed to determine the real effects of strengthening exercises in ALS.

In the meantime, it is reasonable to begin a strengthening program as soon as possible after diagnosis in order to maximize the strength of unaffected or mildly affected muscles. Weight training or strengthening exercises are used with a weight that can be lifted 20 times, a simple way to select a weight that is 20–40% of maximum voluntary contraction; then two or three sets of 10 repetitions are performed. This technique helps prevent overworking the muscles with excessively heavy weights. Another general guideline is that if an exercise regimen consistently produces muscle soreness or fatigue lasting longer than 30 minutes after exercise, it is too strenuous.

Aerobic exercise helps to maintain cardiorespiratory fitness. Only one study has compared the response to aerobic exercise in patients with ALS and healthy controls (Sanjak et al., 1987). The oxygen cost of exercise, at similar intensity levels, was increased in patients with ALS, perhaps due to increased energy consumption from weakness and spasticity. Plasma glucose, pyruvate and lactate levels were not different, but free fatty acids, beta hydroxybutyrate and carnitine did not rise as much as in control subjects, suggesting either a possible defect in lipid metabolism during exercise or physical deconditioning. Overall, heart rate and ventilatory response to exercise were similar to those seen in controls.

We recommend aerobic exercise training for patients with ALS as long as it can be done safely without a risk of falling or injury. In addition to the physical benefits, exercise often has a beneficial effect on mood, psychological well-being, appetite and sleep. Pool therapy is often an ideal place for patients with ALS to do aerobic exercise, which can be as simple as walking in the water. Water exercise is best done in a therapy pool with a flat, uniform depth floor that is heated to 92–95° Fahrenheit. The warmth of the water can help reduce spasticity and facilitate movement (Krivickas et al., 2005).

Drory et al. (2002) studied the effects of a simple, moderate home exercise program in 14 patients with ALS. They randomized 25 ALS patients to receive a moderate daily exercise program consisting of gentle aerobic activity such as walking, stationary bicycling or swimming for 30 minutes or less ($n = 14$) or to normal daily activity ($n = 11$). At 3 months, patients who performed regular exercise showed less deterioration on the ALSFRS and Ashworth scales. At 6 months, there were no significant differences between groups, although there was a trend toward less deterioration in the treated group. In this study, a regular exercise program had a short-lived positive effect.

Two recent studies in the ALS transgenic mouse model reported that aerobic exercise slows disease progression. Kirkinezos et al. (2003) showed that 30 minutes per day of treadmill running 5 days a week for 10 weeks increased life span by 8% in female mice and 4% in male mice. In a study by Veldink et al. (2003), mice were exercised 45 minutes per day; disease onset was delayed and survival prolonged in female but not male mice. In contrast to these findings, a study of high intensity endurance exercise in the mouse showed decreased survival in exercised male mice only (Mahoney et al., 2004). From these studies, it appears that in mice, light to moderate aerobic exercise may have a neuroprotective effect, particularly in female mice, but heavy resistance exercise may have a deleterious effect.

20.5.2. Assistive devices

A large variety of equipment is available to help patients function better and maintain independence. Most patients with ALS eventually need an aid to maintain mobility, either because of muscle weakness, spasticity or balance problems. The type of aid is determined by the degree weakness, extent and rate of progression, acceptance by the patient and by financial constraints. Medicare does not reimburse the cost of many of the devices, but a physician's prescription may allow partial reimbursement from third party payers.

Aids generally need to be lightweight because patients with ALS experience muscle fatigue. Canes give the least amount of support and are usually prescribed in the early stages of ALS for mild leg weakness or balance problems in those with good arm strength (Krivickas et al., 2005). The standard wooden cane is the least expensive, but cannot be adjusted. Aluminum canes can be adjusted for different heights, are lightweight and have several different types of handles. An offset handle allows the weight to be directed over the cane tip when in contact with the floor, rather than anteriorly, as is the case with a curved handle. Quad canes, aluminum canes with four points of floor contact, provide greater stability than straight canes, but are heavier to lift. A variety of sizes of handgrips are available and patients with hand weakness may be better able to grip an enlarged or molded handle.

Patients with ALS rarely use crutches because of the need for very good balance and arm and trunk strength to use them. If crutches are recommended, Loftstrand or Canadian crutches are preferred. These crutches consist of a single upright, a forearm cuff and a handgrip; the hands can be freed for standing tasks without having to release the crutch.

Walkers provide greater support than canes and crutches, but are more bulky and may be cumbersome in confined spaces (Mitsumoto et al., 1998). Various types of walkers are available (e.g. folding, with or without wheels, with brakes, with seating surfaces, etc.) and some walkers can be modified to suit individual needs. Standard aluminum walkers are the least expensive, are very stable and can be adjusted for various heights. However, they are heavy and must be picked up during walking. Walkers with wheels are usually recommended for patients with ALS because they do not need to be lifted and they roll forward easily. Brakes are usually necessary to prevent the wheeled walkers from moving forward too quickly. Push down brakes can be used when hand weakness limits the application of squeeze type brakes. Specialized walkers with large wheels that can move over a variety of terrains are the most expensive.

For those with difficulty arising from chairs, either using higher seats or extra cushions may help. Self-powered lifting cushions, such as the UPlift Seat Assist™ are inexpensive and portable, but the patient must have adequate trunk control and balance. Powered seat lift recliner chairs, although more expensive, are also available for rental or purchase. These chairs enable a person to rise to a standing position or recline by activating an electric control.

Other devices that assist with mobility are transfer boards, belts and swivel cushions or seats. Transfer boards can be used alone, if the patient has adequate arm strength and balance or with the assistance of a caregiver. Transfer belts ease the process of transferring for the caregiver and prevent potential painful traction on the patient's arms. Swivel cushions are lightweight cushioned seats that swivel in both directions and make getting in and out of a car easier. Patients who have difficulty transferring, even with the assistance of a caregiver, may need a mechanical lift, such as the Hoyer Lift. Electric hospital beds also facilitate mobility and transfers, both for patients and caregivers. A variety of mattresses and mattress overlays, such as alternating air pressure pads, help prevent bedsores and improve comfort when in bed.

A portable standing frame can provide muscle stretching, weight bearing for bones and psychological benefit from being upright. These devices can be used at home and integrated into the daily routine.

20.5.3. Wheelchairs

As the disease progresses, most patients develop the need for a wheelchair to maintain mobility. In the early or middle stage of ALS, a lightweight manual wheelchair can be used to conserve energy. The manual wheelchair should be rented or borrowed from a local ALSA or MDA chapter because most insurance companies will only pay for one wheelchair.

As the disease progresses, a power wheelchair tailored to the patient's current and potential future needs can be purchased, or the manual wheelchairs can have a power pack attached directly to the rear wheels. The advantage of this system is that the battery can be disassembled and the wheelchair folded to fit inside a car. Conventional power wheelchairs resemble a manual wheelchair with large powered rear wheels and front casters. Power-base wheelchairs are designed so that the powered section is independent of the seating components. They are categorized according to the placement of the drive: rear wheel drive, mid-wheel drive and four-wheel drive.

Although power scooters may be suitable for patients with good arm and trunk strength, they often become inadequate as the disease progresses, particularly because the legs cannot be elevated. Most insurance companies will not pay for a power wheelchair if the patient has already received reimbursement for a scooter, a factor of great importance because power wheelchairs can cost as much as $30,000.

The optimum wheelchair has a high back with adequate head and neck support, a reclining or tilt-in-space seat that enables postural changes for pain and pressure relief, and the ability to accommodate a ventilator and communication aid. Numerous other features and options are available for wheelchairs that help patients remain independent (Trail et al., 2001). Specialized wheelchair clinics can evaluate which options are best for individual patients.

20.5.4. Orthoses

Orthoses are devices designed to improve function. They provide support to joints and weakened muscles, decrease the stress on compensatory muscles, conserve energy and help prevent contractures.

20.5.4.1. Cervical collars

In the early stages of ALS, weakness may cause neck stiffness, heaviness and fatigue. Patients may notice difficulty in keeping the head upright with unexpected movements. As the weakness becomes more severe, head droop and neck pain become bigger problems. Several different types of collars can support the head,

protect the weakened muscles and reduce pain. A soft, foam collar may help when the weakness is mild. Soft collars are usually comfortable and neck movements are only slightly limited.

