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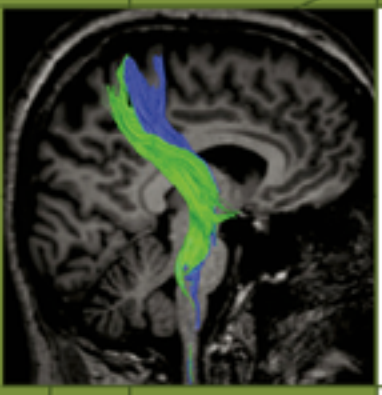
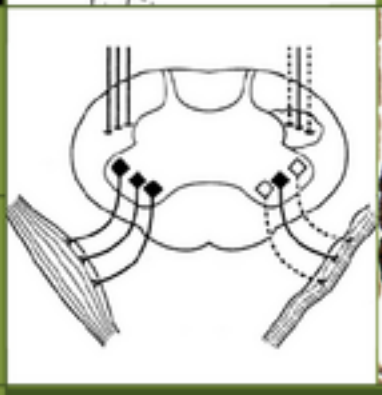
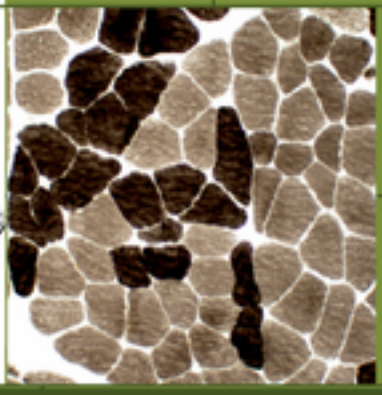
MOTOR NEURON DISEASE IN ADULTS



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MOTOR NEURON DISEASE IN ADULTS

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Preface

My ability to write and edit this book came from my 25-year involvement with motor neuron disease (MND), and amyotrophic lateral sclerosis (ALS) in particular. It started with an invitation to participate in a clinical drug trial, and evolved into starting an MND clinic. Since then, I have participated in many trials, conducted numerous clinical research studies, and started a second clinic in a second institution. Thus, ALS/MND has been my main clinical and research interest. Although the disorder is monumental for patients and family members, it is also challenging for providers who can only give compassionate care due to the lack of a clinically effective treatment. The reward in this endeavor, however, is observing how innate human nature allows patients to manage progression with grace and caregivers to give selfless assistance.

This book would not have been possible without help over the 25 years. My clinical expertise grew as the clinics expanded, and I am appreciative of the roles played by the ALS/MND nurses I have worked with: Dallas Forshew, Barbara Miano, Bernadette Tallon, and Mary Jensen. My best instructors have been the patients and caregivers as I try to answer their questions.

I started my career in basic science, and I acknowledge the mentorship of Sid Gilman in making the transition to clinical neurology and for putting my name forward to Oxford University Press for editorship of this book. One attraction to ALS/MND was the electrophysiologic aspects in the diagnosis and progression, and in particular the technique of motor unit number estimation. I received help along this path from William Brown, Alan McComas, and Erik Stålberg.

I thank Lucie Bruijn for her chapter and help in suggesting contributors. Much of this book is based on the expertise of the contributors and I greatly appreciate their chapters. Craig Panner, the CNS Series editor, gave valuable advice, and most important he was patient with me in completing the book. I also appreciate the final support of the Richard K. and Shirley S. Hemingway Foundation.

Finally, I want to thank my wife, Diane, for her support. We met through the challenges of caring for her mother who had ALS.

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Introduction to "Motor Neuron Disease"

Motor neuron disease (MND) represents a rare group of disorders recognized in the early 19th century that remain a challenge to the understanding of their pathophysiology, and most importantly, their management and ultimate treatment. There have been several extensive reviews of MND, and in particular, the initial volume "Amyotrophic Lateral Sclerosis" in the Contemporary Neurology Series published in 1998. In the ensuing 15 years there have been noteworthy advances in the knowledge base, in particular genetic implications, disease propagation, and the association of amyotrophic lateral sclerosis (ALS) with frontotemporal lobe syndromes and dementia.

This book focuses on adult forms of MND, particularly ALS, progressive muscular atrophy, and primary lateral sclerosis. It is an update of the field since the initial volume in the Contemporary Neurology Series. Advances since 1998 have led to more than 11,000 articles cited in PubMed using search titles "amyotrophic lateral sclerosis" or" motor neuron disease" (Figure FM-1). Given the explosion of articles, some report subtle findings, and only those that represent significant contributions for clinical features, diagnosis, pathophysiology, clinical trials, and patient management are presented. The MND community of researchers and clinicians is small, and I apologize in advance if I have left out a colleague's work.

The book is intended to be "clinically useful." A single-author book is challenging to write given the breadth of topics, and authors with expertise in the scientific areas have been asked to contribute chapters. Writing styles may thus vary among chapters.

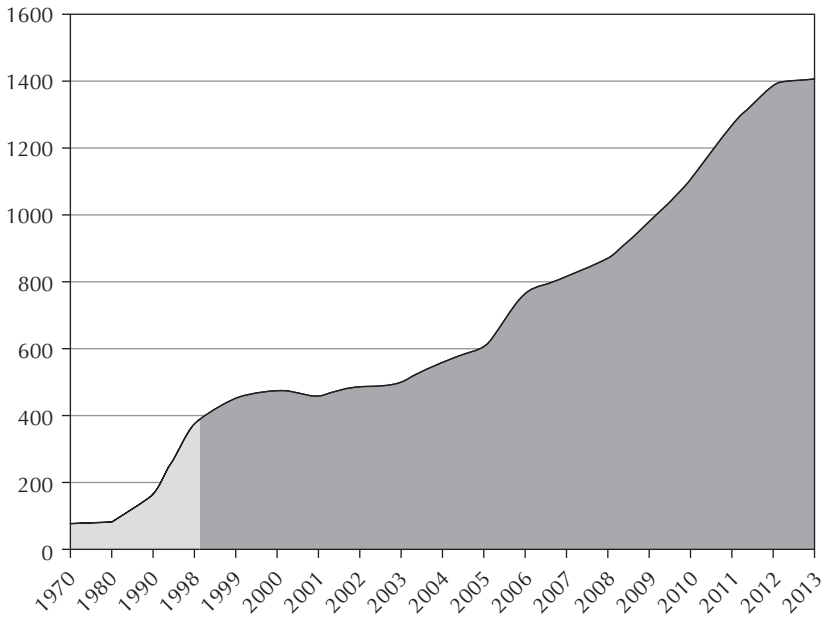

Figure FM–1. Histogram of the number of articles cited in PubMed using search terms "amyotrophic lateral sclerosis" or "motor neuron disease" from 1970 through 2013. The demarcation at 1998 separates the number of publications between the previous edition and the current edition.

The book focuses on evidence-based information, and in some areas relatively little new information has been published since 1998. Thus, chapter length varies. Each chapter includes a paragraph on the background and new points as an aid to linking the current edition with the previous edition.

For the neurologist, ALS is perhaps the most emotionally difficult disease to diagnose due to its inexorable and fatal course. For me, the writing and editing of this book reinforces my resolve as a clinician to provide the best care I can until we have more to offer.

Mark B. Bromberg, MD, PhD

Abbreviations

AAN American Academy of Neurology
AD Alzheimer disease
ALS amyotrophic lateral sclerosis
ALSbi amyotrophic lateral sclerosis behavioral impairment
ALSci amyotrophic lateral sclerosis cognitive impairment
ALSFRS Amyotrophic Lateral Sclerosis Functional Rating Scale

BMI body mass index
BMMA beta-*N*-methylamino-L-alanine
BOLD blood oxygenation level–dependent

CAG cytosine-adenosine-guanosine
CC corpus callosum
Cho choline
CMAP compound muscle action potential
CMCT central motor conduction time
CN cranial nerve
CNS central nervous system
Cr creatine
CSF cerebrospinal fluid
CSMN corticospinal motor neuron
CST corticospinal tract

DTI diffusion tensor imaging

EFNS European Federation of Neurological Societies
EIM electrical impedance myography
EMG electromyogram
ESC embryonic stem cell

FA fractional anisotrophy
FALS familial amyotrophic lateral sclerosis
FDA Food and Drug Administration
fMRI functional magnetic resonance imaging
FTD frontotemporal dementia
FTLD frontotemporal lobe dementia
FUS fused in sarcoma
FVC forced vital capacity

GABA γ-aminobutyric acid
GDNF glial cell–derived neutrophic factor
GWAS genome-wide association studies

HSSC human spinal stem cell

IGF insulin-like growth factor
IL interleukin
iPSC induced pluripotent stem cell
IQR interquartile range

LMN lower motor neuron
LOD log of odds ratio
LOH loss of heterozygosity
LTMV long-term mechanical ventilation

MEP motor evoked potential
MND motor neuron disease
MRI magnetic resonance imaging
MRS magnetic resonance spectroscopy
MSC mesenchymal stem cell
MT motor threshold
MUNE motor unit number estimation

NAA *N*-acetylaspartate
NIV noninvasive ventilation
NLS nuclear localization signal
NMR nuclear magnetic resonance
NO nitric oxide
NPC neural progenitor cell

PBP progressive bulbar palsy
PDC parkinsonism dementia complex
PEG percutaneous endoscopic gastroscopy
PLIC posterior limb of the internal capsule
PLS primary lateral sclerosis
PMA progressive muscular atrophy
PMC primary motor cortex
PNFA progressive nonfluent aphasia

RIG radiologic inserted gastrostomy
ROS reactive oxygen species

SALS sporadic amyotrophic lateral sclerosis
SD semantic dementia
SMA spinal muscular atrophy
SNP single-nucleotide polymorphism
SOD superoxide dismutase
SPECT single-photon emission tomography
STA spike-triggered averaging

TDEE total daily energy expenditure
TDP43 Tar DNA Binding Protein
TMS transcranial magnetic stimulation
TNF tumor necrosis factor
TQNE Tufts Quantitative Neuromuscular Examination
Treg regulatory T cell

UBC umbilical cord blood stem cell
UMN upper motor neuron

VBM voxel-based morphometry
VCP valosin-containing protein
VEGF vascular endothelial growth factor

WFN World Federation of Neurology

Chapter 1

History of the Recognition of Motor Neuron Disease

Mark B. Bromberg

BACKGROUND AND NEW POINTS

The history of the recognition of motor neuron diseases (MND) has been well described. However, it is worth briefly repeating because it is striking how clear and thorough the observations were in the mid-1800s. Furthermore, the question posed at that time, as to whether the different forms of MND represent separate disorders or a continuum, remains relevant today.

Efforts by the World Federation of Neurology to codify the diagnostic criteria for amyotrophic lateral sclerosis (ALS) continue to evolve to make the diagnosis at an earlier stage. However, cognitive features have not been incorporated, nor have criteria for other forms of MND (progressive muscular atrophy [PMA] and primary lateral sclerosis [PLS]) been developed.

HISTORICAL SETTING

The recognition of neurologic diseases evolved during the 19th century as clinical observation was combined with pathologic examination. Early efforts to categorize diseases were frequently based on small numbers of patients and pathologic observations on few postmortem samples using simple methods of histologic preparation. Furthermore, the level of understanding of anatomy, physiology, and pathology was undergoing rapid changes, with major advances on a yearly basis. These changes were incorporated into the setting of different schools of neurologic pedagogy (often with a dominant figure who could be authoritarian and intolerant of competing views), a fluid and evolving vocabulary complicated by descriptions and terminology in different languages, and frequent oral presentations of data and opinions without written documentation. It is not surprising that there were differing views of similar clinical and pathologic data, and an evolution of word usage to describe findings. It is arguable who first described or wrote about the various forms of MND as recognized today. However, it is remarkable that a reasonable number of patients with progressive motor system disorders presented to the small number of major neurologic centers in the United Kingdom and

Europe and that those clinicians were able to recognize and categorize within the spectrum of MND. Furthermore, their observations were remarkably accurate and complete, and their interpretations of pathologic involvement astute for the level of anatomic and pathologic knowledge at that time.

HISTORICAL RECOGNITION OF MND

The chronology of observations and the interchanges have been reviewed (Goldblatt, 1969; Goetz, 2000). Several terms used in the 1800s have different contemporary meanings: "fibrillary contractions" now indicate fascicular contractions; "limb contracture" now means spasticity. It should be recalled that tendon reflexes were recognized in 1875 by Westphal and Erb, and that the significance of the plantar response was described by Babinski in 1896.

Bell

Charles Bell, in 1830, described a patient with progressive weakness of limbs and tongue who had pathologic changes in the anterior portion of the spinal cord.

Aran

François Amilcar Aran, in 1850, described a condition with wasting and weakness of muscles, including intrinsic hand muscles, rounded-shoulder posture, head drop, foot drop, dyspnea, and stiffness that he termed PMA. He mentioned observing "fibrillary movements" of muscle, representing an early recognition of fasciculations. Among the patients described was one who had a sister and two maternal uncles similarly affected, likely representing familial MND. Aran acknowledged communications with Duchenne and study of clinical material he had collected. Duchenne asserted after Aran's 1850 paper that he had described a similar condition a year earlier. Thus, it is not clear who has the honor of the first recognition of MND.

Duchenne and Cruveilhier

Guillaume Duchenne and Jean Cruveilhier, in 1853, described a patient with muscle atrophy and weakness and fibrillary contractions. They used the term progressive bulbar palsy. Cruveilhier concluded that the ventral root atrophy was primary and muscle atrophy secondary, and that the ventral cord is the origin of ventral root atrophy. It is to be noted that early there was disagreement as to the site of primary pathology and Duchenne emphasized the muscle. The tremors and difficulty walking have been interpreted as reflecting upper motor neuron (UMN) loss (Veltema, 1975).

Charcot

Jean Martin Charcot, between 1865 and 1874, stands out as a prominent figure in medicine in general and neurology in particular, and specifically with respect to his observations on MND. Somewhat in contrast to chance clinical observations of patients with PMA in the early 1800s, Charcot was in a position to methodically observe clinical features and pathologic findings at death of patients he followed in the Salpêtrière hospital in Paris (Fig. 1–1). Charcot, in 1865, presented a case of a young woman who developed progressive weakness with increased muscle tone with contractures and no sensory abnormalities, and at autopsy had brownish grey sclerotic changes in the lateral areas of the cervical spinal cord but did not include the anterior horns (Goetz, 2000). Observations on infantile paralysis noted spinal lesions limited to anterior horns with motor nerve cell degeneration (Goetz, 2000). The concept that lateral sclerosis was associated with spasticity and contractures and anterior horn pathology was related to atrophic muscle weakness evolved with further clinical examples. These concepts came together in the French term "sclérose latérale amyotrophique" offered by Charcot in two lectures in 1874. Other contributory work on the anatomic correlates of glosso-labial-laryngeal paralysis and the functional anatomy of the brainstem were incorporated (Goetz, 2000).

Figure 1–1. Drawing of the Salpêtrière Hospital in the late 1800s where Charcot was able to observe patients during life and study their pathology at death. Retrieved from: www.anti que.prints.de

Erb

Wilhelm Erb, in 1875, described a patient with spastic paraparesis who developed muscle atrophy over time, and thus can be considered to represent recognition of transition of PLS to ALS.

Dejerine

Joseph Dejerine, in 1883, integrated progressive bulbar palsy (PBP) and ALS as a single entity.

Gowers

William Gowers, in 1892, postulated that PMA, PBP, and ALS were part of a spectrum of motor system degeneration.

Although the clinical and pathologic descriptions by Charcot and students were clear, there was debate as to primary versus secondary mechanisms of nerve degeneration. The word order in the term "sclérose latérale amyotrophique" in French places lateral sclerosis as the primary lesion and amyotrophy as the secondary lesion. There was ensuing debate in the literature, and Gowers believed that the degenerative processes occurred simultaneously (Deng et al., 2011).

Another debate concerned the designations of PBP and PMA and the relation to ALS. Titles of papers by Charcot and Duchenne included the term progressive muscular atrophy. However, there were other cases described of progressive palsy without atrophy. Charcot described labio-glosso-laryngeal paralysis with atrophy of medullary motor neurons. Ernst Viktor von Leyden noted sclerosis of corticospinal tracts in the medulla in several cases of PBP. Duchenne seems to have linked PMA with ALS based on pathologic findings of anterior horn cell loss and sclerosis of the lateral tracts.

During this time primary spastic conditions were being recognized. Charcot, in 1865, described a patient with contractures of limbs who had sclerosis of the lateral columns and atrophy of ventral roots but anterior horn cells were unremarkable. The term "primary lateral sclerosis" was applied by Erb in 1875, but debate continued based on varying clinical and pathologic features of individual cases.

With the help of other clinicians, Charcot had described most of the clinical and gross anatomicopathologic features of ALS, PMA, and PLS based on 20 cases and five autopsies from the Salpêtrière hospital. From these observations the eponym of "Charcot's disease" is deserved. Thus, in the 62 years from 1830 to 1892, the clinical features and pathology of the forms of MND were established. It has remained a challenge over the ensuing 100 years to understand the pathophysiology.

Brain

Walter Russell Brain, in 1933, introduced the term "motor neuron disease" as a unifying term.

FAMILIAL MND

A familial pattern of MND was described by Aran in 1873 in a case of PMA that included one of three sisters and two maternal uncles dying from a similar disease, supporting an autosomal dominance. In 1880, William Osler described a family in Vermont with 13 individuals in two generations affected by PMA, and a superoxide dismutase 1 mutation was subsequently identified in this family. Since the superoxide dismutase 1 mutation was discovered in 1993 a growing number of genes have been linked to ALS and frontotemporal lobe syndromes.

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Chapter 2

Nomenclature and Classification of Motor Neuron Disease

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Accurate naming of diseases is essential, but is dependent upon a clear understanding of underlying pathophysiology. For the various forms of motor neuron disease (MND), the pathologic and genetic features are incompletely known and the names amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), and progressive muscular atrophy (PMA) are recognized from general clinical features.

The World Federation of Neurology (WFN) put forth diagnostic criteria for ALS, with periodic revisions. The criteria have not incorporated elements of frontotemporal dementia or pseudobulbar affect commonly found in ALS, which are helpful diagnostically because they are not present in mimicking disorders. The criteria do not include PMA and PLS, and diagnosis remains based on loosely defined criteria. The spectrum of frontotemporal dysfunction seen in ALS, and recognition of families with frontotemporal dementia or ALS or both, enlarges nomenclature. The concept of familial ALS has expanded with the discovery of new gene mutations, and the hyphenated linkage of the gene mutation to ALS increases the number of names. Finally, aggregated cellular proteins, identified by immunohistologic techniques at postmortem examination, have their own nomenclature.

INTRODUCTION

Clear and precise nomenclature is important, and for clinical topics nomenclature is usually intertwined with early descriptions and later clarifications with better information on clinical and pathologic features. Furthermore, inclusion and exclusion features vary over time with more experience and new information. The term "motor neuron disease" is a general term for a number of interrelated diseases, or a continuum of diseases, that predominantly affect motor nerves involved in the direct execution of voluntary movements. The term motor neuron disease seems to have been used by Russell Brain in his textbook in 1933 as a synonym for ALS (Brain, 1933). From common usage, MND in the United Kingdom indicates ALS, but is also considered a general term to include all forms (ALS, PLS, and PMA) as it is used in this book. Professor Rowland

summarized difficulties with terminology in 1982 (Rowland, 1982, p. 1): "We have a serious problem of terminology. . . It has to do with the confusion between the singular, 'motor neuron disease,' and the plural, 'motor neuron diseases'." Despite a 30-year interval, challenges remain in MND terminology.

MND refers to a group of clinical motor disorders that can be more easily recognized clinically than by formal definitions. MND is characterized by the following features:

1. Degeneration, to varying degrees, of mainly two types of nerve cells, lower motor neurons (LMNs) and upper motor neurons (UMNs; Fig. 2–1A). It is recognized that other sets of nerve cells are frequently affected, including clinically those in the frontal and temporal cortex, and subclinically in other portions of motor and sensory systems.
2. Focal site of onset and inexorable progression of nerve degeneration. Rates of progression vary markedly, but progression leads to predictable loss of motor function and premature death contributed to by respiratory failure.
3. Onset in adulthood, rarely earlier than 21 years of age. A wide range of motor system disorders have onset in infancy and childhood, such as spinal muscular atrophy (SMA), but there appears to be little overlap clinically and pathophysiologically between adult and childhood degenerative motor diseases.
4. More commonly a sporadic disease, but in 5–10% of patients with MND there is a familial pattern that is mostly autosomal-dominant, and clinically indistinguishable from sporadic disease. The underlying genetic aspects of ALS have expanded (Fig. 2–1B). New gene mutations have been found to genotype patients with a family history; gene mutations with variable penetration have been identified in patients with unsuspected familial ALS; spontaneous mutations have been documented, and patients have been found who have multiple ALS gene mutations whose

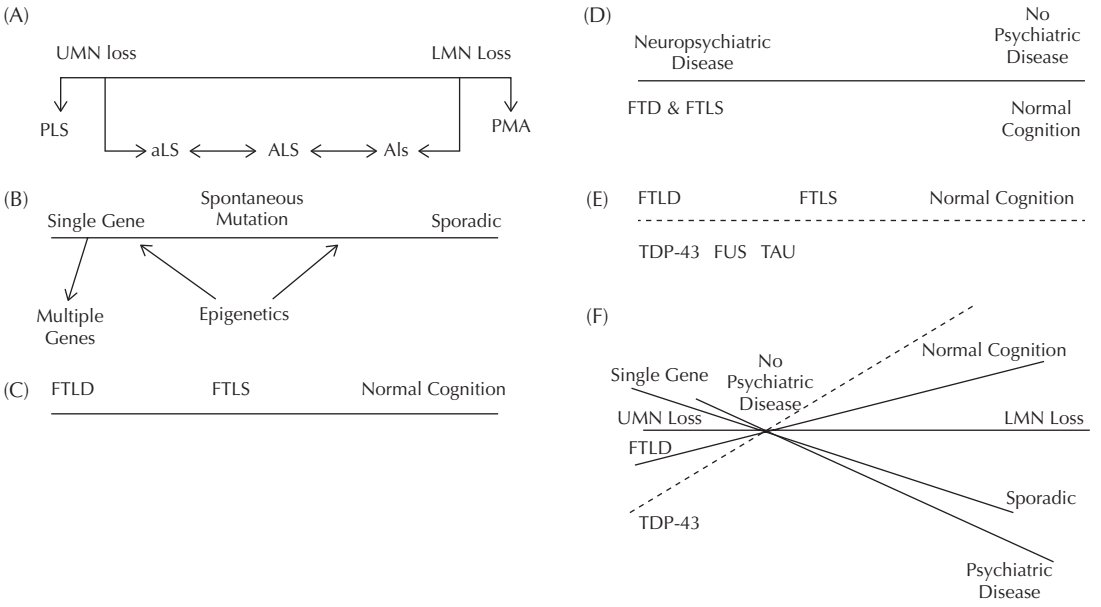

Figure 2–1. Representation of continuum lines for different aspects of MND. (A) Continuum of pure upper motor neuron (UMN) involvement as primary lateral sclerosis (PLS) and pure lower motor neuron (LMN) involvement as progressive muscle atrophy (PMA), with varying combinations as amyotrophic lateral sclerosis (ALS). (B) Continuum of single disease-causing gene (or combinations of genes), possible spontaneous causative or associative mutations, sporadic with no genetic cause, and epigenetics as factors. (C) Continuum of frontotemporal lobe involvement from dementia (FTLD) to a lesser syndrome (FTLS) to normal cognition. (D) Continuum between neuropsychiatric disease with or without FTLD/FTLS and no psychiatric involvement. (E) Continuum of immunohistochemical pattern of protein aggregates determined at postmortem examination (dashed line). (F) Possible combination of continuum lines for a given patient.

combination is thought necessary for clinical ALS.
5. Loss of neurons in the frontal lobes. This results in frontotemporal lobe dementia and frontotemporal lobe syndrome (Fig. 2–1C), and psychiatric conditions psychosis, schizophrenia, suicide; Fig. 2–1D; (Byrne et al., 2013).