For moderate to severe weakness, a semi-rigid collar, such as the Philadelphia™ collar, may be needed, but patients often find them confining, hot or uncomfortable at points of contact. For those with tracheostomy, Miami-J®, Aspen and Malibu collars have anterior openings, but warmth and a sense of confinement are also common. The Headmaster, Executive and Canadian collars offer a compromise between comfort and stability.

Patients with both cervical and upper thoracic weakness may benefit from a cervical-thoracic orthosis or a sterno-occipital mandibular immobilizer. These devices provide a high degree of support, but are expensive and heavy. Custom-made devices may be necessary for those with severe trunk and neck weakness.

20.5.4.2. Arm splints and slings (Fig. 20.1)

An arm sling supports the arm when muscles are weak or the glenohumeral joint is subluxed. These orthoses can decrease pain and prevent soft tissue stretching. A pouch sling or single strap hemisling supports the elbow and wrist, but places the arm close to the body in adduction, internal rotation and elbow flexion; with prolonged use, contractures may develop. The axilla roll sling, which consists of a soft roll fitted under the axilla, holds the humerus slightly abducted, while supporting the shoulder joint. A humeral cuff sling consists of an arm cuff on the distal humerus supported by a figure-eight harness. Studies in patients with stroke have found some supports more effective than others in reducing subluxation (Zorowitz et al., 1995). A therapist can provide guidance as to which sling is best for individual patients.

Splints are hand orthoses and can be purchased commercially or custom-made. They may be static (no moving parts) or dynamic (moving parts). Splinting can help improve hand function and prevent contractures. The Anti-Claw Hand Splint keeps the metacarpalphalangeal (MCP) joints in flexion and can improve grasp strength in patients with intrinsic muscle weakness. The Dynamic Finger-Extension Splint extends the MCP joints, so patients with finger extensor weakness can grasp objects. The Volar Cock-up Splint extends the wrist 20 to 30 degrees and is useful for patients who have wrist and finger extensor weakness. The Opponens Splint supports the thumb in an abducted and opposed position and is useful for patients with prehension difficulties due to abductor pollucis brevis and thumb extensor muscle weakness.

20.5.4.3. Leg orthoses

Ankle-foot orthoses (AFOs) help to maintain ankle stability and can be purchased off-the-shelf (Fig. 20.2) or custom-made. Deciding which version to purchase depends on the rate of progression of weakness and also on financial factors. For those with rapidly progressive ALS who need the orthosis for limited time, the pre-manufactured type may suffice and is less expensive.

A solid AFO, usually made from thermoplastic material, is designed to provide maximum stability to the ankle and foot. Knee stability can be improved by dorsiflexing the ankle a few degrees. Solid AFOs are best for patients who have both ankle and quadriceps weakness. However, the fixed ankle position, in combination with quadriceps weakness, may make arising from chairs and climbing stairs difficult.

A hinged AFO incorporates a mechanical ankle joint between the foot and calf. Hinged AFOs are best for those with good quadriceps strength and only mild ankle dorsiflexion weakness. A stop mechanism can be incorporated in the AFO to prevent excess plantar flexion. Arising from chairs may be easier with hinged than solid AFOs.

Posterior leaf spring (PLS) AFOs have medial and lateral trim lines placed posterior to the midline of the malleolus (Krivickas et al., 2005). PLS braces are more flexible than solid AFOs and allow some plantar flexion during heel strike. Push-off returns provide dorsiflexion assistance. PLS braces are best for patients with mild spasticity and slight footdrop.

An alternative for patients with a mild foot drop is a carbon fiber kevlar AFO that wraps around the front of the leg. The Kevlar AFO is less restrictive than a PLS so it may be easier for patients to become accustomed to it. However, the cost is three to four times that of a plastic AFO and ALS patients quickly outgrow its usefulness.

For patients with severe spasticity, features such as metal uprights can be built into AFOs to help compensate, but at the cost of increased weight; it may be harder for patients to walk with heavy orthoses than with spasticity.

20.5.5. Home modifications

Home modifications range from simple, such as moving the patient's bed to the main floor, to extensive. Physical and occupational therapists can evaluate the home for accessibility and safety. The overall goal when considering recommendations for home adaptations is to create the safest and most supportive environment for the patient, family and caregiver. Unfortunately, the cost of home modifications is usually not reimbursed by insurance companies.

Ramps are necessary for those confined to wheelchairs to get in and out of the house. Ramps need to be sturdily built, with a grade of no more than 1 foot of height for every 12 feet of length (American National Standards Institute, Inc.). Portable ramps and vertical platform lifts can be purchased.

Doorways must be at least 32 inches to allow wheelchairs or walkers through and patients need about three feet of space to turn near chairs or commodes. Furniture rearrangement is one simple solution. Doorways can be widened by removing doors, reversing doors (to open outward) or by installing folding doors. Offset hinges, which swing the door clear of the doorframe, can also be installed.

Bathrooms are often inaccessible to wheelchairs. One option for patients is to transfer to a commode with casters at the door. Various devices can assist with bathing and using the toilet, depending on the layout of the bathroom. A standard shower stall with a three to four inch rim may be made more accessible with a portable ramp. A custom-made shower area that allows the patient to enter the stall on a shower commode, but that has a raised slope that prevents water from running out also may be an option, depending on financial resources.

Inexpensive remote control units, available from hardware stores, can be used to control small appliances or lights. Environmental control systems attached to electronic or electrical equipment, such as household lighting, heating systems, appliances, the television, telephone or stereo are generally more expensive. Patients can operate these systems through a switch, remote-control or computer.

Chair glides and stairway lifts can be used in multi-level homes. These lifts are custom-made and are very expensive. Insurance companies usually do not pay for stairway lifts, but some medical supply companies offer 'rent-to-own' options. In addition, local ALS Association and MDA chapters may have used lifts and may also send a PT or OT to the home to evaluate safety at no charge.

20.5.6. Speech aids and augmentative communication

Patients with mild speech changes due to ALS may use compensatory strategies such as adequate breath support for phrasing and over articulation to improve intelligibility. Many patients suffer from fatigue, with speech being clearer in the morning but deteriorating as the day progresses. Therefore, compensatory strategies must be used judiciously. For an evening engagement, resting or napping in the afternoon or minimizing speaking during the day can allow for greater strength in the evening. Educating family members can also help. Those with hearing loss may need to see an audiologist and speaking face to face in a well-lit environment without competing noise can also help.

For patients with diminished voice volume due to lack of breath support, use of an amplifier may improve communication. Patients with LMN dysarthria may benefit from a prosthetic palatal lift, which elevates the soft palate to occlude the nasopharyngeal port and allows build up of intra-oral pressure (Esposito et al., 2000). Those with active gag reflexes are less able to tolerate a palatal lift.

The role of exercise in improving speech is not well understood (Clark, 2003). Generally exercises other than light range of motion are not recommended for patients with ALS; the exercise obtained during speaking and swallowing in daily use is probably sufficient. On the other hand, range of motion exercise may add to the psychological well being of patients who wish to be proactive in maintaining function.

Even in the early stages of bulbar dysfunction in ALS, education and planning for augmentative communication is indicated. Alternative augmentative communication (AAC) is the term used for any mode of communication other than speech, including gesture, facial expression, writing, symbol or picture boards and computers.

Devices may be categorized as low or non-electronic technology or high technology, which are electronic or computerized. Those with early ALS typically rely on low technology approaches, such as writing. They rely more on high technology during the middle stage (i.e. speech generating device; Fig. 20.3) and then return to low-technology communication methods during the late stage of the disease (Doyle and Phillips, 2001).

Even in the latest stage, when some patients have chosen mechanical ventilation, most patients continue to retain some ability to communicate. Some patients use eye gaze or blinking and infrared technology devices. However, some patients eventually become locked-in and unable to communicate even with eye movement (Hayashi and Oppenheimer, 2003). Techniques are being developed that will allow patients to communicate using EEG signals.

The prescription of the correct device requires referral to a specialized speech-language pathologist. One of the most popular devices is the LightWRITER™, which most people with basic typing skills are comfortable using. For those with impaired hand function, the device can be set to scan the alphabet, allowing patients to select letters or words by the use of a switch. Higher-level AAC technologies include laptop, palm or desktop computers, which use programs that can either be picture or text-based and which have keyboards

or switches. These devices can be mounted at the bedside or on wheelchairs. Many AAC devices cost between $4000 and $6000, and can take several months to acquire due to the slowness of insurance approval.

In January 2001, the AAC insurance designation was changed to Durable Medical Equipment (DME). DME is equipment that is expected to make a meaningful contribution to the treatment of the patient's illness and is partially covered by Medicare. Shortfalls in funding can be made up via a one time $2000 grant from the MDA. Many local chapters of the MDA and ALS Association also have device loan programs.

Patients with dysarthria are referred to a speech-language pathologist who performs an assessment of the patient's needs and orders an AAC device best suited for the individual. Once the device arrives, the speech-language pathologist trains the patients and their family to use the device. In all stages of the disease, speech-language pathologists should be aware that some patients with bulbar onset ALS have cognitive deficits affecting word fluency, working memory and problem solving that can impact the type and complexity of AAC selected.

20.6. Swallowing and nutritional care

Management of dysphagia entails optimizing existing function along with preparation for further decline. Patients with dysarthria also usually have dysphagia, because the same muscles serve both functions. ALS patients with even a mild dysphagia can have calorie imbalance leading to weight loss (Slowie et al., 1983). Monitoring weight is the simplest way to assess caloric balance.

Overt signs of dysphagia include coughing or throat clearing following a swallow, the need for multiple swallows for one bite or sip and a change in voice quality indicating residue in the pharynx. Some ALS patients have a noisy swallow, which tends to occur with all consistencies and may be described as a "clunk" (Krivickas et al., 2005). Patients may report that they are not experiencing swallowing difficulties because they are careful when they eat or drink (Silani et al., 1998), but they may take longer to eat, which may be associated with reduced caloric intake (Morley and Thomas, 1999). Asking about length of meals and food choices and also questioning family members about the patient's ability to eat are all ways to recognize early dysphagia.

A speech therapist's examination provides information about safety or risk of aspiration, ability to maintain adequate nutrition and compensatory strategies. The examination uses a liquid (water), a solid (cookie) and a soft food (pudding or applesauce) to assess all phases of swallowing with different types of food. Between 42% and 50% of aspiration may be missed in the clinical examination (Smithard and O'Neill, 1992), so a modified barium swallow study (or video fluoroscopic swallow study, VFSS) and fiberoptic endoscopic evaluation of swallowing (FEES) may be necessary to more precisely document the presence of dysphagia.

Dysphagia is managed beginning with simple and moving to more complex strategies; the goals change as the disease progresses. Maximizing nutrition and preventing aspiration pneumonia are early goals, while maximizing nutrition remains a late-stage goal only in patients without a PEG. For those with PEG, eating and drinking for pleasure become the goal. Preventing pneumonia due to aspiration may not be entirely possible, even in patients who no longer eat by mouth, because of aspiration of secretions (Langmore et al., 1998). As dysphagia becomes more severe, it is necessary to teach patients about ways to prevent airway obstruction and to encourage caregivers to learn the Heimlich maneuver.

Typically, patients in the early stages report coughing on thin liquids and taking longer to eat meals. Strategies at this stage include teaching the patient to concentrate on eating and drinking and turning off the television or other distracters. Patients are encouraged not to speak or laugh with food in the mouth. Alternating solid and liquid sips may aid in clearing food from both the mouth and pharynx. Education regarding head positioning (i.e. the chin tuck position, in which patients sit up straight, position the head slightly forward with the chin down to help prevent food from going down the airway) (Logemann, 1997) and placement of food inside the mouth to better facilitate control during chewing and swallowing may be helpful.

As dysphagia becomes more severe, changes in food consistency and the use of postural maneuvers are necessary. Interventions include changes in the kinds of food consumed, the consistencies of food and frequency and duration of meals. Adaptive eating utensils from occupational therapists or PEG may be necessary. Soft moist foods (casseroles, well-cooked vegetables) are easier to chew and swallow. Maximizing calories without increasing food volume is also helpful. Patients often begin to eat less in the middle stages of the disease because of fatigue and the time it takes to finish a meal. It is not unusual to find some patients who need more than an hour to finish meals or who abort meals because of fatigue. Using jams, jellies, powdered milk, wheat germ, oil and other additives can boost calories without increasing the amount of food. Changing from three-meals-a-day to smaller more frequent meals may help meals seem less overwhelming. Other diet changes may include cooking foods until very tender, limiting raw vegetables and fruits, cutting food finely, using

pureed and/or strained foods, cooking with ground meats, using foods high in water content, like gelatin, puddings, gravy and sauces, and using beverages that are thick and dense in texture, such as tomato juice, and mixing commercial thickening agents (such as Thick-It®) (Krivickas et al., 2005).

As muscles atrophy, the intra-pharyngeal pressure necessary to swallow becomes insufficient, leaving residue in the mouth after the swallow. When pills become hard to swallow, strategies that help include taking one at a time, changing to liquid forms of medication, cutting pills with consent of the pharmacist, or placing pills in a puree consistency, like applesauce or pudding. Using a PEG tube, if one is in place, is the safest means to take pills for those with dysphagia.

In the later stages of the disease, swallowing may become profoundly impaired. If patients have a PEG tube and continue to eat for pleasure, they are usually only able to manage pureed foods and thickened liquids. Suction may be necessary to clear residue from the mouth and pharynx. Patients and caregivers should be told of the risks of continuing to eat. Treating dental caries may decrease the risk of infection when aspiration cannot be avoided (Langmore et al., 1998).

The overall goal of nutritional care is to match dietary intake to requirements. Recommended Dietary Allowances (RDA) that exist for healthy individuals may be inadequate for complex, progressive disorders like ALS. Dysphagia is one indication for PEG using the AAN Practice Parameters, but ALS patients can have reduced energy intake even without dysphagia (Hardiman, 2000). Conversely, not all patients with dysphagia suffer from malnutrition, but the presence of dysphagia does signify bulbar weakness and risk for inadequate nutrition. Nutritional recommendations should also be tailored to avoid overeating, which can also have adverse consequences. Body weight or body mass index (BMI) provide indirect measures of nutritional status and most ALS clinics now aim for a stable body weight. The ALSFRS includes questions on bulbar weakness, swallowing difficulties and arm weakness that can also be used to search for problems that can interfere with nutrition. Other scales, such as the ALS EATS scale (Luu and Kasarskis, 2004), ask about appetite and the social dimensions of eating. The 24-hour food recall assessment can provide an understanding of energy, protein and micronutrient intake, but it is not generally used in ALS clinics.

If a patient falls into negative energy balance, PEG is the current best alternative for nutrition. Theoretically, using NIPPV can reduce energy loss from overworked respiratory muscles. Therefore, NIPPV may have dual benefits of both supporting respiration and reducing the number of calories spent. This hypothesis is the subject of a current investigation (Luu et al., 2005).

20.6.1. Percutaneous endoscopic gastrostomy (PEG) and radiologically inserted gastrostomy (RIG)

Despite modifying swallowing techniques, altering food consistency and using adaptive equipment, many ALS patients are not able to maintain their oral intake to meet their nutritional requirements. Tube feeding or gastrostomy placement become the only option to deliver adequate nutrition. The types of enteral nutrition available are nasogastric feeding, PEG and percutaneous endoscopic jejunostomy and Radiologically-Inserted Gastrostomy (RIG). Although feeding through a nasogastric tube is the least invasive, it is impractical for the long-term. PEG placement is simple and safe in most instances, although the risks of complications increases as the FVC declines below 50% of predicted (Chio et al., 2004). One of the benefits of PEG is that patients can continue to eat pleasurable foods by mouth, while receiving the majority of their nutrition, hydration and medications via gastrostomy. PEG is generally inserted by a gastroenterologist in an endoscopy suite, with patients admitted and discharged the same day or staying overnight for observation in the hospital.

Nutritional support administered via PEG is effective in stabilizing body weight. Early placement of PEG helps prevent weight loss in most cases and may be associated with improved survival (Desport et al., 2000). Currently, PEG placement is recommended when the FVC is above 50% of predicted in patients with weight loss or dysphagia (Miller et al., 1999a). Although PEG can be successfully placed in patients with a FVC < 50%, particularly when NIPPV is used during and briefly following the procedure, caution is still advised because morbidity and mortality may be higher. RIG, performed by an interventional radiologist, may be a slightly simpler procedure in those with low FVC (Chio et al., 2004), but studies are needed to determine its best role (Shaw et al., 2004). Currently, it may be best to use the procedure with which the ALS center has the most experience, either PEG or RIG. It is also important that the ALS team establishes a care path from the discussion and planning stage to post-procedural nutritional care, so that the care provided by all members of the team, including the gastroenterologist or interventional radiologist and nutritionist, is well coordinated.