New features have been added based on finding protein aggregates in neurons at postmortem examination (Fig. 2–1E). Thus, the nomenclature has markedly expanded, and MND for a given patient can be viewed as the intersection of several continual lines (Fig. 2–1F).

Excluded from the current book are acquired diseases that include motor dysfunction caused by viruses and other infectious agents, toxins, medications, radiation, and electrical trauma. Also excluded are hereditary disorders in adults that include limited degrees of LMN loss, adult-onset SMA, and spinal bulbar muscular atrophy (SBMA) (Kennedy disease; de Jong, 1991).

LOU GEHRIG

In the United States, ALS is synonymous with Lou Gehrig disease. Lou Gehrig was a baseball player with the New York Yankees who played 2,130 consecutive games between 1923 and 1938, and excelled as a first baseman and at bat. Thus, it was a shock that his prowess deteriorated and he left the game in 1939 because of ALS, which was diagnosed that year. He died in 1941 at the age of 41 years. His symptom onset has been analyzed from his batting average, which began to deteriorate in 1938 (Fig. 2–2; Kasarskis & Winslow, 1989). He carried on a thoughtful correspondence with his doctors and tried several therapies (Brennan, 2012). Gehrig displayed a graceful and strong personality as a baseball player that continued with his illness, and is exemplified by his farewell address at Yankee Stadium on July 4, 1939 (Fig. 2–3) (Brennan, 2012, p. 513):

> *Fans, for the past two weeks you have been reading about the bad break I got. Yet today I consider myself the luckiest man on the face of the earth. I have been ballparks for 17 years and have never received anything but kindness and encouragement from you fans.*
>
> *Look at these grand men. Which of you wouldn't considered it the highlight of his career just to associate with them for even one day? Sure, I'am lucky. Who wouldn't consider it an honor to have known Jacob Ruppert? Also, the builder of baseball's greatest empire, Ed Barrow? To have spend six years with that wonderful little fellow, Miller Huggins? Then to have spent the next nine years with that outstanding leader, that smart student of psychology, the best manager in baseball today, Joe Mcarthy? Sure, I'm lucky.*
>
> *When the New York Giants, a team you would give your right arm to beat, an vice versa, sends you a gift—that's something. When everybody down on the groundskeepers and those boys in white coats remember you with trophies—that's something. When you have a wonderful mother-in-law who takes sides with you in squabbles with her own daughter—that's something. When you have a father and a mother who work all their lives so that you can have an education and build your body—that's a blessing. When you have a wife who has been a tower of strength ands shown more courage than you dreamed existed—that's the finest I known.*
>
> *So, I close in saying that I might have a bad break, but I have an awful lot to live for. Thank you.*

WFN RESEARCH CLASSIFICATION

The WFN recognized a need for precise diagnostic criteria for ALS to "provide an algorithm which will enhance clinical studies, therapeutic trials and molecular generic research studies" (World Federation of Neurology Research Group on Neuromuscular Diseases, 1994, p. 96). A meeting, held in El Escorial, Spain (Fig. 2–2) in 1990, led to the "El Escorial Criteria" for ALS, and was published in 1994 (World Federation of Neurology Research Group on Neuromuscular Diseases, 1994). After exclusion of alternate diagnoses, three key clinical features were emphasized for the diagnosis of ALS:

1. Signs of LMN degeneration in one or more of four regions by clinical or electrophysiologic examination. The four

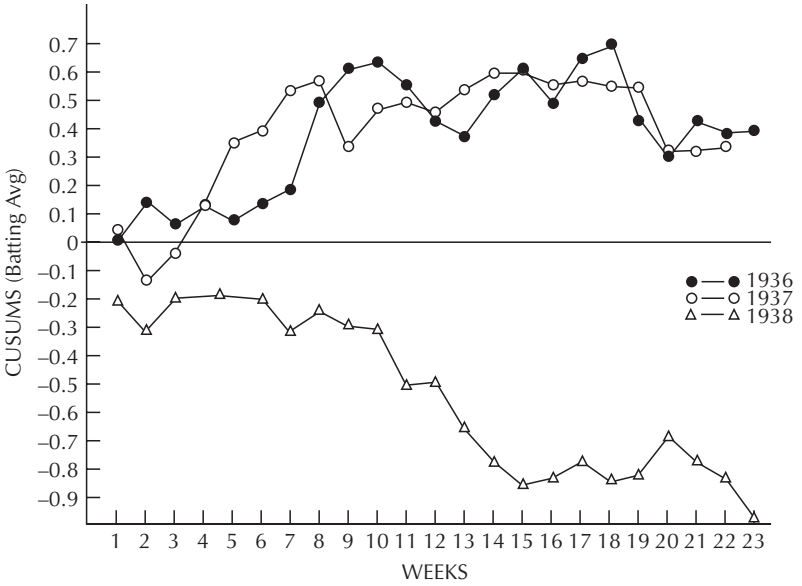

Figure 2–2. Weekly batting averages for Lou Gehrig using CUSUM statistics showing consistent performance at the start of the season and improvement over the season for years 1936 and 1937, but reduced performance and deterioration for the 1938 season. He retired mid-season 1939. (From Kasarskis & Winslow, 1989, with permission.)

regions are bulbar, cervical, thoracic, and lumbosacral.
2. Signs of UMN degeneration in one or more regions by clinical examination.
3. Progression within a region and to other regions.

Electrophysiologic examination for definite LMN degeneration includes active denervation (fibrillation potentials) and chronic denervation (reduced recruitment and large motor unit potentials). Four levels of diagnostic certainty result (Table 2–1): (1) definite ALS, (2) probable ALS, (3) possible ALS, and (4) suspected ALS.

Suspected ALS defines patients with signs of only LMN loss, and is consistent with PMA, but suspected ALS was dropped in a later revision. There is no designation for PLS. The levels of certainty relate to regions of involvement at the time of evaluation, and levels of certainty can increase over time with disease progression.

The WFN research classification has been successful at defining patients with classic ALS for clinical trials. In a study of 388 patients subjected to the El Escorial Criteria, at time of diagnosis 33% had definite ALS, 22% probable ALS, 35% possible ALS, and 7% had suspected ALS (Traynor et al., 2000). After a median follow-up period of 15 months (0.4–68.9 months) 85% fulfilled criteria for definite or probable ALS, whereas 14% remained possible of suspected ALS. Only 40% of patients

Figure 2–3. Watercolor titled "Pride of the Yankees" painted by William Ross, who has ALS and painted the picture with a brush held in his mouth due to limb weakness. (From the MDA art collection, with permission.)

Table 2–1 **World Federation of Neurology El Escorial Criteria for the Clinical Diagnosis of ALS***

Definite ALS	UMN: bulbar region + ≥2 spinal regions		UMN: 3 regions
	+	or	+
	LMN: bulbar + ≥2 spinal		LMN: 3 regions
Probable ALS	UMN: ≥2 regions (1 region above LMN region)		
	+		
	LMN: ≥2 regions		
Possible ALS	UMN: 1 region		
	+	or	UMN: ≥2 regions
	LMN: 1 region	or	LMN: rostral UMN region
Suspected ALS	LMN: ≥2 regions		

* In addition to the bulbar region, the three spinal regions are cervical, thoracic, and lumbosacral.
Modified from World Federation of Neurology Research Group on Neuromuscular Diseases (1994).

with familial ALS fulfilled criteria for definite ALS at time of diagnosis. It is noteworthy that Kaplan-Meier survival curves for the four classes of ALS by the El Escorial Criteria had similar mortality rates (p = .51) with median survival of 27 months for definite or probable ALS, 30 months for possible ALS, and 40 months for suspected ALS (Traynor et al., 2000). Thus, suspected ALS, equivalent to PMA, has a similar progressive degenerative course as ALS.

PMA

PMA has not received WFN codification efforts, other than the possible ALS category included in the El Escorial criteria but dropped in the Airlie House revision. There are diagnostic challenges separating PMA from other LMN disorders (Table 2–2). First, several patients who present with only LMN findings progress to include UMN findings and reach criteria for ALS, usually within 4 years (Traynor et al., 2000; Visser et al., 2007; Van den Berg-Vos et al., 2009). Second, rating tendon reflexes as hyperactive is subjective and is dependent upon several variables, which include positioning of the patient (relative stretch of the muscle being tested) and overall clinical interpretation with possible bias (in either direction) by the examiner. There is a concept born out by clinical experience that an intact reflex in a weak and fasciculating muscle is supportive of UMN pathology (probable UMN signs; Younger et al., 1990). Third, some patients with no clinical signs of UMN involvement are found to have loss of corticospinal motor neurons upon pathologic examination (Ince et al., 2003). Finally,

Table 2–2 **Adult-Onset Lower Motor Neuron Phenotypes**

Name	Clinical Features	Progression
PMA	Asymmetric weakness	May develop UMN signs: ALS
	Rare bulbar onset	Rapid progression: similar to ALS
SMA: slow progression	Asymmetric weakness	Slowly progressive
SMA: distal	Symmetric, arms and legs	Slowly progressive
SMA: segmental distal	Hirayama disease	Usually slowly progressive
	May be widespread	
	May have brisk reflexes	
SMA: proximal	Bi-brachial distribution	Usually slowly progressive

SMA, spinal muscular atrophy. Adapted from van den Berg-Vos et al. (2003) and Visser et al. (2007).

patients can exhibit relatively symmetric distal or proximal weakness due to LMN loss and have very slow rates of progression, with little change over 4 years. Accordingly, patients with clinical features of only LMN involvement have been divided into two groups based on distribution of atrophic weakness and rate of progression: PMA, with diffuse and asymmetric involvement and progression similar to ALS; and adult-onset SMA with symmetric distal or proximal segmental involvement and very slow progression (van den Berg-Vos et al., 2003; Visser et al., 2007).

PLS

PLS has not received WFN codification efforts, and notably is a rare disorder (Table 2–3). A high percentage of patients with only UMN findings at initial evaluation develop LMN findings over 3–4 years and fulfill El Escorial Criteria for definite or probable ALS (Gordon et al., 2006). Another group of patients with only UMN findings at initial evaluation develop limited degrees of LMN loss detected by serial needle electromyogram abnormalities in the form of fibrillation and fasciculation potentials, but continue to progress more slowly than patients with ALS but more rapidly than those with pure PLS (Le Forestier et al., 2001; Gordon et al., 2006).

This, in part, is the basis for the concept that PLS is not a discrete clinical and pathologic entity but is at one end of a spectrum of degrees of UMN and LMN loss (Le Forestier et al., 2001). Independent of how PLS is viewed in the spectrum of MND, a patient with marked UMN signs and no or few LMN signs after observation for 4 years has a prolonged survival over those with greater LMN signs.

Table 2–3 **Relative Frequencies of ALS Phenotypes, Based on Several Studies**

Phenotype	Percentage
Classic ALS	30.3–55.1
Bulbar	26.9–36.8
Flail arm	5.1–11.4
Flail leg	3.0–13.0
UMN predominant	9.1
Mills variant	Rare
Respiratory	1.1
PMA	2.9–4.3
PLS	4.0
PLS plus	<1

Modified from Wijesekera et al. (2009) and Chio et al. (2011).

ALS BY PHENOTYPES

Several clinical phenotypes are recognized that differ from classic ALS, but pathophysiologic differences have not been demonstrated (Table 2–3). Patients may initially appear to have one such phenotype, but with time, progress away from the distinguishing features and change to the classic ALS phenotype:

- Classic ALS: Regional onset with progression to other regions; clinically, LMN greater than UMN findings.
- Bulbar onset ALS: Bulbar onset with UMN findings and limb LMN findings after 6–12 months: rarely remain purely bulbar UMN involvement (Karam, Scelsa, & Macgowan, 2010).
- Flail arm: Bilateral upper limb weakness in a predominant proximal distribution and varying UMN signs with progression to other regions after 12 months (DeJesus-Hernandez et al., 2011).
- Flail leg: Bilateral lower limb weakness in a predominant distal distribution with varying UMN signs (Renton et al., 2011).
- UMN predominant ALS: UMN much greater than LMN findings (difficulty separating from PLS plus pattern).
- Mills variant: Slowly progressive hemiplegia, usually beginning in a leg and ascending to the arm; recent evaluations indicate that it is primarily an UMN disorder with bilateral pathologic reflexes (Turner et al., 2005).
- Respiratory predominant: Marked respiratory weakness at onset with UMN signs and minor spinal involvements.
- Pure UMN: PLS.
- Pure LMN: PMA.
- PLS plus: PLS with electrodiagnostic evidence for minor LMN loss (Gordon et al., 2006).

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Chapter 3

Clinical Anatomy, Physiology, and Pathology of Motor Neuron Disease

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Motor neuron disease (MND) involves primarily death of upper motor neurons (UMNs) and lower motor neurons (LMNs), and the clinical features of amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), and progressive muscular atrophy (PMA) are based on the consequences. Much of the basic anatomic and pathologic information was well established in the 19th century from postmortem examination, whereas most of the basic physiology of the motor system was determined from animal studies.

There is new information in MND on frontotemporal lobe dysfunction from immunohistochemical stains on postmortem examination and on the physiology of corticospinal connections from noninvasive electrodiagnostic studies. The diagnostic process is aided by having in mind anatomic and pathologic features.

INTRODUCTION

Motor symptoms of MND can be divided reasonably well on clinical grounds into ALS, PLS, and PMA. As an aid during the clinical diagnoses, it is useful to have in mind the basic anatomy, physiology, and pathology of the motor system. Information on the motor system and deficits comes from several sources. Dissecting the functional organization of the upper motor system has been aided by lesion experiments. In animals, information has been gained from precise ablations (motor cortex, medullary pyramidal tract) aided by detailed histopathologic examination, and animals can be sacrificed at timed intervals. In humans, information comes mostly from strokes but with minimal histopathologic examination because research-level postmortem examinations are rarely performed. Death from stroke occurs at variable intervals, but patients living with lesions allows for detailed clinical observation. With both animal and human models

of ablations or stroke, the degree of UMN loss is sudden and extensive, whereas in MND neuron loss is progressive and the degree of loss at any stage is not known. New techniques using magnetic resonance imaging provide information from living subjects with ALS and can be performed serially. New electrophysiologic techniques using transcranial magnetic stimulation provides physiologic information on motor connectivity in living subjects. In addition to the motor system, it is useful to have in mind sites of pathology related to frontotemporal lobe dysfunction and pseudobulbar affect.

This chapter focuses on the most clinically apparent deficits.

ANATOMY

UMNs

UMN refers to neurons originating mainly in the primary motor cerebral cortex (Brodmann area 4), which send fibers caudally to the brainstem and spinal cord. UMNs include the large Betz cells in layer V, but they represent only 3% of the 1,100,000 axons of the corticospinal tract, and the majority of fibers are from smaller pyramidal-shaped cortical neurons (Fig. 3–1). Other cortical areas contributing axons to the corticospinal tract are the supplementary motor cortex (area 6), the primary somatosensory cortex (areas 3, 1, and 2) and the secondary somatosensory cortex (area 5; Schieber, 2007). Fibers from these neurons also contribute to the corpus callosum.

The primary motor outputs are the corticospinal and corticobulbar tracts, which are grouped in the posterior limb of the internal capsule, descend caudally in the basis pontis, make up the pyramids, and descend to form the lateral and anterior corticospinal tracts (Fig. 3–2). The lateral corticospinal tracts are largely crossed, whereas the anterior corticospinal tracts are largely uncrossed.

Corticospinal axons terminate primarily in the cervical and lumbosacral enlargements. There are corticospinal axons that terminate in the lumbosacral cord that have collateral branches that first terminate in the cervical cord (Schieber, 2007). Within the cord, axons terminate in the base of the dorsal horn (intermediate zone) in spinal gray matter (Rexed laminae V and VI). There is evidence from comparative animal studies for a large number of axons terminating in laminae IX, where LMN cell bodies are located (Fig. 3–3).

UMNs also project to other motor systems, including the basal ganglia; nuclei giving rise to the rubrospinal, vestibulospinal, reticulospinal projections; and the cerebellum. UMN lesions, therefore, can secondarily affect movements through these systems.

LMNs

LMN refers to alpha motor neurons originating in brainstem motor nuclei and anterior horn of

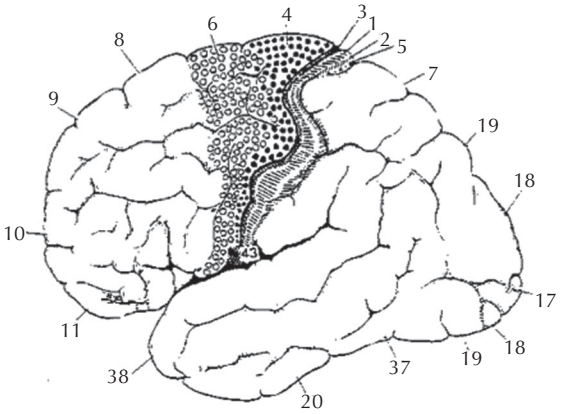

Figure 3–1. Brodmann areas based on cytoarchitecture. The illustration has been simplified to include cortical regions giving rise to corticospinal progressions: the primary motor cortex (area 4), the supplementary motor cortex (area 6), the primary sensory cortex (areas 3, 2, and 1), and supplementary sensory cortex (area 5). (Modified from Brodmann's areas © Nicholas Wade; source http://neuroportraits.eu/, with permission.)

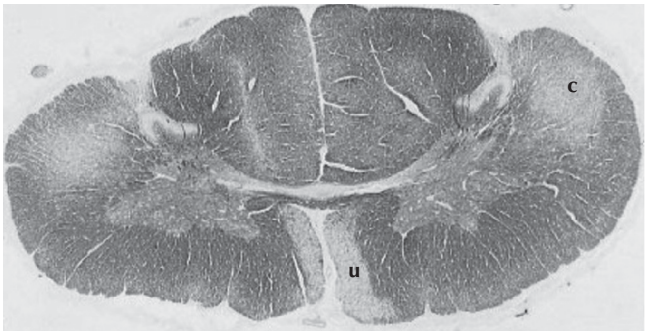

Figure 3–2. Spinal cord cross-section from patient with ALS showing pallor of lateral (c = crossed fibers) and anterior (u = largely uncrossed fibers) corticospinal tracts (Luxol fast blue stain). (From Department of Pathology, University of Utah, Salt Lake City, UT, with permission.)

the spinal cord. Cranial motor nerves are primarily cranial nerve (CN) XII and also CN X and XI, and late in the course can include CN VII and those that move the eyes (CN III, IV, VI). Motor axons exit the cord as ventral roots and distribute to skeletal muscles. The nucleus of Onuf (named after Bronislaw Onufrowicz) is in Rexed laminae IX and represents a small cluster of motor neurons in S1 to S3 roots that innervate skeletal muscle of the external rectal and urethral sphincters.

PATHOLOGY

ALS

In ALS there is variable loss and shrinkage of large and medium-sized pyramidal cells in the primary motor cortex, but also cell loss in the supplemental motor cortex, and to a lesser extent primary and secondary somatosensory cortex. There is also degeneration and cell loss in the temporal lobes and corpus callosum. Loss of UMNs leads to pallor in posterior limb of the internal capsule, basis pontis, pyramids, and lateral and anterior corticospinal tracts (Fig. 3–2).

The large alpha motor neurons in Rexed laminae IX are depleted to varying degrees and ventral roots show thinning to varying degrees (Fig. 3–4). Motor neurons are also depleted in brainstem motor nuclei, primarily CN XII. It is difficult to match the degree of LMN loss in muscle with that in the spinal cord: a postmortem example of early ALS, occasioned by an untimely death, showed fiber-type grouping in muscle but no alpha motor loss in the spinal cord (Fischer et al., 2004). This raises the question of a dying-back pathologic process to account for the greater pathologic changes in

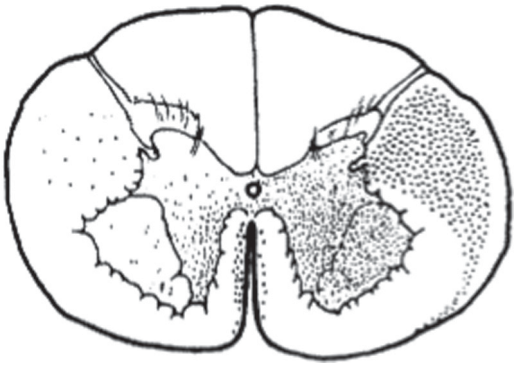

Figure 3–3. Termination of corticospinal tract fibers in the intermedial horn and anterior horn: distribution based on phylogenetic studies showing in the Chimpanzee monkey a high degree of corticospinal fiber terminating in these areas, and presumably greater numbers in humans. (Drawing modified from Kuypers.)