20.6.2. Nutritional formulations

The feeding schedule through gastrostomy is similar to regular oral feeding, generally 3–5 times a day. Although nutritional supplementation is usually administered via

PEG as bolus feeding, patients may report bloating following meals, especially in those with nutritional deprivation prior to PEG. Slowing the bolus or using continuous feeding with a pump may help. Many patients require titration, starting with diluted supplements until the feedings are tolerated and the nutritional goals are met.

There are a variety of commercial nutritional supplements, which range from high calorie to high protein to disease-specific compositions. ALS patients are usually encouraged to focus on high calorie supplements, unless there are other medical conditions such as diabetes mellitus or cardiovascular disease. If weight loss persists or progresses, the daily amount can be gradually increased under the guidance of a nutritionist.

Supplements are easy to use and, for most, will maintain or increase body weight. Commercially available products range in price; most health insurance plans provide coverage, particularly for patients with cachexia. A registered dietician or nutritionist can identify the best formulations for each patient.

20.7. Respiratory care

When respiratory muscles become weak in ALS, symptoms and signs develop that can be used as clues to determine the timing of respiratory support. Early symptoms of respiratory insufficiency are usually due to diaphragm fatigue and may be most prominent during sleep or while supine. Fatigue and weakness of the diaphragm may be noticeable only at bedtime at first, resulting in the inability to lie flat. Later, sleeping upright may be the most comfortable position. Some other common early symptoms include insomnia, frequent awakenings, fatigue, vivid dreams, daytime sleepiness and poor concentration. Nocturnal oxygen desaturation can occur because the diaphragm performs most of respiratory muscle function during sleep and because of the normal predisposition to hypopnea during REM sleep. Airway obstruction can also occur due to a combination of bulbar weakness and diaphragmatic dysfunction, but tends to decrease with disease progression (Santos et al., 2003).

Initial symptoms of daytime diaphragm fatigue may occur only during exertion, giving patients a subjective sensation of inadequate respiration but not necessarily tachypnea. With disease progression there is an increase in breathing effort while active. Eventually, even slight exertion such as bathing and dressing brings on shortness of breath. Since patients may unconsciously adjust their activity to reduce symptoms, early symptoms must be sought by specific questions, particularly those relating to disrupted sleep and daytime fatigue.

In more advanced muscle weakness, hypoventilation results in hypoxemia and hypercapnia. Symptoms of respiratory failure in ALS patients are more often related to hypercapnia, which causes morning headaches, nightmares and yawning, than to hypoxemia. Other factors may also contribute to hypercapnia such as infection with fever and underlying lung diseases such as emphysema. Bulbar weakness and weakened cough can lead to excess secretions and poor airway clearance, which can cause aspiration and pneumonia. In some patients, respiratory function plateaus and stabilizes, but in others respiratory weakness progresses, and shortness of breath and use of accessory muscles eventually occur at rest; CO_2 narcosis may ensue.

Rarely, patients present with respiratory failure, which can also be an early feature in bulbar-onset ALS due to involvement of the phrenic nuclei (Chen et al., 1996). Respiratory failure as the initial symptom of ALS may pose a diagnostic challenge as these patients may first present to pulmonary and critical care physicians before a diagnosis is established. When respiratory failure is the initial symptom of ALS, it usually portends a poor prognosis and rapid progression (Chen et al., 1996).

Physical signs of respiratory weakness include tachypnea, paradoxical breathing, use of accessory muscles, low speech volume, ineffective cough and speech difficulties. Breath-to-breath variations in the inspiratory movements of rib cage and abdomen, 'respiratory alternans,' are a sign of respiratory muscle fatigue and may indicate impending respiratory failure. Percussion of the base of the lung in inspiration and expiration can aid in determining whether the excursion of either hemidiaphragm is impaired; the excursion may diminish with time (Heiman-Patterson and Aboussouan, 2005). Abnormalities may be most evident when lying flat. There may also be coarse breath sounds or inspiratory crackles on auscultation, particularly at the lung bases due to poor bronchial clearance, patchy atelectasis, aspiration or infection.

Despite the importance of respiratory muscle function, there is no consensus regarding the single best spirometry test to measure pulmonary function in ALS. Forced vital capacity (FVC) is the measure most often used and has been shown to decline predictably, but it can be difficult to obtain reliably, requires training to perform (American Thoracic Society, 1995) and measurements may be less reproducible at advanced stages of the disease (Aboussouan et al., 2001). The AAN practice parameter and a consensus statement from the American College of Chest Physicians recommend counseling on the use of NIPPV with the onset of respiratory symptoms or when the FVC drops to about 50% of predicted value (Anon, 1999; Miller et al., 1999a). The FVC may drop from sitting to supine positions, with decreases of more than 20%, and so performing the test while the

patients lie down may increase its sensitivity (Loh et al., 1977).

The maximal inspiratory and expiratory pressures (MIP and MEP) are also often reduced in ALS patients and correlate with respiratory muscle weakness (Jackson et al., 2001; Heiman-Patterson and Aboussouan, 2005). A reduction of MIP to less than 60 cm H_2O is a predictor of reduced survival (Gay et al., 1991) and is a criterion for initiation of NIPPV (Anon, 1999).

Placement of a transesophageal catheter allows measurement of esophageal (Pes, a reflection of pleural pressure) and transdiaphragmatic pressure for more direct estimation of diaphragmatic muscle strength. In one study, a Pes < 30 cm H_2O was associated with increased mortality in patients with ALS (Vitacca et al., 1997). Sniff nasal pressure (SNP), a newer noninvasive measure of inspiratory pressure, estimates intrathoracic pressure and may provide an early marker of respiratory muscle weakness. It decreases predictably over time in ALS patients, predicts survival (Morgan et al., 2005) and may be a better predictor of hypercapnea than either FVC or maximal inspiratory pressure (Lyall et al., 2001).

An awake $PaCO_2 \geq 45$ mmHg is another criterion for initiation of NIPPV (Anon, 1999), but arterial blood gas (ABG) measurements remain near normal until the final stages of ALS (Krivickas, 1998). Arterial blood gases are best used to justify NIPPV in patients who are unable to perform spirometry, and to guide adjustments to NIPPV.

A sleep study with nocturnal desaturation less than 89% for at least 5 consecutive minutes may be a marker of early diaphragmatic dysfunction and is also an indication for initiation of NIPPV (Anon, 1999). Recently, serum chloride, a metabolic marker of respiratory acidosis, was identified as a prognostic factor in ALS patients. Low levels may indicate impending respiratory decompensation (Stambler et al., 1998), but chloride levels are less helpful in early respiratory insufficiency. Controlled prospective studies are needed to determine the most sensitive noninvasive marker of respiratory failure in ALS.

20.7.1. General respiratory care

Respiratory care in ALS begins at diagnosis with a program that is aimed at treating symptoms, preventing infections, improving function and predicting the timing of respiratory support. Symptoms usually begin when the FVC reaches 50%, but when they occur sooner they are indications for additional testing, such as overnight oximetry. As weakness progresses, there is an increasing risk of sudden life-threatening respiratory failure due to aspiration, mucous plugging or infections. Malnutrition can cause more rapid weakening of respiration. Since many of these complications can be managed medically, it is possible that respiratory failure can be delayed by early interventions.

Once diagnosed, ALS patients ideally should receive baseline respiratory evaluations – a baseline evaluation by a pulmonologist can be particularly helpful – and thereafter at least every 3–4 months, with more frequent assessments if weakness progresses rapidly. Other respiratory problems, such as asthma or chronic obstructive pulmonary disease (COPD), are diagnosed and treated aggressively. Patients are instructed to avoid activities that may worsen respiratory function such as cigarette smoking and exposure to dusts, fumes or people with upper respiratory infections. Pneumovax immunization and yearly influenza immunizations help reduce pulmonary infection. Periodic pulmonary function testing is especially important in ALS, since early signs of respiratory impairment can be missed clinically (Fallat et al., 1979).

Bronchitis or lower respiratory tract infection are evaluated with chest X-ray and treated accordingly. Some patients with recurrent infections may need to have antibiotics and instructions available at home so that treatment can be started quickly. Occasionally, temporary ventilator support using noninvasive ventilation or endotracheal intubation is necessary while more severe infections are treated. Some patients benefit from postural drainage and percussion to help clear secretions and the use of an incentive spirometer may help prevent atelectasis. Theophylline may improve diaphragm function and strength (Schiffman and Belsh, 1989). Patients with bulbar weakness are taught techniques, such as the chin tuck, to avoid aspiration.

Maintaining clear airways is an important part of pulmonary care in ALS since secretions can lead to plugging, atelectasis and infection. Treatments are directed both at decreasing and thinning the secretions and increasing clearance. Peak cough flows of greater than 160 liters per minute are necessary to maintain clear airways (Bach, 2002). Once the peak cough flow declines to 270 liters per minute, a program to assist coughing may be helpful. Interventions that facilitate the clearance of secretions consist of air stacking (Bach, 2002; Lahrmann, et al., 2003), assisted cough, either manually or mechanically through an insufflator-exsufflator (Bach, 1993; Winck et al., 2004; Fig. 20.4) and use of a suction machine to remove oropharyngeal secretions (Heiman-Patterson and Aboussouan, 2005). Air stacking, which strengthens the cough by initiating it at higher lung volumes, is achieved by glossopharyngeal breathing (stacking gulps of air behind a closed glottis) or by delivery of air through a manual resuscitator or a volume limited ventilator (Kang and Bach, 2000). Cough can also be

augmented by an abdominal thrust timed with the cough or by application of negative pressure through a mask. The mechanical insufflator–exsufflator combines air stacking and cough enhancement by cycling from positive pressure that enhances lung volumes (insufflation), to negative pressure that facilitates airway clearance (exsufflation) (Bach, 1993; Winck et al., 2004). High-frequency chest wall oscillation may help clear airways and is now being investigated in ALS.

Oxygen may be used to treat hypoxemia and is indicated for persisting PaO2 ≤ 55 mmHg, or an SaO2 ≤ 88% by either ABG or oximetry. However, since oxygen can aggravate hypercapnia, it should be titrated to the lowest possible level using a pulse oximetry target of 92–94%. Ideally, oxygen is used with NIPPV, to avoid hypoventilation or as part of palliative care.

20.7.2. Noninvasive ventilation

As respiratory muscle strength deteriorates, ventilatory support usually becomes necessary. A variety of noninvasive ventilators have been developed to avoid the need for invasive mechanical ventilation in late stages of respiratory insufficiency (Bach, 1995). In recent years, nocturnal noninvasive positive-pressure ventilation (NIPPV) has become the standard treatment for ALS patients with chronic respiratory insufficiency (Hillberg and Johnson, 1997). Noninvasive ventilation can allow patients to continue to speak and eat by mouth. The bi-level intermittent positive pressure noninvasive ventilator closely imitates physiologic function; it is triggered by the patient's inspiratory efforts, reduces the work of breathing and improves gas exchange and sleep quality (Heckmatt et al., 1990).

Those who can tolerate regular use of NIPPV may live longer than those who cannot (Aboussouan et al., 1997) and one retrospective study showed that those using NIPPV have slower decline in vital capacity (Kleopa et al., 1999), although other studies gave conflicting results (Aboussouan et al., 2001). Other potential benefits of NIPPV in ALS include improved cognitive function (Newsom-Davis et al., 2001) and quality of life (Bourke et al., 2003).

Although research has consistently demonstrated the benefits of NIPPV, there is no consensus on which physiologic marker or symptom is best used to trigger its initiation. A treatment algorithm in the evidence-based review of the literature for the AAN suggests that NIPPV should be instituted when patients have symptoms or when FVC reaches 50% (Miller et al., 1999a). The Health Care Financing Administration (HCFA) has established criteria for coverage of respiratory assist devices. Medicare will pay for NIPPV once a patient has documented symptoms of sleep-associated hypoventilation, an ABG with PaCO2 ≥ 45 mmHg, nocturnal oximetry demonstrating oxygen desaturation ≤ 88% for at least five continuous minutes, a FVC < 50% of predicted or a mean inspiratory pressure < 60 cm H_2O (Anon, 1999). Randomized trials should help clarify the best time to start NIPPV.

NIPPV is usually prescribed using inspiratory pressure (IPAP) settings of 6–10 and expiratory pressure (EPAP) settings of 4–6, and then adjusted according to nocturnal oxymetry and patient comfort. Patients can select different masks and can use a chinstrap to keep the mouth closed while using a nasal mask. Symptoms that limit comfort such as nasal congestion, mucosal dryness and claustrophobia may need to be treated. Once the NIPPV is initiated, the ventilator is adjusted periodically based on the patient's symptoms, evidence of increased CO_2 or nocturnal hypoxemia.

Volume-limited ventilation may be used to facilitate air stacking when needed to enhance cough efficacy or to deliver higher volumes when necessary to control hypoventilation. Negative pressure noninvasive ventilators, including the cuirass and tank ventilator, have limited usefulness in ALS because they can exacerbate airway collapse in patients with weakened bulbar muscles.

20.7.3. Tracheostomy/permanent ventilator

As weakness progresses, NIPPV may be inadequate to control symptoms and patients may ultimately need invasive ventilation with a tracheostomy to maintain adequate air exchange and control of the upper airway. The decision to undergo tracheostomy and invasive ventilation is very personal and often difficult. It requires extensive education that includes both the family and patient. Those on ventilators need 24 hour per day supervision from family members and nurses, which can cost up to $335,000 per year (Albert et al., 1999; Lechtzin et al., 2004). Those with ALS who are most likely to choose mechanical ventilation often have a gradually progressive disease that has allowed accommodation, are able to communicate, have a supportive family and the financial resources necessary and derive pleasure from non-physical activities (Moss et al., 1996). Ultimately, only 2–5% of ALS patients choose invasive ventilation (Lechtzin et al., 2004), but, of those cared for at home, 90% are glad they chose to be ventilated and would do so again (Moss et al., 1993). Some patients, who are unable to decide for or against tracheostomy, receive mechanical ventilation on an emergency basis due to respiratory failure and are then cared for at home or in nursing homes.

Once patients have a tracheostomy, a team oversees the needed care. The tracheostomy itself is performed

by an otolaryngologist; a pulmonologist adjusts the ventilator; a gastroenterologist may perform a gastrostomy to ensure adequate nutrition; a speech therapist assists with identifying augmentative communication devices; and physical and occupational therapists help to maintain independence and comfort to the extent possible.

Some patients may wish to end ventilatory support if quality of life is poor or once they become locked in and lose the ability to communicate. It is helpful to know their wishes in advance, so that appropriate decisions can be made for those who lose communication. A careful and thoughtful approach is necessary, with thorough discussions of the decision and its ramifications. Psychiatric counseling may ensure that the patient fully understands and that depression is well treated. Once the decision is finalized, palliative care is initiated as the ventilator is turned off. At the time of ventilator withdrawal, the patient and family should be in a quiet setting with adequate support from the physician, nurse and hospice. Benzodiazepines and opioids are administered to avoid air hunger and anxiety.

20.8. Depression and anxiety

Depression and anxiety are common in ALS and may limit quality of life for patients and caregivers (Ganzini et al., 1999; Lou et al., 2003). Patients who are depressed or anxious may have less reserve to combat the disease and may be less able to make decisions about their care. Fortunately, there are many effective treatments. The challenge is the recognition of subtle signs and relating these to patients in such a way that they accept treatment that may help relieve suffering. Onset of depression is often insidious and patients can minimize symptoms for fear of being labeled with a psychiatric illness. Patients with reactive depression in response to the diagnosis may have few overt signs.

Anxiety may co-exist with depression and feelings of nervousness, insomnia, irritability and restlessness can be treated with medications that relieve both. The treatment of anxiety must also include a search for physical causes such as dyspnea or loss of mobility. Response to medication is often idiosyncratic; drugs may be selected as much for a secondary benefit such as sedation for insomnia or increased appetite for anorexia. Medication trials, in the absence of prominent side effects, should last at least 4 weeks and combinations of medications and counseling may be required.

20.9. Clinical trials

One component of the treatment of ALS is the participation in research. Clinical trials offer patients the opportunity to join in the search for more effective treatments and to have frequent evaluations by the ALS team. These evaluations, which often occur on a monthly basis, provide the patients with a level of attention that could not be obtained otherwise. The patients usually meet with the neurologist, nurse and physical therapist during the research visit and any problems that have arisen since the previous visit are treated. The psychological benefit of participating in research can be great, but patients must understand the overall goal of obtaining clear data so that the trial's conclusions will be valid. Adherence is an important factor in the success of any trial. For this reason, investigators who conduct treatment trials must explain the importance of adherence and the purpose of research to patients before they enroll (Kurtzke, 1986). Patients are prone to dropout of trials if benefits do not occur and side effects develop. They may become depressed and lose interest in the trial as their disease advances. They may dropout of a trial to participate in a new and recently publicized study or they may secretly use other available investigational agents. During clinical trials, it is important to monitor adherence and to implement effective adherence-improving strategies (Gordon, 2005). Depression should be treated aggressively. Study nurses and physicians should spend time personally with patients, discussing difficulties patients face while participating in the study. A discussion of the importance of scientific research and the need for complete data in testing medications, not only prior to enrollment but during follow-up visits, may help patients understand their vital role in the process and let them know that the trial is important to the physician. In the past several years, patients have adopted an activist mode (Miller et al., 1999b). They use the Internet to share information (and sometimes misinformation) on investigational drugs and often advocate multiple medication usage. Patients continue to seek new, ostensibly more effective clinical trials. Under these circumstances, establishing and maintaining a study population becomes increasingly difficult (Mitsumoto et al., 1998).