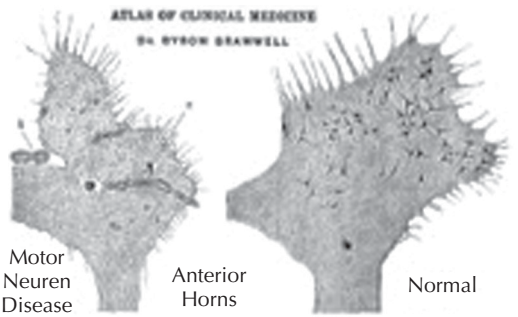

Figure 3–4. Drawing from pathologic studies on patients with ALS showing severe loss of alpha motor neurons. (From Atlas of Clinical Medicine, Neuromuscular Home Page, neuromuscular.wustl.edu, with permission.)

muscle. Clinically there is relative preservation of sphincter strength, and this is matched by neuron loss in the nucleus of Onuf to a lesser degree than alpha neurons innervating limb muscles of the same myotomes (Carvalho, Schwartz, & Swash, 1995).

Muscle fiber denervation is evident by scattered atrophic and angular fibers (Fig. 3–5). With greater degrees of denervation and reinnervation, the normal checkerboard pattern of fiber types is replaced by groups of similar fiber types (fiber-type grouping).

PLS

PLS is characterized clinically as having essentially only UMN loss. Postmortem studies show gross shrinkage of the precentral gyrus, and microscopically loss of large pyramidal cells, similar to ALS, but more striking (Hudson et al., 1993). There may also be pallor in the fasciculus gracilis, and limited gliosis in the anterior horn.

PMA

PMA is characterized clinically as having only LMN loss and no evidence for UMN loss. Anterior horn cells are depleted to varying degrees, leading to fiber-type grouping in muscle. Of note, there is evidence on postmortem examination for UMN loss in about half of one series of 10 patients with pure clinical LMN signs (Ince et al., 2003).

PHYSIOLOGY AND PATHOPHYSIOLOGY

ALS

Fibers from the corticospinal tract are believed to facilitate fine motor control. Anatomic evidence comes from comparative animal lesion studies showing progressively greater numbers of fibers terminating on alpha motor neurons in monkeys compared with cats and rats (Fig. 3–6), and the arrangement in humans of greater corticospinal-to-alpha motor neuron connections is argued from these phylogenetic trends (Schieber, 2007). The distribution of corticospinal tract synapses within a spinal cord motor pool is not uniform, and is higher to alpha motor neurons innervating intrinsic hand and finger extensor muscles than proximal muscles. Physiologic evidence comes from intracellular recordings from alpha motor neurons in monkeys comparing the strength of synaptic input (excitatory postsynaptic potentials) with stimulating the motor cortex. The ultimate efficacy of cortical synaptic input to alpha motor neurons is complex and includes position of the synapses along alpha motor neuron dendrites (those closer to the soma have larger

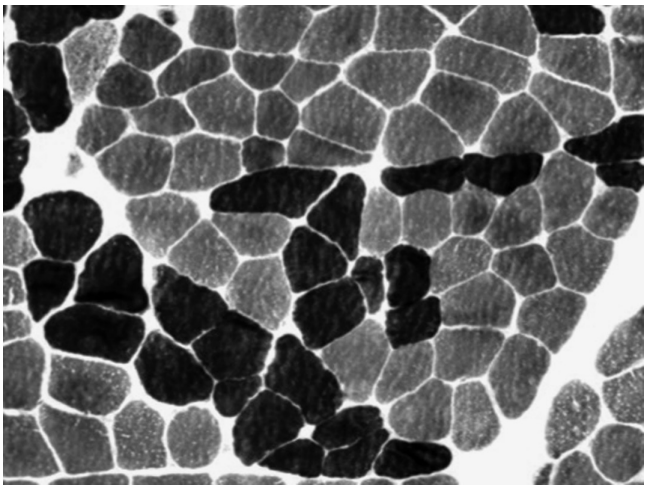

Figure 3–5. Muscle cross-section from a patient with ALS showing marked fiber-type grouping (ATPase pH 9.4 stain). (From Neuromuscular Home Page, neuromuscular.wustl.edu, with permission.)

excitatory postsynaptic potentials), effects of excitatory and inhibitory inputs (from other descending tracts and from spinal cord neurons), and discharge frequency of descending inputs (leading to facilitation of the effects of the input).

Information on the anatomic and pathologic connections in humans is supplemented by electrophysiologic studies using transcranial magnetic stimulation. Transcranial magnetic stimulation is a noninvasive method for activating cortical neurons by magnetic fields, and a variety of stimulation paradigms can be applied to explore cortical motor excitability, conduction time down the spinal cord, relative excitability of alpha motor neurons, and the effects of cortical inhibition (Vucic, Ziemann, Eisen, Hallett, & Kiernan, 2013).

The motor threshold (magnetic field intensity to reliability elicit a small muscle response) reflects the density of corticomotor connections, and is lowest for activation of intrinsic hand muscles. Changes of the motor threshold in patients with ALS over time are variable and may reflect heterogeneity of MND phenotype or stage of the disease. However, early in the clinical course the threshold is low and rises to inexcitability to stimulation. The motor evoked potential (amplitude of the evoked motor response recorded from muscle) reflects a graded muscle potential to increasing magnetic stimulation intensities, and also reflects the density of corticomotor connections. Increases in evoked amplitude are seen early in ALS, and are unique to UMN loss because increases are not observed in disorders in the differential diagnosis of ALS (spinal and

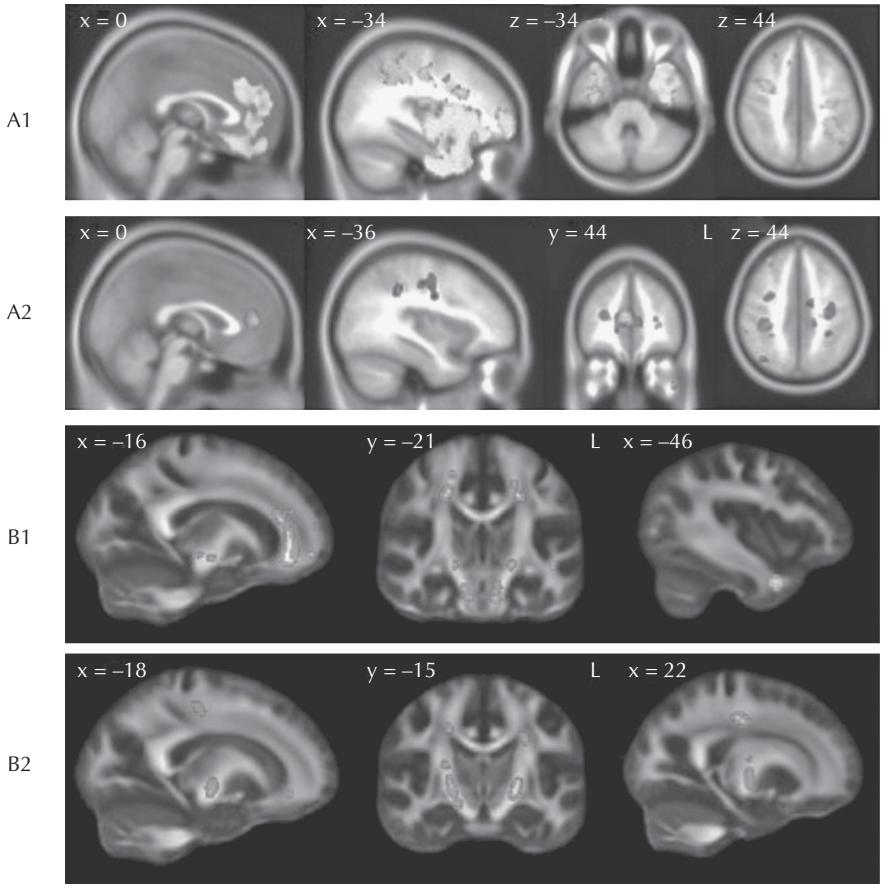

Figure 3–6. Cortical imaging changes in patients with ALS compared with ALS + behavioral variant frontotemporal dysfunction. (A) Grey matter atrophy: A1 ALS + behavioral variant frontotemporal dysfunction compared with control subjects; A2 ALS compared with control subjects. (B) White matter changes: B1 ALS + behavioral variant frontotemporal dysfunction compared with control subjects; B2 ALS compared with control subjects. (Modified from Lillo et al., 2012.)

bulbar progressive muscular atrophy [Kennedy disease], multifocal motor neuropathy with conduction block; Vucic, Cheah, Yiannikas, & Kiernan, 2011). Central motor conduction time (transit time from the motor cortex to the spinal cord) is modestly increased in ALS, thought to reflect loss of corticospinal fibers and desynchronization of descending activity.

PLS

Most transcranial stimulation data are from ALS, but in PLS (one subject) responses are found to be reduced in the arm muscles and absent in leg muscles, consistent with clinical features of greater leg dysfunction (Cruz Martinez & Trejo, 1999).

Frontotemporal Lobe

There is clinical recognition of neuronal dysfunction in ALS beyond the motor system, and involves temporal lobe neurons. The construct of frontotemporal lobe cell and fiber loss is less well organized than for the motor system.

A variety of techniques are available to document neuron and fiber loss, including histopathology and imaging studies (Lillo et al., 2012). Imaging studies in patients with ALS with behavioral variant frontotemporal lobe dysfunction, in contrast to patients with ALS without behavioral dysfunction, show gray matter atrophy frontal gyrus, premotor and motor cortices, anterior insula, temporal poles, thalamus, and striatum, similar to but somewhat less than in patients with frontotemporal lobe dementia but without ALS.

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Chapter 4

Clinical Features of Motor Neuron Disease

Mark B. Bromberg

BACKGROUND AND NEW POINTS

The clinical features of the various forms of motor neuron disease (MND) are important to recognize in the diagnostic process. Major features were well described in the 1800s.

Up to 50% of patients with amyotrophic lateral sclerosis (ALS) have elements of frontotemporal lobe dementia (FTLD), and patients who present with FTLD can be found to have unsuspected ALS. Furthermore, this type of dementia can occur alone in other family members as part of a dementia-ALS hereditary pattern. Recognition of elements of frontotemporal lobe syndrome is important because they have an impact on patient care.

INTRODUCTION

Charcot and contemporaries recognized the clinical features of MND in the 19th century. The constellation of features is unique for ALS and progressive muscular atrophy (PMA), and their recognition is key to making an accurate diagnosis. The features of primary lateral sclerosis (PLS) are less specific, and overlap with other disorders. Clinical features are considered by region of involvement (Table 4–1).

COGNITIVE DYSFUNCTION IN NONDEMENTED PATIENTS WITH MND

Subtle changes in cognition can be recognized in patients with ALS before becoming clinically obvious. Changes documented by neuropsychologic tests cover a range of functional domains: language, immediate verbal memory, delayed verbal memory, visual memory, fluency, executive functioning, attention, verbal intelligence, psychomotor speed, visuoperceptual functions, and visuoconstructive skills. No single study includes all functional domains,

Table 4–1 **Symptoms and Signs by Region of Involvement**

Region/Function	Symptoms	Signs
Frontotemporal lobes: behavior, semantics, executive function	Ease of crying or laughing Increased/wider yawning Poverty of words Social withdrawal Decision-making difficulties Impatience	Emotional lability Increased yawning Word-finding difficulties Quiet demeanor Indecision Impatience Open-eyed appearance
Brainstem: bulbar functions	Excess saliva, frequent throat clearing Slurred speech Deliberate/forced swallowing Choking on liquids Change in diet for swallowing comfort	Sialorrhea Dysarthria Exaggerated swallow Choking Dysphagia Weight loss
Spinal cord: limb functions	Limb weakness Falls Ease of muscle cramping Muscle twitching Loss of muscle mass Slowness of movement Need to concentrate to move Generalized fatigue	Atrophic muscle weakness Fasciculations Slow development of strength Spastic catch Pathologic reflexes Spastic gait
Rostral spinal cord: respiratory function	Shortness of breath with speech Shortness of breath with activity Weak cough Use of additional pillows Shortness of breath lying supine Frequent a.m. headaches Excessive daytime sleepiness Need for naps	Short sentences Weak cough Accessory respiratory muscle use

and a meta-analysis focused on 16 studies that included ALS patients with definite, probable, and possible ALS by El Escorial Criteria and who were not demented (Raaphorst, de Visser, Linssen, de Haan, & Schmand, 2010). The pooled weighted effect indicated medium level severity losses for intermediate verbal memory, visual memory, fluency, psychomotor speed, language, and executive functioning among patients with ALS.

PMA does not involve clinical manifestations of upper motor neuron (UMN) motor loss, but evidence for cognitive changes has been found. In comparisons among 23 patients with clinical PMA, 30 patients with ALS, and normal control subjects, 17% of patients with PMA performed worse on three or more tests, most commonly digit span backward (attention/working memory) and category fluency (naming words/unit time) compared with control subjects, but no differences compared with patients with ALS (Raaphorst et al., 2011).

PLS has been found to include cognitive changes on a par with those found in patients with ALS (Grace et al., 2011). Overall scores on a comprehensive battery of neuropsychologic tests were within normal limits, but 39% of 18 patients with PLS had abnormal scores on tests of executive function and behavioral symptoms. None were severe and in the range of dementia. The pattern of abnormal tests was similar to 13 patients with ALS tested in a similar manner.

FRONTOTEMPORAL LOBE DYSFUNCTION AND DEMENTIA

Identification of frontotemporal lobe dysfunction is important diagnostically because it places lesion sites at a supratentorial level. Symptoms attributed to frontotemporal lobe

dysfunction differ from those in Alzheimer disease, with the former showing a spectrum of changes in behavior, verbal fluency, and executive function, but a relative preservation of memory (Strong, 2008). The features vary from very mild (frontotemporal lobe dysfunction) to a full complement sufficient to meet formal research criteria for FTLD (Neary et al., 2000; Rascovsky et al., 2011; Kreider & Walsh, 1997).

Frontotemporal lobe dysfunction has been divided into three areas based on clinical and pathologic experience: (1) behavioral variant, (2) progressive nonfluent aphasia variant, and (3) semantic dementia variant (Table 4–2; Neary et al., 2000). However, it is recognized that many patients with MND can exhibit subtle behavioral and social features not sufficient to meet formal FTLD criteria, but that can be recognized as a frontotemporal lobe syndrome (Strong, 2008; Strong et al., 2009). Examples of behavioral changes include breakdown of social and personal conduct and emotional blunting. Social conduct changes include disinhibition, emotional blunting, and social withdrawal. Executive dysfunction can be the hardest to detect and is more apparent on neuropsychometric testing, but clinical examples include difficulty attending to details (planning and abstraction) required to make decisions, and the inability to make decisions, which may be apparent only after querying the caregiver (Irwin, Lippa, & Swearer, 2007).

Table 4–2 **Features of Frontotemporal Lobe Dysfunction in MND With Percentages From a Study of 16 Patients With ALS**

Term	Characteristics
MNDbi (bv: behavioral variant)	Deficits in one or more areas: self-centeredness (69%) irritability (63%) apathy (38%) blunted emotional response (25%) reduced personal hygiene (19%) social disinhibition (13%) increased aggression (13%) social disinhibition (19%) excess sociality (13%) aggression (13%)

Note 14 patients had insufficient characteristics to meet Neary criteria for frontotemporal lobe dementia, whereas two did. Modified from Gibbons et al. (2008) and Strong (2008).

In a study of 16 consecutive patients assessed for frontotemporal lobe dysfunction (14 with Airlie House Criteria of definite or probable ALS and two with possible ALS) almost 88% had some change in affect or social behavior with two patients fulfilling criteria for FTLD (Gibbons, Richardson, Neary, & Snowden, 2008). The two patients who fulfilled criteria for suspected ALS exhibited some frontotemporal signs.

Progressive aphasia is characterized by nonfluency with hesitancy with words, effortful word production, and reduced word output. Reduced verbal fluency is most common and usually manifest by reduced speech output and few-word answers to questions. Fluency can be easily assessed in the clinic by word generation tests that include the number of "D" words or animal names recited within 60 seconds. Interestingly, patients with MND with aphasia generate more than 75% of words in the first 15 seconds compared with normal subjects who give words throughout the 60 seconds. Among 100 El Escorial Criteria definite of probable patients with ALS screened as above, 31% had reduced word generation and 6 of 27 patients who had normal word generation scores had behavioral changes meeting FTLD research criteria (Lomen-Hoerth et al., 2003). Conversely, among 36 patients who were initially evaluated for symptoms of FTLD and who met research criteria 14% were also found to meet El Escorial Criteria for definite ALS and an additional 36% with possible ALS (Lomen-Hoerth, Anderson, & Miller, 2002). Several of the remaining patients with FTLD had fasciculations or bulbar dysfunction and one progressed to meet criteria for definite ALS within a year. In general, progressive aphasia is more common than semantic aphasia in the setting of MND. Semantic aphasia is characterized by effortless speech without hesitancies but little information is conveyed and there is increased use of broad generic terms (i.e., "thing" for an item).

Other symptoms of frontal lobe dysfunction may be subtle or occur late. There may be impatience to a request for help from the caregiver when the caregiver cannot comply immediately, and may include overall irritability. A patient with dysarthria may give up with oral communication out of frustration and not

try other forms of communication even when available. A relatively late symptom is a strong wish not to be left alone when the caregiver must leave the room, even briefly for household tasks. Rarely, patients want to be driven in an automobile around their neighborhood. An open question is whether a patient's inability to make a decision regarding an intervention when the need and benefit seems clear, such as use of an item of durable equipment, represents a problem in executive decision making.

A question arises of whether site of ALS onset (bulbar or limbs) is more associated with development of frontotemporal syndrome or FTLD. A prospective study of 355 patients with the diagnosis of definite or probable ALS showed no association with site of onset (Sterling et al., 2010). Another study of 130 patients with ALS compared their level of daily function by the ALS Functional Rating Scale-Revised (ALSFRS-R) scores with their level of behavioral impairment on the Frontotemporal Dementia Rating Scale and found no correlation (Hsieh, Lillo, Kiernan, Hodges, & Mioshi, 2013). This indicates that patients with ALS at any stage of their disease may also have behavioral symptoms.

PSEUDOBULBAR AFFECT AND INCREASED YAWNING

Recognition of pseudobulbar affect, in combination with other motor symptoms, is also important diagnostically because it places a lesion in a supratentorial location. Pseudobulbar affect represents an inability to manage emotional expression and results in outbursts of poorly controlled laughing, crying, or both (Rosen & Cummings, 2007). Alternative terms include pathologic laughing/crying, emotional lability, and emotional incontinence, but pseudobulbar affect is preferred. Pseudobulbar affect must be distinguished from depression, and the key features present in pseudobulbar affect but not depression are (1) an inappropriateness to the situation, (2) precipitation by nonspecific stimuli, (3) lack of a close relationship between the emotional expression and how the patient is feeling, (4) stereotypy of the episodes, and (5) no mood change corresponding to the episodes (sense of relief from expressing the emotion). Some patients have facial expression of wide-open eyes or surprise, which has been observed and discussed most with progressive supranucular palsy (Batla, Nehru, & Vijay, 2010). The lesion site is believed to be loss of anterior descending motor systems. Pseudobulbar affect is observed in PLS (Le Forestier et al., 2001).

Frequent and forced (wide) yawning is common in ALS, and it may have a number of pathologic substrates. It can be considered a UMN release sign (Williams, 2000).

BULBAR DYSFUNCTION

The earliest symptom of bulbar dysfunction is frequently a change in voice quality (dysphonia) followed by a change in speech production (dysarthria). Concurrently, there is usually difficulty swallowing, frequently heralded by sialorrhea. Both speech and swallowing require very fine motor control, and symptoms of dysfunction can be caused by either loss of UMN or lower motor neurons (LMNs) or a combination, and it is difficult to distinguish the relative pathologic contributions in a given patient. Bulbar symptoms may appear as the first symptom of MND or any time later in the course, but about 20% of patients experience no significant bulbar dysfunction.

Dysarthria

The earliest symptoms of dysphonia may be recognized only by the patient, and described as a voice that is hoarse or weak or excessively breathy, and symptoms may vary within a day due to fatigue and from day to day (Robert, Pouget, Giovanni, Azulay, & Triglia, 1999). Dysphonia is localized to disordered vocal fold (chord) contractions during exhalation. Impaired function affects the vibratory function of the folds and changes voice sounds.

Dysarthria implies paralysis or inability to coordinate muscles involved in speech, and can include impairment of planning speech. In the context of bulbar motor involvement dysarthria represents UMN or LMN dysfunction, whereas in the overall context of MND it may include a degree of aphasia from frontotemporal lobe dysfunction. Early symptoms recognized by the patient, before recognition

by listeners, are the need to mentally work harder to form words and sentences. There may also be a factor of listener politeness that delays identification of the onset of dysarthria. Motor and mental fatigue contributing to transient speech deterioration is common. Speaking on the telephone is frequently more problematic than face-to-face speech, likely because there is an element of reading facial expressions and lip movement with the latter. Patients may describe good days when their speech is closer to normal followed by bad days when it is harder to understand, but the overall pattern over time is progression of difficulties. Breath support due to respiratory compromise can affect speech and may lead to low volume or shortened sentences to allow for a breath. Poor breath support is a sign of early respiratory insufficiency.

Quantitative measurements of speech can separate ALS (El Escorial Criteria definite) from control subjects based on vocal instability (Robert et al., 1999). Quantitative assessment was more sensitive than qualitative measures. Furthermore, several quantitative abnormalities were observed in patients with ALS whether they were or were not symptomatic for bulbar dysfunction. Both LMN and UMN lesion sites underlie the quantitative changes noted, and thus dysarthria is complex.

Dysarthria is usually obvious during the clinical interview. Dysarthria results from a combination of UMN and LMN neuron loss. UMN contribution to dysarthria is apparent by spastic speech with a strained and strangled pattern. Primary UMN involvement is further supported by relative preservation of tongue bulk. LMN contribution is apparent with flaccid qualities, supported by loss of tongue bulk and spontaneous or contraction fasciculations noted (true spontaneous fasciculations are when the tongue is at rest in the mouth, and contraction fasciculations are when the tongue is activated). The proportion of UMN and LMN involvement changes over time. UMN and LMN loss results in slowed rapid side-side tongue movements and weakness. Tongue strength is measured most reliably by asking the patient to stick their tongue out to one side and have them press it against a tongue blade while the tongue blade is moved to the midline: normally tongue strength is such that the tongue blade cannot be moved and the blade bends; when weak it is easy to move the tongue with the blade. Weakness of lip function can be frequently elicited when a patient acknowledges that they can no longer purse their lips to whistle with the same control as at an earlier time or by an inability to seal their lips around a straw. Contraction fasciculations can occasionally be observed in the mentalis muscle as a sign of LMN loss.