The best solution to excessive dropout is prevention. A recent Dutch study had no loss of data due to dropouts other than death (Groeneveld et al., 2003b). Investigators traveled to patient's houses when necessary to collect data. Future US trials will emphasize methods of reducing dropout, including easy to administer outcomes, infrequent patient visits and home visits if necessary. Ideally, investigators are very honest with their patients about the lack of effect of current therapies and the need for good trials. Through open and honest conversations, we may see a future where all ALS patients will be enrolled in clinical trials.

20.10. Psychosocial care

The term *psychosocial* refers to the aspects of being that encompass emotional, social and intellectual realms (Borasio et al., 2005). Psychosocial well-being may promote survival in ALS; patients with a low quality of life (psychological distress) are more likely to die sooner than those with a high quality of life (McDonald et al., 1994). Psychosocial care in ALS is complex and requires exploration of psychosocial issues in the context of a progressive illness. The importance of psychosocial care was emphasized in the recent Robert Wood Johnson Initiative to Improve End-of-Life Care in ALS (Mitsumoto et al., 2005b). Educating patients and their families about ALS is the first step in providing psychosocial care. Disclosing the diagnosis and subsequent bad news as the disease progresses so that patients and families feel engaged, supported and even hopeful is an art and requires adequate patient and family education. Foremost is respect for the patient's autonomy, allowing the patient to make decisions about care at the end of life.

20.10.1. Caregivers

Traditionally, care in ALS has focused on the patient. However, family members act as advocates, provide and oversee care, serve as companions and make decisions on behalf of incompetent patients (Levine and Zuckerman, 1999). In the current health care climate, patients are increasingly cared for at home. The financial and physical burdens of care, therefore, have shifted from the hospital to the families, in part to reduce costs. More than half of patients enrolled in the ALS Care Database die at home and, thus, a family member, most often the spouse, usually is the principal caregiver (an 'informal' caregiver as opposed to a professional home care nurse) (Bradley et al., 2001). Consequently, the home may become a place of sickness, even hospital-like, and what once was a place of relaxation and enjoyment is transformed into a place of work and stress for family members.

Family caregivers may experience burden during all stages of the disease. It occurs in part because, at least in the US, formal home health care for patients with ALS often is inadequate to relieve the burden placed on caregivers. Primary caregivers spend a median of 11 hours each day caring for patients with ALS, even despite having home care assistance (Krivickas et al., 1997; Borasio et al., 2005). Accordingly, primary caregivers report feeling either physically (42%) or psychologically (48%) unwell (Krivickas et al., 1997). Their outside activities may be severely curtailed (Gelinas et al., 1998). A recent study found that patients with tracheostomy had a quality of life as good as that of patients with non-invasive ventilation but that the caregivers of the tracheostomy patients had a much greater burden (Kaub-Wittemer et al., 2003). Anxiety and depression in caregivers correlate with the degree of the patient's functional impairment (Goldstein et al., 1998).

Surveys of caregivers and patients indicate that the lack of readily available resources and medical information about the disease, and how to cope as the disease progresses is a great stressor (authors observation based on a focus group study on caregiver issues, June 2004). One source families go to most often for information is the Internet, which may be inaccurate or confusing. Providing ALS-specific literature designed for patients and families, as well as the telephone numbers and websites of reliable voluntary organizations, is a starting point. Educational and support group sessions for patients and caregivers that are organized by the ALS Association, the Muscular Dystrophy Association and other local or regional ALS organizations may also be helpful (Mitsumoto and Munsat, 2001).

Caregiving can also be rewarding. One interview-based study of 56 patients in the terminal stages of ALS and their 31 caregivers found that neither patients nor caregivers had significant depression (Rabkin et al., 2000). Although caregivers were distressed, their perception of the amount of burden was associated with finding positive meaning in caregiving. Thus, clinical depression in caregivers is not inevitable. Concordance between patient and caregiver distress was high, suggesting that attending to the mental health needs of caregivers may alleviate the patient's distress as well (Rabkin et al., 2000).

20.10.1.1. Causes of distress from caregiving

In ALS, the caregiver's perception of burden correlates in part with a loss of intimacy with the patient, a consequence of changes in the patient's cognitive, behavioral and communication functions (Goldstein et al., 1998). Changes in the patient's ability to interact socially also correlate with the extent to which caregivers feel that the illness affects other areas of their lives, the extent to which the patient dominates their thoughts and the extent to which they can control their reactions when thinking about the patient (Goldstein et al., 1998). In a study of other disorders, stress develops in the relationship because of an imbalance of giving and receiving (Grand et al., 1999). The caregiver may feel trapped in a cycle of giving only.

Caregiver distress in other diseases is also influenced by how well the caregiver has mastered care giving (Yates et al., 1999). Tasks that are required for care of the patient may indirectly lead to caregiver

depression through their effect on the caregiver's perception of work overload. Caregivers with high levels of mastery of caregiving and emotional support are at lower risk for depression, regardless of the number of primary caregiving stressors, indicating the importance of emotional support and skills training for the caregivers (Yates et al., 1999).

Among caregivers for cancer patients, those who also have physical problems of their own are at risk for psychological morbidity that may become apparent at a later time (Jepson et al., 1999). Caregivers who have lower subjective burden practice more health-promoting behaviors than those with higher subjective burden, which may be an important factor for caregivers who have physical difficulties themselves (Sisk, 2000). Elderly spousal caregivers may require special consideration, particularly those who have mental or emotional strain, because they are at higher risk for mortality (Schulz and Beach, 1999). Older married couples should be evaluated as a unit, both in terms of their health status as well as the caregiving demands that exist in the home, and ALS clinicians must pay close attention to the spouse's physical well being.

20.10.1.2. Caregiver role and awareness of dying

The awareness that a loved one is dying develops gradually and is characterized by uncertainty and anguish, followed by hope that dying will be far in the future, pretending that nothing has changed, and then preparing for the death (Yates and Stetz, 1999). ALS health professionals try to identify stages in the process for each family and give support accordingly. Support may include helping family caregivers to maintain hope, sustain social relationships and prepare for the patient's death. Further research as to how family caregivers use these strategies for managing their developing awareness of dying is needed in ALS (Yates and Stetz, 1999; Cobb et al., 2001).

Men and women differ in their selection of informal caregivers (Allen et al., 1999), with gender role norms being a main factor. Women are only one third as likely as men to select their spouses as caregivers. However, spouses, regardless of gender, who describe their mates as confidants, meaning that there is mutual trust, are three times more likely to name them as caregivers than those who do not; gender role norms may be the most important determinant of whether the patient selects the spouse to be the caregiver, but an emotionally close marriage is also important. The closeness of a couple's long-term relationship also affects caregiver distress. If the relationship has been close, the caregiver's restriction of activity outside the home is predicted by the degree of loss of intimacy and affection rather than by the severity of patient symptoms (Williamson et al., 1998). The loss of intimacy and affection also predicts caregiver depression. Conversely, among caregivers in less affectionate relationships, activity restriction is influenced more by symptom severity.

Approximately one third of caregivers in the US are male spouses. Caregiver husbands experience changes in their household responsibilities, social integration, relationship and well-being (Kramer and Lambert, 1999). Adult daughters may provide care to elderly parents (Dautzenberg et al., 1999). A major stressor for these women is the lack of any other major social role; the absence of social roles rather than having many roles (e.g. caregiver, mother, working outside the home) is associated with distress.

Use of professional home health care does not necessarily relieve burden from family caregivers. Professional health care is expensive, a stressor by itself, is often of limited duration and the personnel may be inexperienced in caring for those with ALS (Krivickas et al., 1997; Goldstein et al., 1998; Yates et al., 1999). Considering who provides the caregiving may help in anticipating whether a family will use formal home care services. Those most likely to use formal caregiving include physically impaired, employed or male caregivers (Houde, 1998). Patient characteristics that predict the use of formal home care include female sex, recent hospitalization, marked physical impairments, Medicaid insured, older age and low number of household members.

20.10.1.3. Respite service for family caregivers

In the US, respite services are not used frequently even though Medicare covers the expense (Borasio et al., 2005). Spouses who do use respite report less intimacy within the marriage (Braithwaite, 1998). One weakness of the current respite structure is the lack of skills training, education and emotional support that help support relationships and reduce strain after the respite period has ended (McNally et al., 1999). A more care-centered approach could address some of these deficiencies.

20.10.1.4. Financial concerns

In addition to the emotional and physical stresses, terminal illnesses also produce financial strains for families. As much as 10% of family income may be spent on health care (Emanuel et al., 2000). Despite the economic burden, no financial support is available to family caregivers in the US as it is in other countries (Wahner-Roedler et al., 1999). Home care costs for ventilator-dependent patients may be higher than for hospitalized patients, when all aspects of home care are considered, including lost wages for the family caregiver and the costs of professional home care (Sevick and Bradham, 1997). A referral to a social worker early

in the course of the illness can help families make financial plans for the future.

20.10.1.5. Improving caregiver coping

Caregivers need to feel they have both social and medical support. The ability to cope is associated with the number of social groups to which the caregiver belongs and with their satisfaction with formal home health care (Goldstein et al., 1998) and when physicians are perceived to listen to patients' and caregivers' needs (Emanuel et al., 2000). A well-developed religious or spiritual belief system is also associated with lower levels of depression and feelings of submersion in the caregiver role (Chang et al., 1998).

Patients and family members may benefit from formal counseling, ideally participating in therapy as a unit. Family therapy can help the family to maintain a sense of normalcy, develop mutual support and understand mechanisms to reduce stress. Few mental health professionals have experience in ALS, but referral to therapists who specialize in chronic or terminal illness is helpful. ALS support groups are an alternative for those who refuse or cannot pay for counseling. The value of interacting with others in the same situation cannot be underestimated.

20.10.2. Intimacy and sexuality

Even though autonomic function may be unaffected by ALS, sexual dysfunction can occur in ALS. Medications used for depression, spasticity and sialorrhea can all impact sexual function. Patients with ALS also experience a decrease in sexual activity that they report is due to physical weakness and the loss of a positive body image (Mitsumoto et al., 1998; Wasner et al., 2004). Weakness, fatigue, spasticity, communication difficulty and respiratory distress, as well as fear, anxiety and depression may interfere with sexual performance. The loss of self-esteem, fear of losing the affection of one's partner, fear of impotence, loss of interest in sex and fear that the partner will "look elsewhere" may all influence sexuality in ALS (Oliver, 1993).

Patients, caregivers and doctors may think that, when dealing with a life-threatening illness, issues around sexuality may not be as important (Pernick, 1994). Many patients and caregivers do want to discuss the issue with either the neurologist or nurse, however. Neurologists may feel inadequately trained to provide recommendations, but an open honest attitude in soliciting responses about sexual function may be enough to broach the topic and open communication. The issue can be raised indirectly by providing literature containing information on the issue of sexuality in ALS (Mitsumoto et al., 1998).

Most ALS patients have limited physical ability but normal sexual desire and should be able to enjoy sexual intimacy. Methods that have been suggested to work within the limited physical constraints include role changes within the partnership, more comfortable positions during intercourse and other ways to express affection and intimacy, such as mutual masturbation (Oliver, 1993).

20.10.3. Children

While young children may not be directly involved in care and decision-making, they are deeply affected emotionally by the changes occurring in the family. Because children often have difficulty articulating their emotions, changes in their behavior may give clues to how they are feeling and provide insight as to how to give psychosocial support to the child (Borasio et al., 2005). Children benefit from respect and acknowledgment of their emotions. They need clear, simple, information about what is happening and what might happen next. Reassurance will help them learn they did not cause the illness and cannot catch it, and to anticipate what is going to happen to the family and their own care after the person has died. They need to be involved in helping the patient, and to have a chance to talk about feelings to adults with whom they can safely share their feelings. Means for self-expression, such as drawing, writing, playing games and reading material that relate to loss, aging and the natural cycle of life are helpful. Children can be told that life during and after ALS goes on, that it is all right to have fun and that they are not alone in their experience. Children, like caregivers, can benefit from family therapy (Borasio et al., 2005).

20.11. Hospice and Palliative Care

Currently, all of ALS care is palliative. The palliation at the very end of life, when death is near, is a time when specialist care is needed to avoid physical suffering (Sykes, 2005). The transition to this phase is less marked if built on a foundation of continuous palliative care from the beginning. Around 60% of ALS patients die within 24 hours of deterioration in their clinical condition and some die suddenly. Advance directives can help prevent tracheostomy ventilation being instituted in a crisis, but the key to good care is ongoing and open communication between the patient and healthcare team.

Anticipating symptoms before they occur is crucial to proper management. Medications, including opioids, sedatives and anticholinergic agents, can be made available in the home as respiratory capacity declines, so family members can, under the direction of the

neurologist and hospice team, administer the drugs when symptoms develop. Morphine is effective for treating pain, breathlessness and nocturnal discomfort in ALS long before the terminal phase of the illness (Oliver, 1998). Doses can be increased to control new symptoms, but dose escalation may not be necessary and there is no evidence that opioids shorten life. Non-verbal signs of distress, such as groaning, grimacing or restlessness, may need to be monitored and treated. The relief of distress is the goal of dose titration and some sedation may be necessary.

In patients with gastrostomy, the route of administration of medication can remain unchanged toward the end of life. In those who require a change in route of administration, subcutaneous, intravenous, rectal or transdermal dosing are possible, but require recalculation of the equivalent dose (Sykes, 2005). Non-steroidal anti-inflammatory drugs, if previously effective, can be administered by suppository.

Anxiolytics are helpful for symptoms of anxiety and restlessness and a benzodiazepine can be used in combination with morphine. Theoretically, their action is complementary to that of opioids (Leach, 2004). Midazolam is an appropriate choice of drug when patient, family and professionals agree that life is being prolonged by assisted ventilation to a degree that is intolerable. The use of these drugs is also indicated to control of any distress that might occur during ventilator withdrawal (Von Gunten and Weissman, 2003).

It may not be possible to stop noisy breathing. Consequently, the first step in management is to explain to the patient's family the causes of noisy breathing and to reassure them that the patient is not aware of the sounds (Bennett et al., 2002). Anticholinergic agents may help to control rattly breathing in those with reduced ability to cough or unsuccessful treatment of a lung infection. Atropine tends to be arousing, but other anticholinergic medications are used commonly.

The grounds of hope can change for a person with a terminal illness. The quality of a patient's dying is a powerful memory for those left behind and good care at this time is crucial in shaping public attitudes toward disability and serious illness. Hospice teams can provide both adequate symptom management and also counseling and emotional support for patients, families and caregivers.

20.11.1. Spirituality

Part of palliative care is tending to the spiritual needs of the dying (World Health Organization, 1990; Mitsumoto et al., 2005b), a difficult task because health care professionals may not have adequate knowledge of spiritual issues. Patients' spiritual health affects their decisions about medical care. Those with greater spirituality are more likely to have advance directives, less likely to undergo gastrostomy, less fearful of death and dying, less likely to participate in support groups and more likely to choose a natural death rather than mechanical ventilation (Murphy et al., 2000). Religion and spirituality ease adaptation and the ability to cope with the process of dying (Murphy et al., 2000; Borasio, 2001).

Spirituality can be defined as the need to find a sense of meaning in present existence (Sykes, 2000). It may or may not involve a religious framework. Most people engage in this search, particularly as they approach death. Religion may serve as a source of spiritual fulfillment for some, but attending religious services does not necessarily correlate with spiritual well being (Borasio et al., 2005).

Patients may benefit from conversations about spirituality with their physicians (Balducci and Meyer, 2001), but collaboration with chaplains and other spiritual counselors is also important. Cross-cultural differences in the spiritual attitudes and needs of patients from diverse backgrounds should be acknowledged. Sensitivity requires a basic understanding of the different ways death and dying are viewed within the major religious traditions. Hospice staff often have specific training in these issues and also usually have contacts with ministers of various faiths who can be consulted if needed.