Acoustic analysis of patients with ALS (El Escorial Criteria definite or probable) shows difficulties in pronouncing specific syllables for those with clinical dysarthria (bulbar onset) and also those with no clinical dysarthria (limb onset) compared with control subjects (Tomik et al., 1999). The time between consonant and vowel sounds was significantly longer, in descending order, for the bulbar onset group (B > O > I > W > T) and the limb onset group (B > I > T > W > O).

The degree of dysarthria can progress to no intelligible sounds. Short of this stage, listeners accustomed to the speech pattern, such as family members, can understand a sufficient amount for basic communications. There is frequently impatience on the part of the patient and frustration on the part of the listeners. Despite the ability of a patient to write messages, written communication is used less by the patient than might be expected. Likewise, simple communication devices, alphabet boards, or computer-based devices are uncommonly adopted, and cognitive issues maybe a factor (see later in the chapter).

Dysphagia

Dysphagia usually follows dysarthria in disease progression. Patients are the first to be aware of difficulty swallowing. The first sign may be the need to clear the throat more often to manage secretions. Sialorrhea, in the form of wetness of the mouth when inspecting the tongue, is frequently observed. Salivary glands produce 1–1.5 quarts of saliva per day, and when swallowing slows, saliva is not cleared and accumulates. There may be deliberate swallowing prior to speaking to clear pooled saliva.

More specific signs are the need to think about the act of swallowing; to swallow multiple times to clear secretions or liquids or a bolus of food; or the need to begin to

eliminate certain foods, such as lettuce and bread. With progression, liquids become harder than solids to swallow. Pills, especially small tablets, may be particularly challenging, because they cannot flow along with the rapid transit of the liquid bolus and become caught. Tongue weakness during the chewing process becomes apparent with difficulty sweeping food between cheek and teeth and moving it to the posterior portion of the mouth in preparation for swallowing. Although muscles of mastication rarely become weak to the point that chewing is impaired, jaw opening may become reduced.

Swallowing is a complex process with voluntary and reflex initiation and several defined motor stages. In a study using electromyographic markers of muscle activation in patients fulfilling El Escorial criteria and with both UMN and LMN involvement of bulbar function to varying degrees the following defects were noted (Ertekin et al., 2000). (1) There is slowed triggering of the swallowing reflex, more marked in the setting of voluntary swallowing, attributed primarily to loss of UMNs. (2) The crycopharyngeal sphincter muscle in ALS dysphagia relaxes abnormally due to alterations in timing of relaxation and increased and inappropriate activity. This, in turn, is attributed to a lack of coordination with other muscles and due to disinhibition from loss of UMN control. There is progressive loss of voluntary swallowing attributed to loss of UMN to initiate the swallow, but with preservation of reflexive swallowing. However, reflexive swallowing is impaired by loss of UMN control. In a study of 11 patients with bulbar involvement, including tongue fasciculations, patients underwent fiberoptic examination during swallowing of paste, liquid, and solid foods (D'Ottaviano, Linhares Filho, Andrade, Alves, & Rocha, 2013). Eight reported symptoms of dysphagia but all had swallowing dysfunction. The oral preparatory phase was altered in seven patients, whereas oral transit and pharyngeal phase dysfunction was observed in all of them. These difficulties were attributed to LMN impairment, which in turn is attributed to tongue weakness. There is also evidence that up to 50% of patients with ALS have a deficit in laryngeal sensation that may contribute to dysphagia (Amin, Harris, Cassel, Grimes, & Heiman-Patterson, 2006). Swallowing can become markedly impaired and lead to a reduction in dietary intake and weight loss. However, some patients manage to swallow in the setting of no visible tongue movement. A gastric feeding tube can eliminate the need to swallow liquids and food, but the need to swallow saliva remains. Aspiration pneumonia occurs relatively infrequently despite marked swallowing difficulties, but an occasional patient experiences recurrent bouts (Sorenson, Crum, & Stevens, 2007).

Masseter muscle strength has been measured quantitatively in patients with ALS with bulbar symptoms (Ohnuki, Takizawa, & Shinohara, 2002). Occlusal forces were not different from control subjects, indicating that the masseter muscle is not particular vulnerable in ALS. However, in patients with bulbar involvement subclinical denervation changes by various quantitative electromyographic techniques can be detected (Finsterer, Erdorf, Mamoli, & Fuglsang-Frederiksen, 1998).

UPPER EXTREMITY DYSFUNCTION

Upper extremity dysfunction occurs in most patients in all forms of MND. For ALS and PMA, upper extremity weakness is mostly caused by LMN loss, but in ALS there are varying degrees of dysfunction related to UMN loss, primarily manifest by slowness of movements and the need to exert greater mental effort for a movement. In PLS, all dysfunction is associated with UMN loss and movements are slow.

Certain muscles and muscle groups seem to be vulnerable to LMN loss early in the course of ALS and PMA, and can be considered to be "index" muscles during the diagnostic process. Intrinsic hand muscles have received the most attention, in particular those in the lateral hand (anatomic position: first dorsal interosseous and thenar group muscles) with atrophy and weakness affecting fine motor control, such as manipulating buttons. Although the pattern of a dissociated or split hand is also seen in other disorders (normal aging, spinal muscular atrophy, spinocerebellar ataxia type 3; Schelhaas, van de Warrenburg, Kremer, & Zwarts, 2003),

in the appropriate clinical context, atrophy and weakness of these muscles are strongly supportive of ALS and PMA (Voermans, Schelhaas, Munneke, & Zwarts, 2006; Kuwabara et al., 2008). Other index muscles are the third volar interosseous muscle with weakness of fifth digit adduction when the fingers are in an extended position. Digital extension is characteristically affected causing flexor tendon contractions (curled hand posture). Patients may describe ease of muscle cramping with routine activities. Prominent contraction fasciculations are indicators of LMN loss and subsequent collateral reinnervation. Muscle strength may not be weak in a muscle with fasciculations, likely due to the fact that a large number of LMNs must be lost before reinnervation cannot keep up with denervation. Asymmetry of weakness, especially early in the course, is a very common feature.

Clinical signs of UMN loss, demonstrable in some patients with ALS and in all patients with PLS, are spastic catches. In the upper extremities, a catch to rapid extension of the forearm at the elbow or pronation of the forearm may be demonstrated, and in the legs, slowed and reduced swinging when the leg is passively extended at the knee with the patient seated on an examination table. Pathologically brisk tendon reflexes are the most common sign of UMN loss. The term "pathologically brisk reflexes" represents clinical judgment. Perhaps the term "incongruously brisk" is more descriptive of a tendon reflex that is brisker than expected for a weak muscle (Younger et al., 1990). The finding of asymmetry of reflexes, whether or not they are clearly pathologic, is supportive of UMN pathology when other causes of asymmetric reflexes are excluded. Spread of reflex muscle activation to surrounding muscles is unequivocal evidence for UMN loss. Hoffmann responses, especially in asymmetric distribution, are also indicative.

LOWER EXTREMITY DYSFUNCTION

Leg dysfunction occurs eventually in all patients with MND. Gait spasticity is prominent in PLS and occurs frequently in ALS. Muscle weakness in ALS and PMA is due to LMN loss. The anterior tibialis muscle is an index muscle involved early in the course of ALS and PMA, presenting as a mild foot drop leading to an ease of tripping. Mild weakness of ankle dorsiflexion can be verified by asking the patient to stand on their heels. Occasionally, a patient may fall for unclear reasons causing a lower extremity fracture that leads to neurologic assessment and diagnosis of MND due to lack of improvement with rehabilitation after healing of the fracture. Proximal muscle weakness is noted when patients have a difficult time rising from a low sitting position, and rising from the toilet is a good example of a low seat. The leg may give away at the knee as an indication of quadriceps muscle weakness. Patients frequently describe, after a fall to the ground, difficulty getting up without using their arms on a solid support.

Fasciculations are a prominent feature of ALS and are considered an electrophysiologic hallmark. The question of benign fasciculations arises in the setting of exercise. A study using muscle ultrasound to detect fasciculations was conducted in 58 healthy subjects across an age range of 20–93 years (Fermont et al., 2010). When assessed at rest, 43% had fasciculations, and the most common muscle was the abductor halluces, and rare fasciculations were found in muscles above the knee. Among 10 subjects ages 20–40 years tested 30 minutes after strenuous exercise four of five males but no females had fasciculations in the gastrocnemius muscles, which resolved within 24 hours.

Gait instability leading to falls, in the setting of good strength, reflects leg spasticity from UMN loss. Spasticity in the lower extremities can be demonstrated by an increased resistance to passive movement or a catch with rapid bending movements at the knee. Movements that cause postural instability, such as turning, leaning to reach, and crouching, are problematic and lead to frequent falls. Spasticity of gait may be marked and is usually asymmetric. Patients frequently describe more stiffness with the first few steps after sitting for some time that lessens after walking several steps. Pathologic tendon reflexes can be identified with the same attention to incongruous briskness, asymmetry, and spread. Clonus can be frequently elicited at the ankle joint. Interestingly, extensor plantar responses are less frequently encountered than expected for the degree of other signs of UMN.

RESPIRATORY DYSFUNCTION

It is rare for a patient to present with symptoms of respiratory failure that leads to the diagnosis of MND, but in this context the history and examination usually reveal an unsuspected or unreported degree of diffuse muscle atrophy and weakness.

Patients may describe transient shortness of breath, lasting less than 1 minute, in the setting of relatively good levels of pulmonary function, and because these episodes are relieved by taking several deep breaths, they likely reflect a brief episode of anxiety. However, the pathophysiologic trigger for these episodes is not clear. Patients may also mention a transient feeling that they have to work to breath out. Overall, shortness of breath, even in the setting of relatively poor levels of pulmonary function, is a late phenomenon. This is likely due to the fact that pulmonary function usually stays ahead of respiratory demands because limb muscles are very weak and muscle metabolism is overall low. Orthopnea is also a late phenomenon. The activities of bathing and dressing are strenuous and patients frequently describe shortness of breath and the need to lie down before continuing on. This likely reflects fatigue more than respiratory insufficiency.

Clinical assessments of respiratory insufficiency are a shortening of spoken sentences and use of accessory muscles of respiration. Interestingly, the patient infrequently describes distress when accessory muscles are used. Other sign of insufficiency is an ineffective cough, identified in the clinic as a "huff" rather than a "bark." Similarly, patients may describe their sneeze effort as less forceful than in the past.

EYE MOVEMENT ABNORMALITIES

Eye movement abnormalities are generally observed clinically only late in the progression of ALS, mainly in patients who are supported by invasive ventilation and are beyond the normal life span of ALS. However, the overlap of ALS and FTLD broadens the range of central neuron involvement and detailed studies of eye movements reveal early involvement (Sharma et al., 2011). Pursuit abnormalities, slowed saccades (mainly vertical saccades), and saccadic intrusions are noted mostly in association with frontotemporal lobe dysfunction and are ascribed to supranucular neuron loss.

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Chapter 5

Frontotemporal Dysfunction and Dementia in Amyotrophic Lateral Sclerosis

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BACKGROUND AND NEW POINTS

Although cognitive changes were noticed early on, they were not widely recognized or characterized. Recently, cognitive or behavioral impairment has been found to occur in up to half of individuals with amyotrophic lateral sclerosis (ALS), and is severe enough in approximately 10% to meet criteria for frontotemporal dementia (FTD). The most common presentation is the behavioral variant (bvFTD), characterized by personality changes. Definitive diagnosis requires detailed neuropsychological testing, but brief examination instruments are available for clinical use. Imaging techniques to date lack the sensitivity, specificity, and availability to be used as routine clinical tools. Pathologic, genetic, and biomarker studies are leading to a better understanding of biology and pathogenesis. FTD shortens lifespan in patients, and increases caregiver strain, distress, and burden. Education of, and support for, the caregiver are essential because pharmacologic interventions for the patient are limited.

INTRODUCTION

Classically, ALS has been considered to be a motor neuron disease. It is now known that a more appropriate conceptualization of ALS is that of a broader neurodegenerative disease

of the central nervous system. Of particular importance among the extramotor abnormalities of ALS are cognitive and behavioral dysfunction, which if sufficiently severe may manifest as FTD. Cases of ALS associated with dementia have been reported sporadically since the late 1800s but until recently this was considered a relatively rare occurrence. Since the mid-1990s the association between ALS and FTD has been increasingly recognized and reported (Neary, Snowden, & Mann, 2000; Lomen-Hoerth et al., 2003; Ringholz et al., 2005).

EPIDEMIOLOGY

Reports of the prevalence of cognitive and behavioral impairment in ALS vary, depending upon the definitions and instruments used. It is important to keep in mind that not all patients with cognitive or behavioral impairment demonstrate deficits that are severe enough to meet criteria for FTD. Thus, approximately 5–15% of patients with ALS meet the formal criteria (Neary et al., 1998) for the diagnosis of FTD, whereas an additional 33–51% of patients have some degree of cognitive or behavioral impairment (Raaphorst, de Visser, Linssen, de Haan, & Schmand, 2010; Phukan et al., 2012). Conversely, when patients who present clinically with FTD are screened for motor neuron dysfunction, 12–15% meet criteria for the diagnosis of ALS, and some additional patients are found to have otherwise unexplained signs of motor dysfunction by examination or electrophysiology (Lomen-Hoerth, Anderson, & Miller, 2002; Burrell, Kiernan, Vucic, & Hodges, 2011). Attempts to determine if there are specific subsets of patients with ALS that are likely to present with or develop dementia have led to mixed results. For example, bulbar-onset disease is more likely to be associated with cognitive or behavioral changes in most series (Lomen-Hoerth et al., 2003; Raaphorst, Beeldman, De Visser, De Haan, & Schmand, 2012a; Chio et al., 2010; Zimmerman, Eslinger, Simmons, & Barrett, 2007) but not all (Ringholz et al., 2005). Patients with clinically isolated upper motor neuron disease (Kobayashi et al., 2010; Grace et al., 2011) or lower motor neuron disease (Geser et al., 2009) may also manifest cognitive and behavioral abnormalities.

CLINICAL FEATURES

FTD may present either primarily as a behavioral dysexecutive disorder or as a language disorder, both in the setting of relatively preserved memory function. Consensus diagnostic criteria (Neary criteria) for FTD were published in 1998 and address the subtypes of the disorder (Neary et al., 1998). The term FTD is used to refer to the global clinical syndrome that encompasses both the behavioral/executive and language variants; frontotemporal lobar degeneration (FTLD) is the pathologic correlate of most cases of FTD. The original Neary criteria refer to the behavioral/executive form of FTD as FTD. Subsequently, to avoid confusion, this subtype of FTD has often been termed bvFTD (Strong et al., 2009). bvFTD is a disorder of social comportment characterized by personality change, impaired interpersonal relationships, apathy, emotional blunting, poor insight, and lack of empathy. Patients may exhibit a change in eating habits with new-onset carbohydrate craving and impulsive eating that often leads to weight gain; there is often a decline in personal hygiene and grooming. The language variants of FTD can be subsumed under the term primary progressive aphasia and can present as progressive nonfluent aphasia (PNFA), semantic dementia (SD), or logopenic progressive aphasia (LPA). In PNFA, speech is effortful and agrammatic with prominent paraphasic errors; this syndrome may progress to muteness. Speech in SD is fluent and empty with impaired confrontational naming and object knowledge; word and object comprehension are similarly affected. LPA, which was not included in the Neary diagnostic criteria, is characterized by difficulty with word retrieval and repetition; the pathology in this disorder may be that of FTLD or Alzheimer disease (Mesulam et al., 2008; Grossman et al., 2008). There may be considerable overlap among these syndromes. For example, supportive features of the diagnosis of bvFTD include changes in speech output that may lead to mutism. Both PNFA

and SD may be associated with behavioral changes later in the course of disease.

DIAGNOSTIC CRITERIA AND CATEGORIZATION

The cognitive and behavioral abnormalities seen in patients with ALS do not always easily map onto the diagnostic criteria for FTD and may extend beyond the usual deficits seen in FTD to include memory (Raaphorst et al., 2010, 2012a). Patients with ALS may have a frontal dysexecutive syndrome with prominent impaired mental flexibility without the severe behavioral abnormalities required for the diagnosis of bvFTD; the Neary criteria does not allow for a diagnosis in this subset of patients. In fact, the most common pattern of frontal lobe impairment seen in the ALS population is likely a combination of mild cognitive and behavioral deficits (Murphy JM et al., 2007). Deficits in verbal fluency, as measured by word generation tasks, are the most commonly documented cognitive abnormality in patients with ALS; this dysexecutive feature may be seen in patients who meet clinical criteria for dementia and in those who do not (Murphy J, Henry, & Lomen-Hoerth, 2007). The key concept for clinicians to grasp is that not all patients with ALS who have frontotemporal dysfunction necessarily have FTD. Some have cognitive or behavioral impairments that place them outside of the normal range, but are not sufficiently severe to fulfill "dementia" criteria.

The need to recognize the continuum of cognitive and behavioral presentations led to the development of consensus criteria and the creation of clinically relevant diagnostic terms by a group of experts in ALS and FTD that included both neurologists and neuropsychologists (Strong et al., 2009). Patients who meet the Neary criteria for full blown dementia are termed ALS-FTD. This group can be subdivided by FTD subtype into bvFTD, PNFA, or SD. In contrast, the term ALSbi is used to describe those with ALS and behavioral impairment who do not meet all criteria for FTD but who fulfill at least two supportive diagnostic features from the Neary criteria or Hodges criteria (Gregory, Serra-Mestres, & Hodges, 1999), such as a decline in personal hygiene, mental inflexibility, distractibility and impersistence, hyperorality, stereotyped behavior, utilization behavior, loss of insight, disinhibition, restlessness, distractibility, reduced empathy, impulsiveness, social withdrawal, reduced verbal output, perseveration, poor self-care, and sexual hyperactivity. These behaviors should not be explained by any other conditions, including Axis I and Axis II psychiatric disorders and pseudobulbar affect. ALS-cognitive impairment (ALSci) is appropriately diagnosed when the patient scores below the fifth percentile on a minimum of two tests of executive function. Again, exclusionary criteria include premorbid and comorbid conditions that better explain the abnormalities.

ASSESSMENT OF COGNITIVE AND BEHAVIORAL DEFICITS

Correct assignment of patients into these diagnostic categories is a somewhat tricky process that requires standardized evaluation. Patients may meet criteria for both ALSbi and ALSci. The evaluation of behavior and cognition in ALS is complicated by the need for completeness while limiting the motor component for tasks and speech to minimize their influence on scoring, and limiting the length of the evaluation to avoid patient fatigue. Adjustments for timed verbal responses are required for patients with dysarthria or for those who communicate by other means. Agrammatism and aphasia should not be overdiagnosed in patients who write, type, or use eye-gaze systems, because a change in language patterns may simply reflect energy conservation. Importantly, pseudobulbar affect should not be interpreted as a manifestation of behavioral pathology. The bare minimum requirements for a screen, as defined at the consensus, include a word generation task to assess executive function and a caregiver interview to assess emotional and behavioral function (Strong et al., 2009). A screen alone should not be used to diagnose ALS-FTD, ALSbi, or ALSci. Formal neuropsychological evaluation is the gold standard to make these diagnoses; however, such assessments take several hours, and routine clinical use requires a shorter assessment.

The most sensitive test to detect executive dysfunction in this population is a verbal fluency task, which can be given in oral or written format. Verbal fluency tests can be administered as phonemic or category exercises. Phonemic tests involve production of words that fill certain letter criteria (e.g., words that begin with the letter "S" or four letter words beginning with the letter "C"), whereas category tasks require production of words within a category (e.g., "animals" or "colors"). The most accurate normal values for these tests are derived from control groups of similar age and education level (Abrahams et al., 2000). Spoken and written verbal fluency tests can be adapted for individuals with motor impairments affecting their ability to speak or write, through use of a fluency index (Abrahams et al., 2000). There is not a validated analogous single item screen for behavioral impairment, and assessment should involve observation of the patient and discussion with the family.

A number of brief assessment batteries have been proposed for use in the ALS population. Ideally such a battery would take 5–20 minutes and minimize motor requirements, or provide adjustments where necessary. These are best categorized by whether they evaluate cognition, behavior, or both.

Cognitive Instruments

The Abrahams Written Verbal Fluency Test is sensitive to cognitive impairment, specifically executive dysfunction. It is a well-validated test that can be scored so as to compensate for deficits in speaking and/or writing speed, and has been used in patients with ALS who have motor impairments and dysarthria (Abrahams et al., 2000, 2005). The Penn State Screen of Frontal and Temporal Dysfunction Syndromes is a 20-minue brief examination that focuses on verbal fluency, abstract reasoning, and judgment, and was designed specifically for assessing executive function in patients with ALS (Flaherty-Craig, Eslinger, Stephens, & Simmons, 2006; Flaherty-Craig, Brothers, Dearman, Eslinger, & Simmons, 2009). Other published batteries that evaluate executive function may have utility in ALS, but their use for this patient population is still being explored. The Montreal Cognitive Assessment (Nasreddine et al., 2005) has been studied in patients with ALS, but cannot always be completed by those with physical impairment and there are no controls for those with dysarthria or motor weakness (Osborne, Sekhon, Johnston, & Kalra, 2013). The Frontal Assessment Battery has been used in some preliminary studies of cognition in ALS (Ahn et al., 2011; Raaphorst et al., 2013), but seems to have limited use in patients with ALS who are unable to perform some of the required motor tasks (Raaphorst et al., 2013). Similarly, Addenbrooke's Cognitive Examination (Mioshi et al., 2006) has no controls for motor weakness or dysarthria. The Mini Mental State Exam is not sensitive for the cognitive and social deficits in FTD and has limited utility in this evaluation (Hodges et al., 2004; Hutchinson & Mathias, 2007).

Behavioral Instruments

Batteries that evaluate behavioral function are administered to the caregiver and usually take around 10 minutes. The most common reported behavioral abnormalities in patients with ALS are perseveration, apathy, and disinhibition (Raaphorst et al., 2012a). Commonly used tools include the Frontal Behavioral Inventory (Blair et al., 2007; Kertesz, Davidson, & Fox, 1997) the Neuropsychiatric Inventory (Blair et al., 2007; Cummings et al., 1994), and the Frontal Systems Behavior Scale (Grossman, Woolley-Levine, Bradley, & Miller, 2007). Each of these tests has been used in ALS populations, but not specifically validated for them (Ahn et al., 2011; Grossman et al., 2007; Gordon et al., 2007; Raaphorst et al., 2012b; Tsujimoto et al., 2011). The Amyotrophic Lateral Sclerosis-Frontotemporal Dementia-Questionnaire is a caregiver interview developed from neurobehavioral changes documented in the ALS population (Raaphorst et al., 2012a) and constructed with questions to minimize the relevance of motor and speech impairments (Raaphorst et al., 2012b); this instrument has been partially validated in an ALS population.

Combined Cognitive and Behavioral Instruments

The Amyotrophic Lateral Sclerosis Cognitive Behavioral Screen is a 5-minute instrument,

that assesses frontal executive and behavioral functions and has been validated against the gold standard of formal neuropsychological testing in an ALS population (Woolley et al., 2010). The executive portion of the Amyotrophic Lateral Sclerosis Cognitive Behavioral Screen includes items for the evaluation of attention, concentration, mental tracking and monitoring, and word initiation and retrieval; the behavioral component is a 19-item caregiver questionnaire. The Edinburgh Cognitive and Behavioural ALS Screen is a combined battery to assess behavioral, executive, and language function with minimal motor requirements (Abrahams, Newton, Niven, Foley, & Bak, 2014). Cognitive tasks are included for the evaluation of language, verbal fluency, executive function, memory, and visuospatial function. A caregiver questionnaire addresses the behavioral domains of disinhibition; apathy or inertia; loss of sympathy or empathy; perseverative, stereotyped compulsive or ritualistic behavior; hyperorality and altered food preferences; and psychosis. This instrument attempts to correct the exclusion of formal language tests from prior batteries, because language dysfunction can occur in the absence of executive dysfunction and may be as common (Phukan et al., 2012; Taylor et al., 2013).

The cognitive and behavioral assessment instruments are summarized in Table 5–1. It is important to bear in mind that these brief examinations are not definitive assessments. If one of them is suggestive of a diagnosis of cognitive or behavioral dysfunction in ALS, then formal neuropsychological assessment is recommended if possible.

IMAGING

The clinical overlap of ALS and FTD has led to a search for other commonalities between the two disorders, including imaging characteristics, pathologic findings, genetic causes, and biomarkers. Traditionally, standard magnetic resonance imaging (MRI) has not aided in the diagnosis of ALS, whereas frontotemporal atrophy is often seen during the diagnostic phase in FTD (Borroni et al., 2007; Chow et al., 2008). Patients with ALS-FTD may demonstrate atrophy on routine MRI but for most patients with ALS, imaging findings are more subtle and identification may require the use of advanced techniques (Lillo et al., 2012).

Such advanced techniques have successfully demonstrated abnormalities in patients with ALS who do not meet criteria for FTD, and who may not have clinically significant cognitive impairment, but who may have measurable executive dysfunction by neuropsychological testing. In one study, functional MRI paradigms demonstrated cognitive deficits during word-retrieval processes (verbal fluency and confrontation naming) in patients with ALS who were not demented, but who demonstrated deficits in letter fluency, a sensitive test of executive function. Specifically, during a letter fluency task, nondemented patients with ALS were found to have areas of reduced activation in middle and inferior frontal gyri and anterior cingulate gyrus along with the parietal and temporal lobes. Also demonstrated was reduced activation in the inferior frontal gyrus along with areas of temporal, parietal, and occipital lobes with a confrontation naming task (Abrahams et al., 2004). Similarly, positron emission tomography studies using flumazenil as a marker of neuronal dysfunction have shown a relationship between cognitive deficits in nondemented patients with ALS, as measured by abnormal word retrieval on verbal fluency and confrontation naming, and reduced binding in several cortical areas, including the right inferior frontal gyrus, superior temporal gyrus, and anterior insula that correlated with performance on a verbal fluency task and reduced binding in the left middle frontal gyrus and left cuneus (Wicks et al., 2008). Functional MRI has also shown abnormal patterns of activation in nondemented patients with ALS during tasks that require the executive function of cognitive inhibition (Goldstein et al., 2011). Although this latter group did not undergo detailed neuropsychological testing, they had no clinical evidence of cognitive dysfunction, thus supporting the role of advanced imaging techniques in detecting subclinical cognitive deficits in patients with ALS. Diffusion tensor imaging has largely been used to demonstrate changes in white matter tracts associated with the motor system and motor deficits in patients with ALS. Diffusion tensor imaging also shows changes in the corpus callosum, corticospinal tract, and white matter association tracts that correlate with performance in tests of executive function and attention (Sarro et al., 2011).

Table 5–1 **Brief Assessment Instruments Commonly Used for Cognitive and Behavioral Testing in ALS**

Instrument	Cognition	Behavior	Comments
Abrahams Written Verbal Fluency	x		Scoring can compensate for motor impairment and dysarthria
Penn State Screen of Frontal and Temporal Dysfunction	x		Designed specifically for ALS; not all parts available in public domain
Montreal Cognitive Assessment	x		No compensation for motor impairment or dysarthria
Frontal Assessment Battery	x		No compensation for motor impairment or dysarthria
Addenbrooke's Cognitive Examination	x		Not used in ALS; no compensation for motor impairment or dysarthria
Mini Mental State	x		Not sensitive for FTD
Frontal Behavioral Inventory		x	
Neuropsychiatric Inventory		x	
ALS FTD Questionnaire		x	Designed to minimize effects of motor and speech impairments
ALS Cognitive Behavioral Screen	x	x	Validated against formal neuropsychological testing
Edinburgh Cognitive and Behavioural ALS Screen	x	x	Includes evaluation of language

Additionally, changes in diffusion tensor imaging metrics in the right frontal gyrus (Tsujimoto et al., 2011) and specifically the anterior cingulum (Woolley, Zhang, Schuff, Weiner, & Katz, 2011) are correlated with the presence of apathy in patients with ALS. Apathy is also correlated with atrophy in the orbitofrontal and dorsolateral prefrontal cortices as measured by voxel-based morphometry (Tsujimoto et al., 2011). Magnetic resonance spectroscopy has shown a correlation between performance on the Wisconsin Card Sorting test and neuronal integrity of the frontal lobe (Abe et al., 2001) and similarly between F-word generation and neuronal integrity in the dorsolateral prefrontal lobe (Quinn et al., 2012). Voxel-based morphometry demonstrates somewhat widespread gray matter atrophy that is similar in patients with ALS and ALS-FTD with the exception that the frontal regions are significantly more atrophic in the ALS-FTD group (Fig. 5–1; Chang et al., 2005). Dividing patients into groups of cognitively normal, ALS-Plus (ALSci and ALSbi), and ALS-FTD yielded a continuum for cortical atrophy as measured by voxel-based morphometry (Mioshi, Lillo, et al., 2013), with ALS showing atrophy only in the brainstem, ALS-Plus showing atrophy in the motor and somatosensory areas, and ALS-FTD showing additional atrophy in the frontal and temporal regions. Longitudinal cortical atrophy in the motor and premotor cortex and parietal lobes has been demonstrated in one small series of patients with ALS-FTD (Avants, Khan, McCluskey, Elman, & Grossman, 2009). In summary, with the use of advanced neuroimaging techniques, and particularly when patients with ALS are divided into groups based on their cognitive status, it is possible to identify areas of abnormality. The applicability of these techniques for clinical use and for use as a surrogate marker of disease has not yet been demonstrated.

GENETICS AND PATHOLOGY

Mutations in the genes encoding copper/zinc superoxide dismutase (SOD1), TAR DNA-binding protein (TDP-43), and fused in sarcoma (FUS) account for approximately 30% of familial ALS (Al-Chalabi et al., 2012). Importantly, a hexanucleotide repeat expansion in the C9ORF72 gene is the commonest cause of familial ALS and is also associated with approximately 6–10% of apparently sporadic ALS (DeJesus-Hernandez et al., 2011l Renton et al., 2011; Dobson-Stone et al., 2012; Snowden et al., 2012; Sha et al., 2012; Majounie

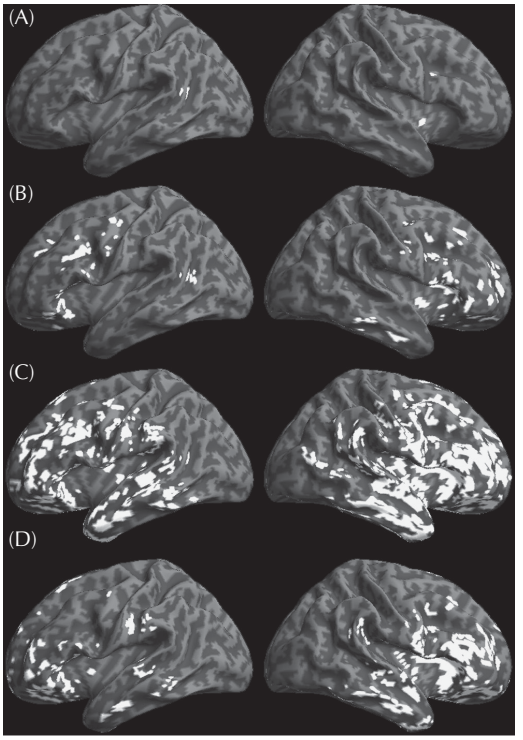

Figure 5–1. Grey matter density derived from T1-weigh ted magnetic resonance imaging. (A) Regions of atrophy in ALS with normal cognition relative to elderly control subjects. (B) Regions of atrophy in ALS with mild cognitive impairment relative to elderly control subjects. (C) Regions of atrophy in ALS-FTD relative to elderly control subjects. (D) Regions of atrophy in ALS-FTD relative to ALS with normal cognition. (Courtesy of Penn Frontotemporal Degeneration Center.)

et al., 2012). There are other, less common, causes of familial ALS and mutations that are known to be associated with sporadic disease. A brief discussion of the genetics and pathology of a few of these genes is illustrative of the relationship between ALS and FTD.

The pathology of FTLD is divided into two large groups. FTLD-tau is characterized by abnormal tau pathology in neurons and glia and may be sporadic or associated with mutations in the MAPT gene. Progressive supranuclear palsy may be seen with FTLD-tau, but associated motor neuron disease has not been reported. FTLD-U is an alternative pathologic form characterized by tau-negative and α-synuclein–negative ubiquitinated intraneuronal inclusions. Likewise, ubiquitinated intraneuronal and intraglial inclusions is a common pathologic feature of ALS. The first pathologic link between ALS and FTD was reported in 2006 with the identification of the 43 kDa TAR-DNA binding protein (TDP-43) as the major protein component of ubiquitinated inclusions in most cases of ALS and FTLD-U (Neumann et al., 2006). Subsequently, mutations in the gene encoding the protein TDP-43 were identified in cases of familial ALS (Van Deerlin et al., 2008). Since then knowledge of the links between the genetics and pathology of ALS and FTD, and of the overlap between the two, has rapidly expanded. TDP-43 positive inclusions are found in extramotor cortical areas in patients with ALS; similarly, TDP-43 pathology is not limited to the frontal and temporal regions in patients with FTD. The distribution of TDP-43 pathology within the central nervous system in patients with ALS, ALS-FTD, and FTD is reflective of the clinical phenotype (Fig. 5–2; Geser et al., 2009).

Mutations in C9ORF72 account for approximately 35–40% of familial ALS and at least 10% of familial FTD; families may have members with ALS, FTD, or ALS-FTD (Dobson-Stone et al., 2012; Snowden et al., 2012; Sha et al., 2012; Majounie et al., 2012; Mahoney et al., 2012). The most common pattern of cognitive impairment seen is a behavioral variant presenting as apathy and loss of initiative (Mahoney et al., 2012). Other reported presentations include language abnormalities, anxiety and agitation, memory impairment, and psychotic symptoms (Dobson-Stone et al., 2012; Snowden et al., 2012; Mahoney et al., 2012; Boeve & Graff-Radford, 2012). The pathology of C9ORF72 expansion-related disease is emerging as distinct from that of other forms of TDP-43–positive ALS. In addition to the typical TDP-43–positive inclusions in frontal and temporal cortex, hippocampus, and motor system, there is an excess of TDP-43–negative inclusions that are ubiquitin-positive and p62-positive distributed throughout the neocortex, hippocampus, and cerebellum (Mahoney et al., 2012; Mackenzie, Frick, & Neumann, 2013). Additionally, imaging in patients with C9ORF72-associated FTD is more symmetric and shows less temporal lobe involvement than in FTD related to either a mutation in progranulin or microtubule-associated tau (Mahoney et al., 2012).

Although most ALS cases are characterized by the presence of TDP-43 pathology, there are

some exceptions. A search for TDP-43 homologs in the region on chromosome 16 identified by linkage studies in ALS-FTD families led to the identification of causative mutations in FUS (Kwiatkowski et al., 2009). The pathology of FUS-mediated ALS shows FUS-positive cytoplasmic inclusions in spinal cord motor neurons and dystrophic neurites, and is negative for TDP-43 (Kwiatkowski et al., 2009; Vance et al., 2009). The clinical phenotypes of FUS-mediated ALS include ALS, ALS-FTD, and FTD.

Mutations in the SOD1 gene were the first described genetic cause of ALS in 1993 (Rosen et al., 1993). SOD1-mediated ALS demonstrates ubiquitinated inclusions containing SOD1 but these inclusions are TDP-43–negative (Mackenzie et al., 2007). Dementia is not commonly found in patients with SOD1-mediated ALS (Wicks et al., 2009).

The identification of mutations in the gene UBQLN2, which encodes the protein ubiquilin-2, as causative for X-linked–dominant ALS and ALS/dementia led to description of a potentially unifying pathologic pattern among different genetically mediated and pathologically distinct forms of ALS (Deng et al., 2011). The ubiquitinated inclusions in UBQLN2-associated ALS contain ubiquilin-2 and are also immunoreactive to TDP-43, FUS, and optineurin (mutations that are associated with sporadic ALS), but not SOD1. Ubiquilin-2 immunoreactivity is also found within the inclusions in cases of sporadic ALS; familial ALS without mutation in SOD1, TDP-43, or FUS; ALS with dementia; familial ALS with SOD1 mutation; and familial ALS with TDP-43 mutation. Dementia may be present in patients with UBQLN2 mutations and ALS in the form of FTD with eventual progression to more global dementia. Patients with this mutation and dementia without motor neuron disease have not been described. Ubiquilin-2 pathology was identified in the hippocampi of patients with ALS/dementia with and without UBQLN2 mutations; these inclusions were negative for FUS and only variably positive for TDP-43. Ubiquilin-2 pathology in the hippocampus was absent in nondemented patients with ALS. This suggests that an interaction between TDP-43 and ubiquilin-2 may increase the likelihood of protein aggregation and may be involved in the pathogenesis of cell death (Deng et al., 2011).

BIOMARKERS

Identifying biomarkers to aid in diagnosis and to act as surrogate markers for disease severity and progression has proved extremely difficult in the field of neurodegenerative diseases, but important progress is being made. A reduced cerebrospinal fluid (CSF) ratio of Tau phosphorylated at threonine 181 (pTau) to total Tau (tTau) differentiates patients with FTLD-TDP from patients with FTLD-tau, Alzheimer disease, and healthy seniors (Hu et al., 2013). Similarly, reduced CSF pTau:tTau differentiates patients with ALS from patients with tauopathies (Grossman et al., 2014). In

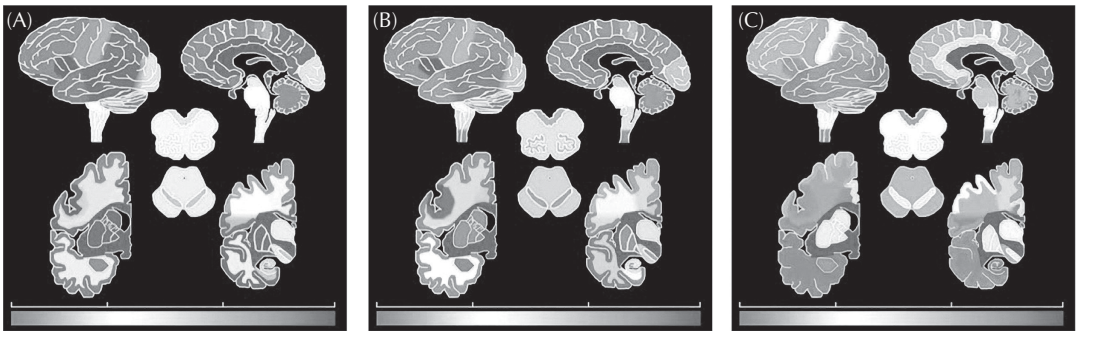

Figure 5–2. Whole-brain heat map of TDP-43 pathology in the central nervous system. The color scale represents quantity of TDP-43 pathology from green (zero) to red (highest). Blue areas were not examined. The clinical phenotypes are (A) FTD, (B) FTD-ALS, (C) ALS. (Adapted from Geser et al., 2009, Copyright © 2009 American Medical Association. All rights reserved.)

addition, CSF pTau:tTau in ALS is correlated with clinical measures including the Mini Mental State Exam and the ALS Functional Rating Scale-Revised, and with imaging findings in the corticospinal tract and prefrontal cortex; this may be a reflection of the relationship between a biomarker (CSF pTau:tTau) and disease burden. The importance of biomarkers cannot be overstated because drug development aimed at protein-specific underlying mechanisms of disease requires in vivo knowledge of the underlying pathology to appropriately direct trials and eventual therapy.

CLINICAL RELEVANCE

The clinical relevance of identifying the presence of cognitive and behavioral dysfunction in patients with motor neuron disease is two-fold. First, education about the clinical features of FTD is helpful and often validating for baffled caregivers, particularly in light of the many statements in the lay press and on the internet that patients with ALS remain cognitively unimpaired. Second, the presence of cognitive impairment may inform the discussion of advance directives with respect to when the topic is approached, the interventions offered, and the degree of involvement of the patient and caregiver in these decisions. This is particularly relevant in that patients with ALS-FTD have shorter survival than that seen in isolated ALS or FTD (Hu et al., 2009; Olney et al., 2005); this may be related to noncompliance with recommended medical interventions in the setting of dementia (Olney et al., 2005).

EFFECT OF FTD ON COURSE AND PROGNOSIS

Cognitive and behavioral dysfunction in patients with ALS has direct implications for patients with regard to clinical course and interventions. Noncompliance with recommendations for noninvasive ventilation and feeding tubes was much higher in patients with ALS who had executive or behavioral dysfunction than in those who were cognitively and behaviorally unimpaired (Olney et al., 2005). Perhaps (or perhaps not) related to this, executive or behavioral dysfunction in patients with ALS, including those whose impairments are not severe enough to meet criteria for FTD, is associated with significantly shorter survival than that seen in patients who are cognitively and behaviorally normal (Olney et al., 2005; Elamin et al., 2011). This is particularly true in those patients in whom cognitive and motor symptoms begin simultaneously, or in whom motor symptoms precede cognitive symptoms (Hu et al., 2009). However, in a potentially large subset of patients in whom subtle neurobehavioral deficits precede the onset of motor symptoms and do not meet criteria for FTD, the presence of these symptoms does not carry with it a worsened prognosis (Mioshi et al., 2014).

IMPLICATIONS OF FTD FOR THE CAREGIVER

Most of the effects of cognitive and behavioral dysfunction on caregivers have been studied and reported in patients without ALS, but the information is instructive and important for guiding clinical care for patients with ALS-FTD. Caregivers of patients with FTD had a higher burden than caregivers of patients with Alzheimer disease. Equally important, caregivers of those who were demented for shorter periods of time had lower health-related quality of life, a finding that is particularly relevant for those with ALS and FTD, who often have faster rates of progression than patients with FTD alone (Nunnemann, Kurz, Leucht, & Diehl-Schmid, 2012; Riedijk et al., 2006). Those caring for patients with bvFTD (the most common in ALS) experience a higher burden than those caring for patients with PNFA and SD (Mioshi, Foxe, et al., 2013). They demonstrate greater strain and distress, more depressive symptoms, and a lower perception of control (Wong et al., 2012). For patients with FTD, apathy correlated with caregiver emotional distress (Merrilees et al., 2013). This does not mitigate over time. On the contrary, caregiver burden increases with disease progression (Mioshi, Foxe, et al., 2013). Studies specific to caregivers of patients with ALS and FTD are rare but confirmatory of the overall picture in FTD. In patients with ALS, neurobehavioral symptoms as measured by the

Frontal Systems Behavior Scale were related to lower caregiver quality of life, higher caregiver depression, and higher caregiver burden (Chio et al., 2010).

NONPHARMACOLOGIC MANAGEMENT OF FTD

Nonpharmacologic management of the patient must occur through the caregiver, and should begin with caregiver education about FTD in general and about its relationship to ALS. There is a poor understanding overall of cognitive and behavioral change in ALS among caregivers. A few years ago, more than half of ALS caregiver respondents to an online ALS forum reported not knowing that a form of dementia can sometimes occur with the disease (Boutoleau-Bretonniere, Vercelletto, Volteau, Renou, & Lamy, 2008). Patients and caregivers alike have indicated a preference to have more information from their healthcare providers regarding cognitive symptoms associated with ALS (Wicks & Frost, 2008).

There are no randomized trials of nonpharmacologic interventions. Such interventions have included structured caregiver support groups, respite care, daycare programs, and advance-practice nursing involvement (Nunnemann et al., 2012). Management strategies have been discussed in a descriptive manner, and provide a broad framework for helping these patients and their caregivers. Education about FTD, as discussed previously, is critical. Safety concerns should be addressed. Verbal interactions with the patient should be guided by knowledge of their limitations in judgment. Financial interactions must be supervised or co-opted by the caregiver. Guidance in making end-of-life decisions is crucial. General guidelines are summarized in Box 5.1 (Houseman et al., 2013; Merrilees et al., 2010). A structured group program for caregivers of people with FTD has been piloted and shows promise (McKinnon, O'Connor, Savage, Hodges, & Mioshi, 2013). There is a need for ongoing emotional support by the ALS team to the caregiver. A recommendation has been made that those caring for patients with FTD be offered more support than those caring for individuals with Alzheimer disease. Because passive coping strategies have been associated with increased caregiver burden and decreased caregiver quality of life, there is also a need for identifying and teaching coping strategies to these caregivers (Riedijk et al., 2006).