Spiritual care involving the whole family can ease bereavement, which can be severe and prolonged in ALS (Martin and Turnbull, 2000), possibly because of the burden of care in the months preceding death. Screening scales are available that can be used to assess patients' spiritual needs (Hickey et al., 1996; Salmon et al., 1996; Hatch et al., 1998; van Wegberg et al., 1998; Dal Bello-Haas et al., 2000). Although the instruments have not been formally validated in ALS, they can be an opportunity for the physician and patient to discuss spiritual issues in a non-threatening way. Most patients appreciate the attempt. Because some patients may interpret the discussion to mean that the end is near, it is best performed early in the course of the disease.

Achieving a feeling of completion is important for dying patients, as it is easier to leave a life when spiritual, psychosocial, family and financial issues have been addressed. Regardless of a patient's religious beliefs or spirituality, closure is an integral part of the dying process and can begin any time before the actual process of dying. Closure may include a life review, looking back as to what has been achieved or left behind. A sense of reaching closure can be an indicator of the patient's readiness for death.

Spiritual counseling may also help patients come to terms with missed opportunities. If counseling does not

help, and the patient dies in despair, health care providers must remember that it is not their fault. The professionals' task is to remove obstacles that might interfere with such acceptance and to provide gentle counseling for the patient.

20.11.2. Bereavement

Bereavement is a process that begins when something is lost or someone dies (Borasio et al., 2005; Mitsumoto et al., 2005b). Grief is the feeling of sadness associated with the loss. Mourning is the expression of sorrow and grief. Bereavement is usually considered in the context of death and focuses on the loss of an individual. However, one can also mourn the loss of physical function and independence, as occurs in ALS. Thus, bereavement affects both the patient with ALS and the family (McMurray, 2000). Recognizing that bereavement can encompass more than mourning the loss of life can help the patient, caregiver and family members understand many of their feelings and may lessen distress.

Because grieving is experienced not just by survivors, but also by the patients as they lose abilities, it is necessary to regard bereavement on a long time scale, starting with the diagnosis, extending through the course of the disease and lasting beyond the time of death. The progressive nature of ALS allows family members to prepare for the death of their loved one. The bereavement process in ALS, therefore, differs from bereavement accompanying a sudden and unexpected death. Bereavement has been divided into stages (Kubler-Ross, 1969), but it may be more practical to view the resolution of grief as a series of tasks: (1) accepting the reality of the loss, (2) working through the pain of grief, (3) adjusting to the loss and (4) relocating emotionally and moving on (Worden, 1991; McMurray, 2000).

Issues unique to chronic terminal illnesses such as ALS include anticipatory grief and final grief. Anticipatory grief can begin at diagnosis (Ackerman and Oliver, 1997) and may be magnified by imagination and inaccurate or limited knowledge concerning the manner of death in ALS. Bereavement may develop gradually, with the sense of loss growing as specific functional losses occur. Bereavement also may subside but then recur when the health care professional periodically evaluates the patient's loss of abilities. Anticipatory grief may help the family and caregiver prepare for the patient's death and may even lessen the intensity and duration of bereavement after death. Final grief starts at the time of death. Because the progressive loss of the patient's functional abilities leads to a loss of the caregiver's independence, physical and emotional relief may temper the intensity of final bereavement when the patient dies. On the other hand, the relief from the burden of care can cause caregivers to feel resentful because their work and identity as caregivers no longer exist (Murphy, 2001).

Family relationships influence anticipatory and final bereavement. The illness may bring family members together. Family reunions are an important part of preparing for the end-of-life process because a united family is likely to be stronger and able to provide mutual support (Murphy, 2001). Support may be greater when multiple generations are involved. For example, grandchildren can provide a sense of balance between aging and death and youth and promise. Exposing a child to infirmities and death as natural aspects of life may help them cope in the future with deaths of family members and friends (Borasio et al., 2005).

Varying responses to death among religions and cultures may not be obvious to an outside observer. Health care providers should respect the patient's customs and religious beliefs and should inquire about the patient's beliefs as a means of opening the initial discussion of bereavement. Other factors, such as financial and social resources, can have an impact on the bereavement process. Division of property can generate strife; if the patient and family discuss how property is to be distributed, conflict may be reduced during bereavement. Monetary loss because of the illness can lead to financial struggles. The financial burden of ALS can cause hardship, bitterness and guilt (Bromberg et al., 1996; Moss et al., 1996).

20.11.3. Resources for the patient and family

Informally, bereavement counseling can start with discussing how death is a consequence of living and that ALS gives the patient and family time to come to closure. Patients and families can understand the relationship between bereavement and disease progression by reading, attending lectures and engaging in discussions with other patients, families and health care providers (Curry, 1990; Worden, 1991; Fitzgerald, 1994; James and Friedman, 1998).

The actual manner in which patients with ALS die may be a concern for the patient and family, and it is important to describe the dying process for them. Death is peaceful in most cases, without evidence of choking or pain (Neudert et al., 2001; Miller et al., 2005). The fears surrounding the end of life may interfere in the family's and patient's attempts to deal with other emotional aspects of the disease and should be addressed before the patient is near death. This information may need to be repeated at various stages of the disease, particularly when the end of life is near. It is essential that the patient and family are made to feel that they are not alone. In the past, a diagnosis of ALS was associated

with abandonment by the health care profession. The proliferation of ALS clinics has changed this approach. Although the neurologist and ALS nurse may not provide formal psychosocial care, their ongoing therapeutic relationship with the patient and family often is close and supportive.

The patient's death is a point at which health care providers can easily distance themselves from the bereavement process. This is the time, however, when formal bereavement begins for the family, caregiver and even for the health care providers. A personal phone call and card or letter to the family are extremely important and their absence can leave a long-lasting emptiness (Bedell et al., 2001). When health care professionals attend the memorial service, it provides a chance for the whole family to come to closure with the medical team and the medical aspects of the disease.

Structured bereavement programs can address a spectrum of issues. Structured programs include written handouts, professional counseling and support groups; counseling and support group participation can continue as long as necessary to meet an individual's needs. Written material about death and bereavement can be helpful to the patient and family, particularly for family members who cannot attend clinic visits. The material, written in language that is easy to understand, includes information that can raise awareness of feelings and fears about the dying process. The information can be discussed and questions answered at follow-up appointments.

The complexity of psychological processes in bereavement may not be fully recognized by health care providers who are not mental health professionals. Accordingly, social workers, psychologists and psychiatrists can be very helpful to the patient and family. Formal bereavement support groups led by mental health professionals can also help family members reach closure.

Continued involvement as volunteers in ALS clinics and support groups can be useful for some family members. Ongoing involvement may be important for family members who have been intensely involved with patient care because their routine comes to a stop when the patient dies, and these individuals may benefit by "winding down" their emotional and physical energy by helping other families with ALS. Receiving periodic ALS clinic newsletters is another method of staying in touch.

Providing health care to ALS patients and their families is emotionally taxing. Strong relationships and attachments to patients are not unusual in ALS. The care team at an ALS clinic may experience the deaths of several patients within a short period. The health care providers must also face their own mortality every time they prepare a patient and family for death. It is therefore important for members of the ALS team to come to terms with their own feelings about loss of function, death and grief and to understand their emotional involvement with their patients. Mental health counselors can also help the ALS team come to terms with these issues (Murphy, 2001).

20.12. Conclusion

While ALS is still an incurable condition, great strides have been made in helping patients manage the multitude of symptoms that arise as the illness progresses. The AAN has published parameters on the symptomatic care of ALS patients and the majority of patients now receive care consistent with the guidelines; studies suggest that we are doing better each year. Many symptomatic therapies can improve quality of life in patients with ALS and may even extend survival. The first randomized controlled trials of symptomatic therapies in ALS have just been published and large prospective studies are underway assessing the best timing and impact of PEG and NIPPV. Depression and anxiety are common in ALS, but are highly amenable to therapy. The multidisciplinary care clinic, where patients can have access to specialists who perform evaluations for assistive devices, orthoses, speech aids and home modifications, has become the standard of care in many parts of the world. By participating in clinical research, patients may not only receive increased attention from the ALS team, but also participate in the ongoing effort to find better treatments and refine current therapy. Palliative care, particularly at end of life, remains one way we can best help patients by relieving suffering. Helping to ease bereavement is done on a personal level by the ALS team, but research continues in how best to manage grief and bereavement, both from the families' and patients' perspective. We may not yet have struck on the therapy that truly impacts disease progression in a meaningful way, but until then, symptomatic therapies and palliative care help our patients meet the challenges of this disease with dignity and comfort.

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