PHARMACOLOGIC MANAGEMENT OF ALS-FTD

To date, there are no medications approved by the Food and Drug Administration for the treatment of the underlying disease process of FTD. Treatment strategies in FTD have centered on neurotransmitter augmentation. Acetylcholinesterase inhibitors are used with success in Alzheimer disease, although there is no evidence of deficiencies in the cholinergic system in FTD that are likely to respond to this therapy. The only placebo-controlled trial of an acetylcholinesterase inhibitor in FTD was conducted using galantamine and was negative (Kertesz et al., 2008), and all current recommendations are to avoid these drugs because there is some risk of worsening behavior (Mendez, Shapira, McMurtray, & Licht, 2007). A consensus statement from the British Association for Psychopharmacology cites type I evidence that cholinesterase inhibitors are not recommended for the treatment of FTD (O'Brien & Burns, 2011). From a symptom management perspective, additional saliva that would likely result from acetylcholinesterase inhibitors is not a side effect that would be well tolerated by a patient with bulbar dysfunction from motor neuron disease. Memantine, an *N*-methyl-D-asparate receptor antagonist, has commonly been used off-label in the FTD population but there has been a recent negative phase 3 trial of memantine in FTD (Boxer et al., 2013), so this will likely fall out of favor.

Similarly, there are no treatments approved by the Food and Drug Administration for the behavioral symptoms in FTD. In large part, clinicians have used psychoactive drugs off-label to treat these symptoms. There is some evidence that the serotonergic system is involved in the pathophysiology of FTD. Serotonergic agents have been used with some success to treat the symptoms of disinhibition, depression, carbohydrate craving, and ritualistic behaviors (Pasquier et al., 2003; Chow & Mendez, 2002; Moretti, Torre, Antonello, Cazzato, & Bava, 2003a; Wang, Shen, & Chen,

Box 5.1 Guidelines to Assist Caregivers of Individuals with ALS/FTD

- Medication management
  - Assist initially, control if needed
- Driving
  - Assess whether patient is driving safety
  - Remove driving privileges or schedule formal driving evaluation
- Financial
  - Oversee the patient's financial transactions
  - If money is not being managed properly, take action: close credit card accounts, take over the management of savings and checking accounts
  - Obtain power of attorney for financial decisions when appropriate
- Safety
  - Monitor or prohibit use of power tools, stoves, ovens
  - Lock up firearms
  - Prevent access to some foods
  - Supervise meals to avoid choking due to food stuffing
- Alter verbal interactions
  - Use simple language
  - Provide direction rather than asking questions (e.g., "we are going to get dressed" rather than "would you like to get dressed?" or "you are going to be eating this food for lunch, and we will be giving some of your calories through your feeding tube" rather than "what would you like for lunch?")
  - Avoid arguing and trying to reason: change the subject or distract
- Assist with decision-making
  - Priorities include invasive treatment decisions and end-of-life issues: feeding tube, tracheostomy, and ventilation
  - Help with developing advance directives, such as a living will and durable power of attorney for health care
  - Address critical issues as early in the disease as possible, before cognitive and behavioral impairment prevent meaningful discussions

Adapted from Wicks & Frost (2008) and Houseman et al. (2013).

2013; Huey, Putnam, Grafman, 2006; Swartz, Miller, Lesser, & Darby, 1997; Mendez, 2009). One study, however, has shown some negative effects of paroxetine on cognition (Deakin et al., 2004). Trazadone seems to be beneficial in improving behavioral symptoms, such as irritability, agitation, depressive symptoms, and eating abnormalities (Lebert, Stekke, Hasenbroekx, Pasquier, & Lebert, 2004).

Antipsychotics may improve behavioral symptoms and irritability (Mendez, 2009; Fellgiebel, Muller, Hiemke, Bartenstein, & Schreckenberger, 2007; Moretti et al., 2003b). Quetiapine was effective in reducing agitation (Chow & Mendez, 2002). Although atypical antipsychotics are often used in this population, somnolence and extrapyramidal signs limit their use. It is also important to remember

that typical and atypical antipsychotic agents are associated with increased risk of death in the elderly population (Wang et al., 2005).

CONCLUSION

It is now clear that ALS is a heterogeneous neurodegenerative syndrome in which cognitive and behavioral dysfunction, ranging from subclinical to FTD, occurs in about half of all patients. Genetic and pathologic evidence has established clear ties between the motor and cognitive/behavioral aspects of the ALS syndrome. Clinicians should become familiar with this aspect of ALS to provide the best possible care to their patients and the patients' caregivers. Relatively short cognitive screening batteries can be administered in the outpatient setting to assist with diagnosis. Imaging holds promise, but lacks sensitivity, specificity, and availability needed to be a standard part of the diagnostic armamentarium. FTD has many ramifications, including shorter survival of the patient and greater burden on the caregiver. Effective management must be multifactorial, including the education and training of the caregiver. Some behavioral aspects of FTD may respond to pharmacologic treatments, particularly with serotonergic agents. There is no effective treatment for the cognitive impairment.

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Chapter 6

Laboratory Tests for Motor Neuron Disease

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Efforts to find biomarkers to confirm the diagnosis of the forms of motor neuron disease (MND) have not been successful to date, and the diagnosis remains clinical. A list of laboratory tests to detect an alternative diagnosis was included in the El Escorial Criteria.

The most important laboratory test is the electromyogram (EMG) to confirm lower motor neuron (LMN) loss as the cause of weakness in clinically affected muscles and demonstrate subclinical LMN loss in a wide distribution. The Awaji Criteria raised the presence of fasciculation potentials on EMG examination as the earliest and most sensitive sign of LMN loss and can serve to document a muscle's involvement. Ultrasound imaging of muscle is sensitive for fasciculation potentials and other changes supportive of LMN loss. Blood tests help exclude other diagnoses, but most tests listed are not pertinent to identifying a true mimic disorder. Cognitive and behavioral abnormalities are common and several screening and brief tests are available. The number of genes associated with amyotrophic lateral sclerosis (ALS) and frontotemporal dysfunction has grown. The role of genetic testing is more important because a family history of dementia may be linked to ALS, and the penetrance for some genes is less than 100%.

INTRODUCTION

There are no distinct laboratory features or biomarkers for the various forms of MND. Electrodiagnostic testing, consisting of nerve conduction studies and needle EMG, are the

most important laboratory tests to demonstrate LMN degeneration in addition to assessing for alternative diagnoses. All laboratory studies, and electrodiagnostic studies in particular, are extensions of the neurologic evaluation, and test results are to be interpreted in the clinical context and do not stand alone. Laboratory tests for upper motor neuron (UMN) degeneration are not sensitive or specific.

NERVE CONDUCTION TESTING

Sensory nerve studies are generally within normal limits (de Carvalho & Swash, 2000). However, reduced sensory nerve amplitudes are found in 27%, and morphologic evidence for axonal loss in 91%, but some of the abnormalities were subclinical and not associated with symptoms of a neuropathy (Hammad, Silva, Glass, Sladky, & Benatar, 2007). Motor nerve studies are normal in strong muscles but show reduced compound muscle action potential (CMAP) amplitude proportional to strength in weak muscles. Nerves with reduced CMAP potentials are associated with mildly slowed conduction metrics (prolonged distal latency, slowed conduction velocity, prolonged F-wave latency) attributed to axonal loss (de Carvalho & Swash, 2000; Daube, 2000).

A length-dependent small fiber sensory neuropathy has been demonstrated in ALS. Twenty-eight patients with ALS were studied by skin biopsy an average of 34 months after disease onset (Weis et al., 2011). There was a significant loss of intraepidermal nerve fibers compared with control subjects, and corresponding sensory symptoms were noted in seven patients and most had normal sural nerve responses.

A defect in neuromuscular junction transmission can be demonstrated by a decrement of CMAP amplitude to low-frequency repetitive nerve stimulation (4–9 shocks at 2–3 Hz), most often in muscles that are weak and have low CMAP amplitudes. The pathophysiologic mechanism is thought to be a reduction in readily available quantal stores of acetylcholine in the presynaptic terminal leading to failure at neuromuscular junctions (Henderson & Daube, 2004).

NERVE CONDUCTION TESTS FOR UMN LOSS

Efforts to assess change in LMN excitability as a measure of UMN loss show that F-waves (persistence) and H-waves (H/M ratio) vary between patients with predominantly UMN or LMN loss, but there is marked overlap with normal subjects, and it is not diagnostically discriminative (Drory, Kovach, & Groozman, 2001).

NEEDLE EMG TESTING

The most important laboratory test to assess for LMN degeneration is the needle EMG study (Daube, 2000). Criteria for electrodiagnostic studies supportive of progressive and diffuse LMN degeneration were formalized by Lambert in 1957 and 1969 (Lambert & Mulder 1957; Lambert 1969) (Box 6.1) as active denervation (positive waves and fibrillation potentials) and chronic denervation (enlarged motor units with increased duration with decreased recruitment) denervation and were later incorporated into the El Escorial Criteria. However, operational issues with the Lambert and El Escorial Criteria as applied to ALS have been critiqued:

1. Rapid motor unit discharge rates (reduced recruitment) is the electrodiagnostic hallmark of neurogenic denervation, but this pattern may not be apparent in the setting of prominent UMN degeneration, and lower discharge rates may be observed in the setting of ALS (Wilbourn, 1998).
2. There is a major effort to identify subclinical denervation to aid in the early diagnosis of ALS (Krarup, 2011). Abnormal spontaneous activity in the form of positive waves and fibrillation potentials is a reliably recognized sign of active denervation (Daube, 2000). However, positive waves and fibrillation potentials may not be present in the setting of LMN loss as manifest by enlarged motor units with increased duration and reduced recruitment. Accordingly, there is a focus on the diagnostic role of fasciculation potentials as a very early (earliest) sign of subclinical denervation, and fasciculation potentials have been incorporated into the

Box 6.1 Review of Lambert Criteria for Findings in ALS (Lambert & Mulder, 1957; Lambert, 1969)

- Normal sensory nerve conduction studies
- Normal motor nerve conduction velocities when recording from relatively unaffected muscles and ≥70% of age-matched normal values when recording from severely affected muscles
- Fibrillation and fasciculation potentials
- Motor unit potentials reduced in number and increased in duration and amplitude
- Distribution of fibrillation and fasciculation potentials in limb and head muscles

Awaji Criteria (de Carvalho et al., 2008). Although there is controversy over the origin of fasciculation potentials (representing UMN or LMN pathology) there is agreement that they are common, diffusely distributed, and occur early in ALS, and may have complex waveforms (Wilbourn, 1998; de Carvalho et al., 2008; Shiga et al., 2000; Krarup, 2011).

The frequency of fasciculation potentials (i.e., time period of observation) has been investigated (Mills, 2011). From 19 patients with a diagnosis of definite ALS the number of fasciculation potentials and the time to occur was recorded from 53 muscles. The first dorsal interosseous, biceps brachii and anterior tibialis muscles showed the most potentials (~4 per min), and the longest observation time for one fasciculation to occur is about 90 seconds and for two potentials about 120 seconds.

3. Documentation of widespread LMN loss as set forth in the El Escorial Criteria (in at least two muscles of different root or spinal nerve and different cranial or peripheral nerve innervation in two or more of the four regions) is somewhat arbitrary (Wilbourn, 1998). The distribution of LMN loss in limb muscles at time of diagnosis varies among patients. In a retrospective study of 73 patients who ultimately met El Escorial Criteria, receiver operating characteristic curve analysis was used to determine the sensitivity and specificity of the number of abnormal muscles identified (Makki & Benatar, 2007). Analysis indicated that the best combination of sensitivity and specificity was achieved by requiring EMG changes in two muscles in cervical and lumbosacral regions and in one muscle in cranial and thoracic regions.

EMG ABNORMALITIES IN INDIVIDUAL MUSCLES

One goal of the EMG study is to confirm LMN loss in both clinically atrophic and weak muscles but also subclinical denervation in strong muscles. The likelihood of finding evidence of denervation in MND in individual muscles at initial diagnostic evaluation has been studied.

Cervical and Lumbosacral Muscles

The selection of muscles to study depends upon the clinical presentation (distribution of weakness) and the electromyographer's preferences. In one study in the cervical region, the frequency

of muscles studied was deltoid, first dorsal interosseous, biceps brachii, flexor carpi radialis, and triceps; in the lumbosacral region the frequency of study was anterior tibialis, vastus lateralis, and medial gastrocnemius. However, the sensitivity for finding denervation of each muscle is not indicated (Makki & Benatar, 2007).

Cranial Muscles

Demonstration of denervation in muscles innervated by cranial nerves is strong evidence for extensive distribution of LMN loss. The El Escorial Criteria requires demonstration of denervation in a single cranial muscle. The tongue is an obvious muscle to assess for denervation but it can be difficult to identify abnormal spontaneous activity due to poor relaxation. A study of 17 patients demonstrated spontaneous activity in 82% of patients with and without bulbar symptoms (Tankisi, Otto, Pugdahl, & Fuglsang-Frederiksen, 2013). A study of nine patients without clinical evidence of LMN degeneration in cranial muscles documented six who had positive fibrillation or fasciculation potentials in frontalis, masseter, or sternocleidomastoid muscles (Finsterer, Erdorf, Mamoli, & Fuglsang-Frederiksen, 1998). The sternocleidomastoid muscle has been shown to be involved as often as the tongue in ALS, and is easier to evaluate (Li, Petajan, Smith, & Bromberg, 2002). The upper trapezius muscle has been shown to have positive waves and fibrillation potentials more often (45%) than the sternocleidomastoid (13%) and tongue (8%; Sonoo et al., 2009).

Diaphragm

Denervation of the diaphragm muscle is unique to ALS (and progressive muscular atrophy), and in a study of 52 patients with the El Escorial Criteria diagnosis of definite ALS, 44% had abnormal spontaneous activity (positive waves, fibrillation potentials, or fasciculation potentials) at time of diagnosis (Stewart, Eisen, Road, Mezei, & Weber, 2001).

Thoracic and Rectus Abdominis Muscles

Thoracic paraspinal muscles have been assessed for denervation in ALS and positive waves and fibrillation potentials were found in 44% of patients, 93% who met El Escorial Criteria for definite or probable ALS (Makki & Benatar, 2007). Another study compared 38 patients with El Escorial Criteria definite and probable ALS with 28 patients evaluated for other disorders and found fasciculation potentials in 44.7% and positive waves and fibrillation potentials in 21.1% of patients with ALS and none in control patients (de Carvalho, Pinto, & Swash, 2009).

The rectus abdominis muscle has also been considered when relaxation of thoracic paraspinal muscles is difficult. Sixty-seven patients with definite ALS were compared with 110 healthy control subjects, and abnormal spontaneous activity was found in the rectus muscle in 75% of patients with ALS and none in control subjects. Furthermore, motor unit potentials were of higher amplitude, longer duration, and greater waveform complexity (polyphasia) (Xu et al., 2007).

MUSCLE BIOPSY

Muscle biopsies can be used to document neurogenic denervation and exclude alternative diagnoses, usually myopathies. Histologic changes supportive of neurogenic denervation are angular atrophic fibers of fiber types 1 and 2, and fiber-type grouping with individual groups containing the same fiber types, whereas associated findings are scattered hypertrophic fibers and necrotic fibers (Baloh, Rakowicz, Gardner, & Pestronk, 2007). These features can be present in a muscle prior to changes observed on EMG (Blijham, Schelhaas, Ter Laak, van Engelen, & Zwarts, 2007). The disadvantage of muscle biopsies is that it is invasive and thus limited, usually to observations in a single muscle.

SEROLOGIC AND SPINAL FLUID LABORATORY STUDIES

A large number of blood and spinal fluid tests have been proposed for the evaluation of MND, primarily looking for alternative causes. A review of the literature indicates that most tests have only class IV supportive evidence (anecdotal evidence) (Andersen et al., 2005), Despite this

lack of supportive data a large number of basic tests of homeostatic function, and not directly related to MND, are frequently recommended (full metabolic panel, complete blood type, serum electrophoresis, and creatine kinase).

Serum Creatine Kinase

Elevated creatine kinase levels are primarily associated with myopathic disorders, but can also be elevated in ALS. Creatine kinase levels are elevated in 23–43% of patients with ALS, with values up to 7 times the upper limit of normal at time of diagnosis (Chahin & Sorenson, 2009; Ilzecka & Stelmasiak, 2003). Values tend to be higher in male patients with ALS and those with limb onset. There is no correlation between creatine kinase levels and degree of denervation on EMG study (fibrillation and fasciculation potentials), overall degree of weakness and dysfunction, or survival (Felice & North, 1998). Serial levels do not significantly change over time (Lima, Evangelista, & de Carvalho, 2003). The underlying pathologic reason in ALS is not known.

Cerebrospinal Fluid

A neuroinflammatory factor has been proposed as part of ALS pathology, and analysis of cerebrospinal fluid for evidence of breakdown of the blood-brain barrier (elevated protein) or intrathecal synthesis of immunoglobulins has been performed (Ticozzi et al., 2013). Among 246 patients, oligoclonal bands were noted in 3.5% (other fluid values were normal), and one-third had ALS-associated gene mutations.

CENTRAL NERVOUS SYSTEM IMAGING

Conventional magnetic resonance imaging (MRI) of the brain is usually unrevealing for evidence for UMN degeneration and is most commonly used to exclude other conditions that might account for hyperactive reflexes. With conventional MRI, using T1, T2, and FLAIR sequences, hyperintensity (defined as signal changes observed over several sections) can best be sought on coronal scans showing bilateral increased signal intensity from the centrum semiovale down into the brainstem (Agosta et al., 2010). In a research MRI setting studying patients with ALS early in the course of their disease, sensitivity was 17.1% for corticospinal tract hyperintensity, but rose to 63.4% for patients with ALS diagnosed with El Escorial Criteria definite of probable ALS. Among patients with the diagnosis of primary lateral sclerosis sensitivity was 71.9% (Charil et al., 2009). In routine radiologic practice, the sequences used, level of scrutiny, and experience vary, and the sensitivity is likely much lower. Furthermore, patients without neurologic disease can have such signal changes. There are many possible sequences available for research-focused MRI studies and sensitivity is likely higher with diffusion-weighted imaging and other sequences

MUSCLE IMAGING

Imaging muscle with MRI or ultrasound has not been routinely used diagnostically. Neither MRI or ultrasound findings are specific for LMN loss. MRI required a separate visit but ultrasound could be incorporated with an electrodiagnostic study.

MRI

Reports of MRI of skeletal limb muscle in ALS are sparse. A distinct pattern in ALS (two subjects) compared with Kennedy disease (three subjects) has been reported with T1-weighted images showing more generalized atrophy of leg muscles in ALS and more fatty replacement in Kennedy disease (Hamano et al., 2004). Another study of 11 patients with ALS showed in half a "moth-eaten" fatty infiltration pattern that was either symmetric or asymmetric in the anterior tibialis muscle and some degree (but not significant) of atrophy compared with control muscles (Bryan et al., 1998).

A report of a routine brain MRI scan on a patient with symptoms and signs of ALS, including bulbar dysfunction, shows atrophy and T1 hyperintensity of the tongue consistent with fatty infiltration (Fox & Cohen, 2012).

Box 6.2 Hierarchical Cognitive and Behavioral Tests to Assess Frontotemporal Lobe Function, and Elements to Query Family Members About

Screening Tests

Verbal (written or oral) fluency measures

Brief Tests

Penn State Rapid Screening Battery (Flaherty-Craig et al., 2006)
ALS Cognitive Behavioral Screen (Woolley et al., 2010)
UCSF Brief Screening Battery (unpublished)
Addenbrooke's Cognitive Examination (Mioshi et al., 2006)
Montreal Cognitive Assessment (Nasreddine et al., 2005)
Frontal Behavioral Inventory (Blair et al., 2007)
Frontal System Behavioral Scale (Stout et al., 2003)
Edinburgh Cognitive & Behavioral ALS Screen (Abrahams et al., 2014)

Observed Cognitive and Behavioral Abnormalities

Decline in personal hygiene and grooming
Mental rigidity and inflexibility
Distractibility and impersistence
Hyperorality and dietary changes
Perseverative and stereotyped behavior
Loss of insight, disinhibition
Restlessness, distractibility
Reduced empathy or unconcern for others
Lack of foresight or planning
Apathy or loss of spontaneity
Reduced verbal output
Sexual hyperactivity

Modified from Strong et al. (2009).

Ultrasound

Ultrasound has been used to demonstrate fasciculation potentials and changes in muscle volume and echo intensity, and all three features are common in multiple muscles in the clinical setting of ALS (Arts et al., 2008), although it is not possible to distinguish among neuropathic disorders (Maurits et al., 2003). A study of 81 patients, with 48% meeting definite of probable by Airlie House Criteria, compared ultrasound with needle EMG to detect fasciculation potentials (Misawa et al., 2011). Ultrasound was much more sensitive, particularly in the tongue, but also in limb muscles. Using the presence of fasciculation potentials in the setting of the Awaji Criteria the diagnostic certainty increased to 79%.

Ultrasound has also been shown to be useful as a diagnostic tool to distinguish patients with ALS from those with other neuromuscular diseases (Arts et al., 2012). Based on detecting fasciculation potentials and changes in echogenicity (Arts et al., 2008), in a study of 27 patients with ALS (including those with El Escorial Criteria definite, probable, possible, laboratory supported, and progressive muscular atrophy) compared with 32 patients with other diseases (including benign fasciculation

potentials and cramps, polyneuropathy, multifocal motor neuropathy, and myopathy) false-positive ultrasound findings occurred in five patients without ALS, axonal neuropathies, and fasciculation syndrome, and false-negative in one patient with bulbar symptoms who later progressed to a diagnosis of ALS. In a second part of the study, ultrasound findings were found more often than EMG abnormalities.

The diaphragm can also be imaged with ultrasound with the probe at an intercostal space between the anterior and mid-axillary lines (Hiwatani, Sakata, & Miwa, 2013). Measurements of diaphragm thickness, which changes with respiration, can be expressed as an index (minimum/maximal thickness), and was found to be reduced when forced vital capacity was less than 80% of predicted and was correlated with other respiratory measures.

Ultrasound is sensitive to peripheral nerve cross-sectional area, and the median nerve at mid-arm was found to be reduced in 20 patients with ALS compared with control subjects (Cartwright, Walker, Griffin, & Caress, 2011).

FRONTOTEMPORAL LOBE TESTING

There is a broad spectrum of symptoms of cognitive and behavioral dysfunction associated with frontotemporal lobe dementia, and in the setting of MND they can vary in magnitude from those meeting dementia criteria to elements of a behavioral syndrome. There is also a spectrum of neuropsychological testing. A consensus meeting in 2009 laid out a hierarchical testing paradigm based on time, resources, and the underlying clinical question (Box 6.2; Strong et al., 2009). A screening assessment can be performed in 2–5 min, and is based on empirically supported questions. A brief assessment can be performed in 5–20 min, and is based on established cognitive screens or a combination of such. A formal neuropsychological assessment requires several hours and the skills of a neuropsychologist. It was emphasized that for all assessments, the spouse and other family members should be present to provide observational information (Table 6–1). It was also emphasized that the screen and brief assessments are not definitive for the diagnosis of frontotemporal dementia or syndrome, and are not a substitute for formal testing.

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Chapter 7

Biomarkers for Motor Neuron Disease

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BACKGROUND AND NEW POINTS

Biomarkers are important tools for diagnosis, prognosis, monitoring progression, and as aids in understanding pathophysiology. Early efforts were limited and based on relatively simple observations.

Recent biomarker efforts have been enhanced by technological advances in genetics, proteomics, electrophysiologic techniques, and imaging. Candidate markers require extensive testing for verification across laboratories and clinical forms of amyotrophic lateral sclerosis (ALS) and longitudinally.

INTRODUCTION

There is a great need to establish biomarkers that can be used to aid diagnosis, provide prognostic indications, and assist in evaluating treatment effects in clinical trials (Table 7–1). The search for biomarkers of ALS has seen considerable advances during the past 15 years. Biomarkers are defined as any genetic, physiologic, protein, or biochemical measure that can be accurately quantified and related to disease onset, progression, or therapeutic response of a drug treatment (Zerhouni, 2003; Group, 2001). The discovery of ALS biomarkers has progressed as technology advancements created new opportunities to quantify nucleic acid and protein alterations, as well as improvements in imaging techniques. The failure of many ALS clinical trials may in part be caused by initiation of the treatment relatively late in the disease course, because the average diagnostic delay is 1 year (Mitchell et al., 2010). Although ALS is currently diagnosed by clinical opinion of an experienced neurologist to rule out mimic disorders, clinical symptoms may occur much later than initial pathologic changes because of primary cellular events. Earlier diagnosis would permit introduction of therapies much closer to these primary disease events and potentially impact treatment-induced disease

Table 7–1 **Types of Biomarkers, Potential use in ALS, and Current Gold Standards**

Biomarker Type	Potential Use	Current Gold Standard
Diagnostic	Rule out ALS; initiate therapy earlier	Neurologic history and examination Electromyography (revised El Escorial/Awaji criteria; Douglass et al., 2010)
Prognostic	Identify patterns of disease progression Estimate survival time Patient stratification for clinical trials Timely intervention and optimize care for gastrostomy, noninvasive ventilation, cognitive support	Diagnostic latency Neurologic evaluation (e.g., clinical phenotypes) Cox modeling of clinical variables (Haverkamp et al., 1995; Turner et al., 2002)
Surrogate	Determine effectiveness of drugs in trials	Revised ALS Functional Rating Score (electrical impedance myography emerging)

modifications. Biomarkers that can monitor disease progression and provide prognostic indications are needed to facilitate drug development efforts. Pharmacodynamic biomarkers are necessary to demonstrate the ability of a drug to "hit" its target, and are used in drug development and clinical trials. Ultimately, biology-based biomarkers may provide surrogate markers in clinical trials to provide faster and improved decision making during clinical trials. This chapter reviews genetic, protein, biochemical, imaging, and physiologic biomarkers of ALS, focusing on human-based studies, and how these biomarkers may ultimately be used in clinical practice.

GENETIC BIOMARKERS

Approximately 10% of patients with ALS exhibit a family history of the disease (Majoor-Krakauer, Willems, & Hofman, 2003), although a recent meta-analysis of prior studies suggests the figure is closer to 5% (Byrne et al., 2011). Although we do not discuss all the genes that have been linked to ALS, we highlight a few specific gene/nucleic acid biomarkers for ALS. Mutations in the superoxide dismutase-1 (*SOD1*) gene were identified in 1993 by DNA sequencing techniques as causative in approximately 10% of familial ALS (FALS; 1–2% of patients with ALS; Rosen et al., 1993). Although present in all cells, SOD1 is not an essential gene product, suggesting that inhibition of mutant *SOD1* gene expression may be an effective treatment for patients that harbor *SOD1* mutations. Recent studies have demonstrated that delivery of SOD1 antisense oligonucleotides into the central nervous system reduces SOD1 protein levels and in a mutant SOD1 transgenic mouse model and retards disease onset and extends survival (Miller et al., 2005; Ralph et al., 2005; Raoul et al., 2005). For studies involving SOD1 antisense treatment, monitoring SOD1 protein levels within the cerebrospinal fluid (CSF) has been used as a pharmacodynamic biomarker for drug treatment (Winer et al., 2013). Current clinical trials testing antisense technologies to eliminate expression of SOD1 may prove effective as a treatment for patients harboring *SOD1* mutations and could potentially be initiated prior to onset of clinical symptoms. This would require genetic testing of those with a family history of ALS to identify families harboring SOD1 mutations, with subsequent antisense oligonucleotide treatment and monitoring reduction of mutant *SOD1* protein levels in the CSF.

As DNA sequencing technologies improved and polymorphisms were identified throughout the genome many additional genetic causes and risk factors of ALS were identified by linkage analysis, candidate gene approaches, exome sequencing, and high-density mapping techniques using DNA microarrays (Table 7–2). These findings highlight various pathways altered in ALS, including axonal transport, RNA metabolism, protein stability, and environmental stress/inflammation, underscoring the importance of these pathways in the pathogenesis of ALS. The discovery of a repeat expansion in an intron of the *C9ORF72* gene in

Table 7–2 **Genetic Markers for ALS and Molecular Pathways**

	Implicated Molecular Pathway			
	DNA/RNA Processing	**Axonal Transport/ Trafficking**	**Protein Aggregation/ Degradation**	**Environmental Stress/ Toxin Susceptibility/ Infection/Inflammation**
Gene				
SOD1		√	√	√
TDP-43	√		√	
FUS	√		√	
C9orf72	√			
TAF15	√			
Senataxin	√			
Ataxin-2	√	√		
Matrin 3	√			
VCP			√	
Angiogenin	√			
SMN	√			
Ubiquilin-2			√	
Optineurin	√	√		√
Dynactin		√		
VAPB		√	√	
Alsin		√		
NEFH		√		
D-amino acid oxidase (DAO)			√	√
Peripherin		√	√	
PON1-3				√
Sigma-R1				√
SQSTM1/p62			√	
ELP	√			√
KIFAP3		√		
CHGB		√		
UNC13A		√		

List of genes linked to ALS (genetic mutations, expansions, or risk factors) and biologic pathways in which they function.

2011 opened up new areas of research and identified the most common genetic cause of ALS, present in approximately 40% of FALS cases and 4–7% of apparently sporadic ALS (SALS; DeJesus-Hernandez et al., 2011; Renton et al., 2011). The repeat expansion of *C9ORF72* represents the best genetic biomarker for ALS. Many questions remain regarding the normal function of the *C9ORF72* gene product and how the repeat expansion causes loss of motor neurons. A family history of ALS or other neurodegenerative diseases warrants sequencing of at least the *SOD1, TDP-43, FUS*, and *C9ORF72* genes to determine if the patient harbors any of these most common genetic alterations associated with ALS.

The discovery of extracellular RNA released from cells that can signal neighboring or distal cells has ushered in a new era of nucleic acid–based biomarkers for health and disease (Rao, Benito, & Fischer, 2013). Recent studies have identified altered levels of microRNAs (miRNAs) in microglia from transgenic mouse models of ALS (Parisi et al., 2013), and global miRNA malfunction causes a motor neuron disease phenotype (Haramati et al., 2010). Circulating blood monocytes were found to have a distinct miRNA signature that was similar in both the mutant G93A SOD1 mice and human patients with ALS (Butovsky et al., 2012). The miRNA profile included miR-27a, miR-155, miR146a, and miR-532-3p and was similar in both the humans and transgenic mice expressing mutant SOD1. miR-27a was highly expressed in patients with ALS but not in healthy control subjects or patients

with multiple sclerosis. These findings must be repeated in a much larger number of patients and additional disease controls to further validate and explore the use of these monocyte-derived miRNAs, or potentially miRNAs identified from other cells or in biofluids, as biomarkers for ALS. Another recent study used tissue-based microarrays to discover biomarkers common between the spinal cord of transgenic mouse models of patients with ALS and SALS (Kudo et al., 2010). Continued use of miRNA- and microarray-based studies will provide additional genetic biomarkers that originate from circulating cells or cells within the tissues affected by disease.

Butovsky and colleagues (2012) also identified a gene profile from $CD14^{+}CD16-$ peripheral monocytes in patients with ALS. This study implicated specific inflammatory pathways in ALS and the ability of peripheral monocytes as a source of biomarkers for ALS. Modulation of the specific peripheral immune miRNA and gene signature detected in ALS may provide a more effective treatment strategy for ALS, using these candidate biomarkers to evaluate immunomodulators in clinical trials. Although general anti-inflammatory and immunosuppressive drugs have failed in ALS clinical trials (Cudkowicz et al., 2006; Smith et al., 1994), specific modulation of the inflammatory signature detected in patients with ALS provides a more selective immune-based therapy that may be effective in patients. Stratification of the patient population based on activation of peripheral monocytes by aggregated SOD1 protein is also the premise for clinical trials of the humanized monoclonal antibody tocilizumab (Acterma) to modulate interkeukin-6 receptor signaling in the nervous system (Mizwicki et al., 2012). If these studies prove successful, the peripheral immune signature could then be used to select patients to receive these specific immunomodulation therapies and serve as a model for using biomarker signatures from peripheral monocytes to guide drug development and patient recruitment into clinical trials.

PROTEIN BIOMARKERS

The search for protein biomarkers of ALS has also significantly advanced in the past decade. The starting material used to discover biomarkers is extremely important to the ultimate success of subsequent experimental studies (Table 7–3). Standard operating procedures have been established for the collection, processing, and storage of biofluids and tissue for ALS biomarker investigations (Otto et al., 2012). Blood and CSF have been the most common biofluids used for protein biomarker research, although muscle and spinal cord tissue have also been used to discover protein biomarkers. Although blood offers minimum invasiveness and is a well-studied proteome, it contains a complex mixture of proteins in a huge dynamic range that typically dilutes the release of any tissue-specific biomarkers. CSF is near the tissue containing the degenerating motor neurons but is more challenging to collect and limits the sampling volume. Saliva and urine are noninvasively collected but very little has been performed with these biofluids in ALS research. Postmortem and biopsy tissues are critically valuable because they contain the cell type and area affected by the disease but there are few tissue banks with high-quality postmortem ALS samples. Skin biopsies are

Table 7–3 **Sources of Biomarkers**

	Biopsy or Postmortem Tissue	CSF	Urine	Saliva	Blood	Imaging
Specificity	+++	+++	+	+	++	+++
Clinical relevance	+++	+++	+	+	+++	++
Availability	+	++	+++	+++	+++	+
Invasiveness	+++	++	+	+	+	+

The location of each biomarker source with respect to disease pathology (specificity), clinical relevance, degree of availability, and invasiveness of procedure. + = low; ++ = moderate; +++ = high.

becoming a valuable resource for the generation of fibroblast cell lines that can be used to generate induced pluripotent stem cells (iPS) and offer new opportunities in ALS biomarker research.

Most of the proteomic studies to date have focused on distinguishing ALS from either healthy controls or other neurologic diseases. These studies therefore are aimed toward diagnostic biomarkers, although many have also tried to determine if these same proteins can provide any prognostic indications for rate of disease progression or patient survival. Antibody-based methods remain the most common approach for protein biomarker discovery efforts, although mass spectrometry has been used by some groups to perform large, unbiased proteomic screens for biomarkers or to sequence proteins eluted from two-dimensional gel electrophoresis experiments (Brettschneider et al., 2008; Ranganathan et al., 2005; Nardo et al., 2011). Many individual proteins or protein panels have been proposed as candidate ALS biomarkers (Table 7–4). Later we further discuss the recent advancements in proteomic biomarkers for ALS and the strengths and limitations of these studies.

Many proteomic studies have focused on select, targeted proteins to study based on the known pathogenic mechanisms of disease. Simple immunoblot or enzyme-linked immunosorbent assays have been used to compare individual protein levels in the blood, CSF, or tissue between ALS and control subjects (Table 7–4). The most well-established single protein biomarkers for ALS are the neurofilament proteins, a marker of axonal degeneration and injury when released into CSF or blood. Both phosphorylated neurofilament heavy chain and light chain have been shown to be elevated in the CSF and blood of patients with ALS by several laboratories located throughout the world (Boylan et al., 2009; Brettschneider et al., 2006; Ganesalingam et al., 2012; Kuhle et al., 2010; Reijn et al., 2009). The ratio of phosphorylated neurofilament heavy chain to complement c3 in the CSF was shown to increase the specificity of predicting ALS (Ganesalingam et al., 2011). A recent paper suggests that patients with ALS have circulating antibodies to neurofilament proteins that could be used to predict and monitor disease progression (Puentes et al., 2014).

Cystatin C levels have been shown to be reduced in the CSF and increased in the plasma of patients with ALS, with reduced functional activity in the CSF (Tsuji-Akimoto et al., 2009; Wilson, Boumaza, & Bowser, 2010; Wilson & Bowser, 2013). ApoE plasma levels measured in 400 patients with ALS were shown to correlate to rate of disease progression and a risk factor for decreased survival time (Lacomblez et al., 2002). Others noted that a ratio of two proteins can help increase the sensitivity and specificity for distinguishing ALS from various control groups or correlate to patient survival (Ganesalingam et al., 2011; Sussmuth et al., 2010). Tetsuka and colleagues (2013) reported that a ratio of creatinine to cystatin C in the blood was a biomarker for residual muscle mass and assessed disease severity and progression in patients with ALS. Studies with muscle biopsies identified increased levels of Nogo-A in ALS muscle (Pradat et al., 2007). Nogo-A functions to impede neurite outgrowth and therefore increased levels may influence the ability of neurite outgrowth and regeneration during ALS. Although it was expected that Nogo-A levels would also increase in the blood because of muscle wasting during disease, this was not observed (Harel et al., 2009). Therefore, Nogo-A is not a blood-based biomarker candidate. Several other growth factors, inflammatory proteins, and extracellular proteins have been shown to be altered in the CSF or blood of patients with ALS (Table 7–4). Please note examples in Table 7–4 where results for individual proteins differ between publications. This may be due to the fact that antibodies used to detect and measure each protein often differ between laboratories, and differences in how patient samples are collected, processed, and stored, highlighting a challenge for properly interpreting the data. It is important to remember that follow-up studies must be conducted to verify any biomarker candidate and the assay used to quantify the protein must go through a stringent validation process before evaluating the clinical use for any of the biomarkers.

More recent methodologic developments permit the simultaneous measurement of many proteins within one sample (multiplex). Two general approaches are used for these multiplex immunoassays: planar arrays that have several capture antibodies to different proteins attached at defined spots on the

Table 7–4 **Protein Biomarkers for ALS**

Individual Proteins	CSF	Blood	Tissue	References
Angiogenin		↑		Cronin et al., 2006
$A\beta^{42}$	↓			Sjogren et al., 2002
APOE		↓		Lacomblez et al., 2002
sCD14	↑			Sussmuth et al., 2010
Creatine kinase		↑		Sussmuth et al., 2003; Ilzecka & Stelmasiak 2003
Cystatin C	↓	↑	↓	Tsuji-Akimoto et al., 2009; Wilson et al., 2010, 2013; Mori et al., 2009
Erythropoietin	↓	↓		Janik et al., 2010; Brettschneider et al., 2007
FGF-2	↑	↑		Johansson et al., 2003
Fibronectin		↓		Ono et al., 2000
GH	↓	–		Bilic et al., 2006
GDNF	↑			Grundstrom et al., 2000
HGF	↑		↑	Kern et al., 2001; Nomura et al., 2012
Insulin	↓	↓		Bilic et al., 2006
IGF-1	↓	↑		Bilic et al., 2006; Hosback et al., 2007
IL-6	↑	↑	↑	Ono et al., 2001; Moreau et al., 2005
MCP-1	↑	↑		Wilms et al., 2003; Nagata et al., 2007
MMP-2	↑	↑		Niebroj-Dobosz et al., 2010
MMP-9	↑	↑		Niebroj-Dobosz et al., 2010; Beuche et al., 2000
pNfH	↑	↑		Brettschneider et al., 2006; Ganesalingam et al., 2012
pNfL	↑	↑		Norgren et al., 2003; Zetterberg et al., 2007; Tortelli et al., 2012
Nogo-A		–	↑	Pradat et al., 2007; Harel et al., 2009
PEDF	↑			Kuncl et al., 2002
RANTES	↑	↑		Rentzos et al., 2007
S100β	↑		↑	Sussmuth et al., 2003, 2010; Migheli et al., 1999
SOD1	↑			Winer et al., 2013
Tau	↑/–	↑/–		Brettschneider et al., 2006; Sussmuth et al., 2010; Paladino et al., 2009
TGFβ1	↑	↑		Ilzecka et al., 2003; Houi et al., 2002
TIMP-1	↑	↑		Niebroj-Dobosz et al., 2010; Lorenzl et al., 2003

Table 7–4 (Cont.)

Individual Proteins	CSF	Blood	Tissue	References
TNF-α		↑		Moreau et al., 2005; Poloni et al., 2000; Cereda et al., 2008
TNF-RI		↑		Poloni et al., 2000; Cereda et al., 2008
TNF-RII		↑		Poloni et al., 2000; Cereda et al., 2008
VEGF	↓/–	↑/–	–	Devos et al., 2004; Moreau et al., 2006; Gupta et al., 2011

Protein Panels	Biofluid or Cells—Proteomic Method Used in Study	Reference
Transthyretin, cystatin C, 7B2	CSF—SELDI-TOF MS	Ranganathan et al., 2005
VGF, cystatin C, 6.7-kDa mass peak	CSF—SELDI-TOF MS	Pasinetti et al., 2006
Ceruloplasmin, transferrin, α_1-antitrypsin, α_2-glycoprotein, β_2-microglobulin	CSF—Two-dimensional gels and MS	Brettschneider et al., 2008
IL-2, IL-6, IL-10, IL-15, GM-CSF	CSF—Multiplex bead assay	Mitchell et al., 2009
Cystatin C, transthyretin, CRP, and 38 other spectral mass peaks	CSF—SELDI-TOF MS	Ryberg et al., 2010
Heat shock protein 1, α_1-antitrypsin, fetuin-A, transferrin, transthyretin, nebulin-related anchoring protein	CSF—Two-dimensional gels and MS	Brettschneider et al., 2010
Cystatin C, α_1-antitrypsin, VGF, chromogranin A, secreted phosphoprotein 1	CSF—MALDI-TOF MS	von Neuhoff et al., 2012
MCP-1, GM-CSF, L-ferritin, transferrin	Blood plasma—Multiplex bead assay	Mitchell et al., 2010
MCP-1 and IL-8 in CSF; eotaxin in serum	Blood serum and CSF—Multiplex planar assay	Kuhle et al., 2009
Calreticulin, peroxiredoxin-2, GSTO1, CLIC1, HSC70, CypA, PDI, ERp57, PA28a, IRAK4, FUBP1, ROA2, actin, TDP-43	Peripheral monocytes—Two-dimensional gels and MS	Nardo et al., 2011
Complement c3, integrin α-IIb, zyxin, thymosin β4, and three peptides of platelet factor 4	Blood plasma—MALDI-TOF MS	Conraux et al., 2013
IP-10, IL-5, L-ferritin in plasma, IL-8 and MCP-1 in CSF, and a ratio of IFN-γ (plasma/CSF)	Blood plasma and CSF—Multiplex bead assay	Su et al., 2013

Individual proteins are listed with corresponding levels measured in the CSF, blood (serum or plasma), or tissue of patients with ALS when compared with control subjects. ↑ = increased in ALS relative to control subjects; ↓ = decreased in ALS relative to control subjects; – = no change in ALS relative to control subjects; ↑/– = differences between results reported in the publications. Results for protein panels include a list of the biofluid and methodology used in the study. MALDI-TOF MS = matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SELDI-TOF MS = surface-enhanced laser desorption/ionization time-of-flight mass spectrometry.

array, and the luminex technology that contains capture antibodies attached to the surface of microbeads and are distinguished by their fluorescence emission by flow cytometry. The ability to multiplex within a single sample has enabled scientists to generate panels of protein biomarkers that are altered in patients with ALS compared with control subjects (Table 7–4). However, the microbead approach has technical difficulties with respect to potential antibody interference when many microbeads containing different antibodies are used in the experiment, and lot-to-lot variability of the antibody-labeled microbeads that would influence reproducibility of the results.

Using this microbead-based multiplex approach to examine multiple cytokine and inflammatory proteins, Mitchell and colleagues

reported a CSF signature for ALS containing five cytokines (Mitchell et al., 2009). In subsequent studies from the same laboratory, a distinct inflammatory signature was detected in the blood (Mitchell et al., 2010). By combining CSF and blood inflammatory profiles generated using a planar multiplex assay, a group of cytokines was discovered for ALS in both biofluids (Kuhle et al., 2009). Finally, Connor and colleagues used a combination of plasma and CSF cytokine profiles, HFE genotyping, L-ferritin measures, and enzyme-linked immunosorbent measures for transferrin and β_2-microglobulin to generate a signature of ALS progression and prognosis in approximately 30 patients when correlated to follow-up clinical information for each patient (Su et al., 2013). Overall, altered levels of monocyte chemoattractant protein-1 and interleukin-8 in the CSF or blood were identified in multiple studies, indicating these proteins may represent a more robust signature that can be further validated in future studies.

The mass spectrometry–based methods to identify panels of proteins that could distinguish ALS from various control groups were first reported in 2005 (Ranganathan et al., 2005), with subsequent verification and validation studies performed by separate laboratories using the same surface-enhanced laser desorption/ionization time-of-flight mass spectrometry technology (Pasinetti et al., 2006; Ryberg et al., 2010). More recent studies using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to identify biomarkers in CSF or blood from a small number of ALS and control subjects or other neurologic disease control subjects confirmed the surface-enhanced laser desorption/ionization time-of-flight results in the CSF for cystatin C and VGF (Table 7–4). In addition, novel biomarker candidates discovered in the CSF suggest that vesicle secretion pathways are altered in ALS and biomarker candidates in the blood suggest that coagulation factors are altered and may contribute to the disease process. These findings must be further validated in large subject cohorts in future studies.

As noted previously, many of the mass spectrometry–based proteomic studies identified the same proteins within the CSF or blood-based signatures for ALS, and many of these proteins were also identified in prior studies using targeted proteomic methods (Table 7–4). Candidate biomarkers identified across different proteomic platforms represent the most robust biomarkers and efforts should be made to further validate and develop these biomarkers for clinical use. To date, most studies to identify prognostic biomarkers used a single biofluid sample with longitudinal clinical information as a measure of disease progression. Future studies must include longitudinal biofluid samples collected from individual patients to demonstrate the ability of these protein biomarkers to monitor disease progression within patients.

METABOLIC BIOMARKERS

Metabolomics is a more recently developed methodology that typically uses mass spectrometry or nuclear magnetic resonance (NMR) spectroscopy to explore the thousands of small molecules generated by metabolism within cells, tissues, or biofluids (Kaddurah-Daouk, Kristal, & Weinshilboum, 2008). The use of metabolomics can help generate new biomarkers of disease and enhance the understanding of disease pathogenesis and drug effects.

NMR spectroscopy of blood samples confirmed prior findings that particular amino acids are altered in the blood of patients with ALS, including increased glutamate, hydroxybutyrate, acetone, and acetate, and decreased levels of glutamine, histidine, and *N*-acetyl derivatives (Kumar et al., 2010).

Mass spectrometry–based metabolomics has advantages over NMR spectroscopy of detecting metabolites at lower concentrations and the ability to resolve more small metabolites, although these remain complementary techniques to explore the metabolic signatures of disease or drug response. The first metabolomics study comparing ALS and control subjects was performed by Rozen et al. (2005) and examined 300 metabolites in the plasma of 28 ALS and 30 healthy control subjects. Over 100 metabolites appeared altered, although the identity of these metabolites was not known. In a subsequent study using gas chromatography/mass spectrometry and liquid chromatography tandem mass spectrometry, Lawton and colleagues (2012) performed two separate substudies and identified additional metabolic changes in the plasma of patients with ALS. Twenty-three identified metabolites were

altered in the plasma of patients with ALS, including biochemical pathways associated with hypermetabolism, oxidative damage, lipid metabolism, and mitochondrial dysfunction. Among the most significant individual metabolites exhibiting alterations in ALS were creatine (increased in ALS) and creatinine (decreased in ALS).

Wuolikainen and colleagues (2011) also reported significant metabolic differences between the CSF of control subjects and patients with ALS, but they also detected differences between FALS, SALS, and patients with FALS carrying a mutation in the SOD1 gene. This suggests that mechanisms underlying the disease differ within subpopulations of patients with ALS. A total of 120 metabolites were detected and used in their analysis, with known identity of 40 metabolites. Among the known metabolites exhibiting differences between the groups were creatinine (decreased in all ALS compared with control subjects and lower in FALS compared with SALS). Another study using CSF-based metabolomics used 66 ALS and 120 control subjects to identify a group of four metabolites that could predict ALS with 80% accuracy (Blasco et al., 2013). Overall, these studies reveal that a metabolic signature can be identified in patients with ALS, and possibly could be used to stratify patient populations. However, many of the metabolites detected in these studies remain unknown and additional metabolomics studies using a large number of patient samples are required to verify and ultimately prospectively determine the ability of metabolomics to provide diagnostic or prognostic use for ALS.

NEUROPHYSIOLOGIC BIOMARKERS

Although serologic or biofluid biomarkers are undeniably powerful and aid greatly in research and clinical trials, clinical biomarkers are also robust and have the advantage of being readily available to the neurologist via easily accessed resources. Most clinical biomarkers in clinical trials have been nonbiologic surrogate markers, such as ALS Functional Rating Scale; manual muscle testing; handheld dynamometry; and respiratory parameters, such as forced vital capacity. Neurophysiologic techniques have been a mainstay of ALS clinical care for the last 50 years but have not been used as biomarkers until relatively recently. The last 20 years have seen a renewed interest in clinical biomarkers, including motor unit number estimation (MUNE), electrical impedance myography (EIM), and transcranal magnetic stimulation (TMS).

MUNE

The pathophysiologic cascade in ALS, regardless of genetic or phenotypic subtype, inevitably ends in the final common pathway of motor neuron death. As such, the ability to ascertain the number of motor units for a given muscle is of great interest as a clinical biomarker. The ability to track this information is extremely useful as a surrogate marker of disease progression, as a prognostic biomarker, and potentially as a biomarker for monitoring therapy.

For many years, the only available technique that could estimate motor unit numbers was histopathology. Muscle biopsies revealed denervation atrophy that could be semiquantified and pathologic alterations at autopsy demonstrated loss of anterior horn cells. Unfortunately, this kind of postmortem information is of little benefit in clinical studies. The development of neurophysiologic techniques has allowed for in vivo quantification of motor neurons and has been instrumental in ALS biomarker research. In 1971, McComas and coworkers (1971) described a simple, elegant method that could be used to estimate motor unit numbers (MUNE). Since that time, several different methods have been investigated but the fundamental concept of all methods is the same (Fig. 7–1). A compound muscle action potential (CMAP) is recorded from a muscle after supramaximal stimulation of the nerve supplying it. This response is assumed to represent the summation of all motor units in the muscle. Next, a series of submaximal stimulations are performed and used to calculate the average size of individual motor units. This average is divided into the supramaximal response to arrive at an estimated number of motor units.

Conceptually, MUNE is a powerful way to track in vivo disease progression as a biomarker in ALS. It is more attractive than other clinical measures of disease for several reasons. In

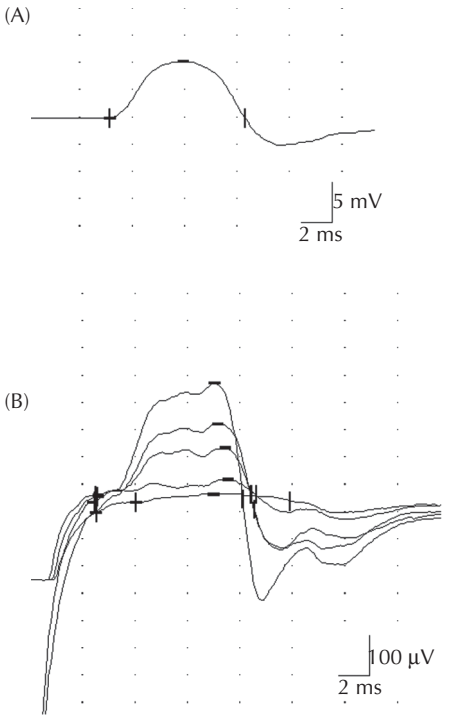

Figure 7–1. This example of the incremental MUNE technique illustrates the concept underlying MUNE. (A) Supramaximal compound muscle action potential is obtained, here in the median nerve. (B) Stimulation is then begun at low levels and slowly increased until all-or-nothing step-wise responses are obtained. Here five increments are seen. Each increment is assumed to result from the addition of a single motor unit. The amplitude of the largest one is divided by the number of increments to give the average size of a motor unit in this sample. The average size is then divided into the amplitude of the waveform in (A) to give the motor unit number estimate.

contrast to other neurophysiologic techniques, such as motor unit recruitment or interference pattern analysis, MUNE is much less susceptible to the dynamic effects of reinnervation and is more amenable to quantitative measurements rather than qualitative or semiquantitative measurements (Shefner, 2001; Carleton & Brown, 1979). It is also superior to force-based measures, such as forced vital capacity or manual muscle testing, because it is less affected by the phenomenon of reinnervation (Bromberg et al., 1993a; Felice, 1997). Comparison studies of MUNE and these clinical measures have shown that motor unit loss in the clinical measures is masked early in the disease because reinnervation is able to compensate and preserve force. MUNE, however, is able to detect this motor unit loss. Another attractive feature of MUNE is that it demonstrates more rapid change than other clinical measures (Felice, 1997; Dantes & McComas, 1991; Mitsumoto et al., 2007). Measures that change rapidly with disease progression are coveted as biomarkers because they allow for trials to be performed more quickly and can provide increased sensitivity in detecting a treatment effect. Another useful feature of MUNE is that it is able to detect motor unit loss earlier than other clinical measures. Studies of mutant SOD1 ALS mice have shown detectable loss of motor units even in a presymptomatic state (Shefner et al., 1999; Hegedus, Putman, & Gordon, 2009). More recently, studies of presymptomatic familial patients with ALS showed that although baseline motor unit numbers were normal, an abrupt drop in motor unit numbers was seen several months before symptom onset in some patients (Aggarwal & Nicholson, 2001, 2002). These studies indicate that MUNE is poised to be an ideal biomarker early in the disease course, which is when experimental agents may be the most efficacious. Finally, MUNE is attractive because most methods are relatively simple to learn and can be performed on standard electromyography machines. This makes it a feasible biomarker for multicenter studies (Shefner et al., 2011).

Unfortunately, MUNE techniques also have some characteristics that have led to criticism of their validity (Shefner, 2001). The first is the fact that the normal anatomic numbers of motor units in specific muscles of controls is not known. This limited MUNE techniques to studies that looked at disease progression, which only require data on the relative change in motor neurons, rather than disease severity, which would compare the absolute number of motor neurons in a diseased patient with normative data. Some studies have attempted to anatomically determine the number of motor neurons in fetal and newborn tissues based on animal data showing that approximately half the large, myelinated axons in a motor nerve are sensory axons (Feinstein et al., 1955; Eisen et al., 1974). However, these studies had sufficiently statistically discordant results that the normative data were not widely accepted. Another criticism of MUNE is that the estimates become less reliable with greater numbers of motor neurons, a scenario that is often encountered early in a disease when a

biomarker is most needed. Next, assumptions that all motor units have similar stimulation thresholds and that the motor units sampled to calculate the average motor unit size are all single motor units are to a certain extent untrue and probably result in overestimation of motor unit numbers (Milner-Brown & Brown, 1976; Brown & Milner-Brown, 1976; Feasby & Brown, 1974). Finally, there is some variability of motor unit numbers estimated between different MUNE techniques leaving uncertainty as to which should be used as the gold standard. Since McComas' description there have been several methods used for MUNE. They include the McComas incremental technique (McComas et al., 1971), multiple point stimulation (Kadrie et al., 1976; Hansen & Ballantyne, 1977), the F-wave method (Stashuk et al., 1994; Felice, 1998), spike-triggered averaging (STA; Strong et al., 1988), and the statistical method (Daube, 1995; Henderson et al., 2007). Each method has characteristics that are advantageous and disadvantageous for use in ALS studies that have been reviewed previously. Modifications to some of these methods have been made to alleviate some of the disadvantages. Over the last 15 years, ALS researchers have focused on several techniques that are most suited to ALS studies.

Multiple point stimulation technique was developed to address one of the major drawbacks to the incremental method; namely, the ambiguity of whether the all-or-nothing quantal response with incremental stimulus increases contains one or more than one motor unit, a phenomenon known as alternation (Kadrie et al., 1976). Similar to the incremental technique, it involves stimulation of nerve at submaximal stimulation to record the amplitude of individual motor units. Unlike the incremental technique, it uses various points along the nerve course to allow for a more varied sample of motor units. Multiple point stimulation has been extensively studied (Kadrie et al., 1976; Doherty & Brown, 1993; Felice, 1995; Wang et al., 2002; Wang & Delwaide, 1995, 1998). More recently, the technique has been modified to involve a combination of the incremental method and the multiple point method in the hopes that bias from the alternation of motor units with similar thresholds could be reduced (Wang & Delwaide, 1995). It seems to be a highly reproducible measure in control subjects and patients with ALS, is well tolerated, and is simple to perform on any electromyogram machine (Shefner et al., 2011; Felice, 1995). Nonetheless, the technique is still hampered by potential bias in that only motor units of relatively low threshold are sampled. This technique is best suited to Phase II ALS therapeutic trials where rapid assessment of a drug effect on motor neuron loss is desired (Shefner, 2001).

STA technique is the only MUNE technique that uses voluntary motor units rather than stimulated ones (Strong et al., 1988; Brown, Strong, & Snow, 1988). A needle electrode records single voluntary motor units. Meanwhile, surface electrodes concurrently record the motor units. The electromyogram motor unit acts as a trigger to time lock the surface recorded motor units. After 10–15 motor unit samples are captured, the average motor unit size is calculated and divided into the supramaximal CMAP of the muscle.

This technique was developed for two specific reasons. First it has the advantage that it can be performed on proximal muscles. Whereas other MUNE techniques require stimulation of relatively inaccessible proximal nerves, the use of volitional motor unit activity in STA obviates this need. Second, the technique was developed to presumably provide a more representative sample of motor units for use in calculating the average motor unit size. Because it does not use electrical stimulation, it is free from the bias introduced by the phenomenon of similar motor unit sizes requiring similar stimulation levels. Unfortunately, the technique is subject to a different kind of bias in that physiologic recruitment recruits smaller motor units first and artificially raises the MUNE. It is also time consuming and requires patient tolerance and cooperation.

A modified technique called decomposition enhanced STA (DE-STA) was developed to address these problems with STA (Boe, Stashuk, & Doherty, 2004; Doherty & Stashuk, 2003). As a potential biomarker, DE-STA seems to have high test-retest reproducibility and good intrarater reliability (Ives & Doherty, 2014; Boe et al., 2009; Calder et al., 2008; Boe, Stashuk, & Doherty, 2006).

Statistical technique was developed to address the problems of sampling bias that plagued other methods (Daube, 1988, 1995). Namely, the technique uses computerized software to sample motor units at different levels

of stimulation and uses Poisson statistics to calculate an average motor unit size. Thus, the sample is not composed of motor units of the same stimulation threshold as in other MUNE methods. Unfortunately, the method is still subject to artificially low motor unit number estimates because of the effect of unstable, varying motor units (Shefner, 2009). It also seems to provide significantly lower motor unit number estimates than other methods, at least in healthy subjects (Shefner, 2001). More recently, a new statistical MUNE method was developed that uses Bayesian statistics instead of Poisson statistics to try to account for some of the biologic variables that negatively affect traditional statistical MUNE (Henderson, et al., 2007; Ridall et al., 2006).

MUNE in ALS Studies

As an ALS biomarker, MUNE has shown great promise. First, it has shown the ability to detect lower motor neuron loss in ALS compared with healthy control subjects. The initial incremental MUNE studies by Carleton and Brown (1979) and Hansen and Ballantyne (1978) found that motor unit numbers in patients with ALS were reduced by 80% and 62% in the upper and lower extremities, respectively. Subsequently, studies with various other methods have consistently demonstrated this as well (Shefner et al., 2011; Felice, 1995; Wang & Delwaide, 1995, 1998; Boe, Stashuk, & Doherty, 2007; Bromberg, 1993b). Next, MUNE is able to demonstrate a rate of decline of motor units over time. This can be useful for stratifying patients for clinical trials based on rate of disease progression and expected prognosis. Early studies demonstrated the nonlinear rate of motor neuron loss in patients with ALS (Dantes & McComas, 1991). During the first year, motor neuron numbers dropped by 70% and then seemed to decline more slowly after that (Arasaki & Tamaki, 1998). Later, studies of serial MUNE in patients with ALS demonstrated that the rate of loss was closely correlated with prognosis (Olney, Yuen, & Engstrom, 1999; Armon et al., 2000; Liu et al., 2008). Next, MUNE techniques have been shown to have good test-retest reliability in patients with ALS with test-retest variability generally less than 20% (Shefner et al., 2011; Felice, 1995; Boe et al., 2004, 2006, 2009; Ives & Doherty, 2012, 2014; Bromberg, 1993b). The reliability is slightly lower in healthy control subjects perhaps because of the fact that there is a higher chance of sampling the same motor unit more than once in patients with ALS (Felice, 1995; Bromberg, 1993b). Finally, MUNE has been successfully used as an exploratory outcome measure in a clinical trial demonstrating that it is simple enough to perform to be appropriate for multicenter clinical trials (Shefner et al., 2011).

MUNE has the potential to be a powerful biomarker that can be used to track disease progression or therapeutic effects of an investigational agent, as a stratification tool for patients with ALS in clinical trials, and as a marker of early disease when therapeutic agents may have the most promise. No MUNE technique has been shown as clearly superior to others and the needs of each future study or trial will determine which method of MUNE is best suited as a biomarker. Before MUNE can be used reliably in clinical trials, further study, and perhaps new techniques, is necessary.

EIM

EIM is an emerging technique that has the potential to be a powerful biomarker in ALS research. Unlike other neurophysiologic biomarkers, EIM does not require muscle contraction or inherent electrical activity from the muscle or nerve. It is based on the field of bioelectrical impedance analysis, which has long been used in studies to assess lean body mass (Lukaski, 1987). The earliest EIM studies demonstrated that the longitudinal orientation of muscle tissue and myocytes resulted in anisotropic (direction-oriented) impedance that could be measured via surface electrodes when an electrical current was applied to the skin (Aaron, Huang, & Shiffman, 1997; Shiffman & Aaron, 2000). Conceptually, the anisotropy of the muscle results in distortion of the characteristics of the applied current that can be measured by the voltage-sensing electrodes. Changes in muscle morphology, such as fibrosis, denervation, or edema that develops in diseased states, influence the muscles impedance characteristics and thus result in altered EIM measurements (Fig. 7–2). Unfortunately, skin and subcutaneous fat can also contribute to this and alter the measurements (Tarulli et al.,

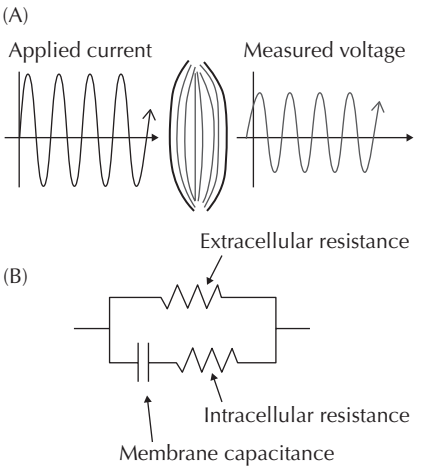

Figure 7–2. Effect of muscle anisotropy. The applied current traverses muscle tissue and is sensed by voltage recording electrodes. Note the changes in amplitude and frequency. These changes are analyzed in electrical impedance myography. (Adapted Rutkove SB. Electrical impedance myography: background, current state, and future directions. Muscle Nerve. 2009;40(6):938. Copyright 2009 Wiley Periodicals. Reprinted with permission.)

2007). This understanding of tissue anisotropy was soon applied to the study of human disease (Rutkove, Aaron, & Shiffman, 2002; Tarulli et al., 2005; Rutkove et al., 2005a). In this application, a painless, low-intensity, high-frequency current is applied to the limbs via surface electrodes. Typically, four or more voltage-sensing surface electrodes are positioned over the muscle to be studied with an interelectrode distance of 2.5 cm (Fig. 7–3). Early studies of this technique, termed linear-EIM, used a single current frequency of 50 kHz and recorded several impedance variables (Rutkove et al., 2002, 2005a, 2005b, 2006). Using an array of current-injecting electrodes at distant sites (e.g., both hands), referred to as a "far-current electrode" montage (Fig. 7–4), the recorded impedance data were found to be mostly free of the potentially confounding influence of skin and subcutaneous fat (Tarulli et al., 2007). A "near-current electrode" montage (Fig. 7–5) was eventually studied given the desire to have the current-injecting and voltage-sensing electrodes in the same region (Ogunnika et al., 2008). This montage was appealing because it made possible a compact, handheld device containing all the electrodes for an efficient and standardized application of EIM (Ogunnika et al., 2008). More recently, studies have investigated the use of multifrequency current instead of constant 50-kHz current to gather more robust data on diseased muscle (Esper et al., 2006; Shiffman & Rutkove, 2013). In addition, varying the angle of the EIM apparatus has also been investigated to assess for the optimal parameters of data collection (Chin et al., 2008; Ogunnika et al., 2010). Of the major impedance measurements (reactance X, resistance R, and phase θ), phase seems to have emerged as a leading candidate biomarker.

EIM is an attractive candidate biomarker for ALS studies for a variety of reasons. First, it is painless, rapid, noninvasive, and simple

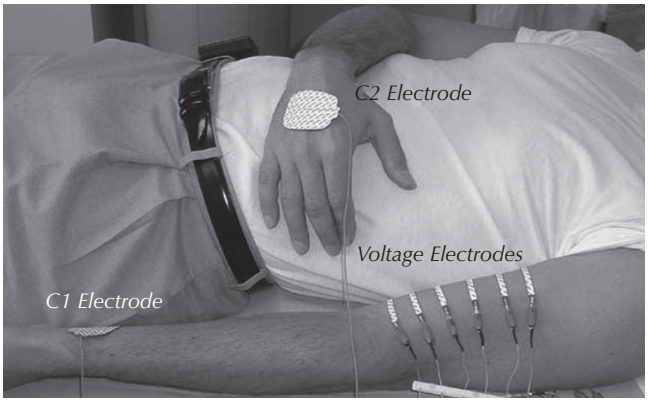

Figure 7–3. "Far-electrode" montage. C1 and C2 represent the current-injecting electrodes over contralateral distal extremities. The voltage-sensing electrode array is arranged over the biceps muscle of the patient and provides impedance data. (From Rutkove SB. Electrode position and size in electrical impedance myography. Clin Neurophysiol. 2005;116(2):291. Copyright 2005 Elsevier. Reprinted with permission.)

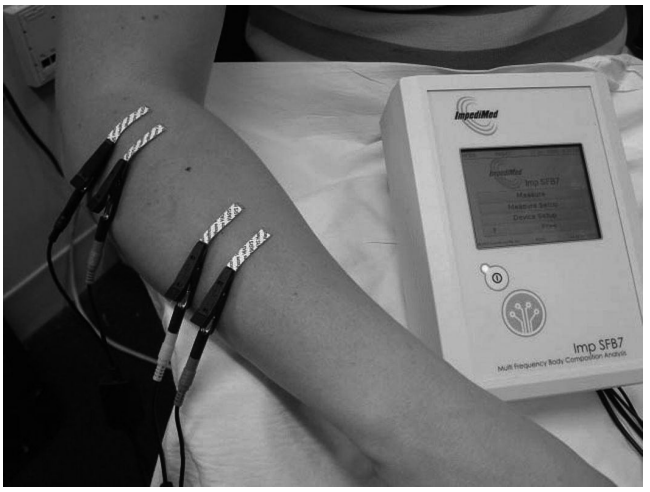

Figure 7–4. "Near-electrode" montage to study the extensor digitorum communis muscle. In this montage, the far right and far left electrodes are the current-injecting electrodes. The center electrodes are the voltage-sensing electrodes. (Adapted from Rutkove SB. Electrical impedance myography: background, current state, and future directions. Muscle Nerve. 2009;40(6):937. Copyright 2009 Wiley Periodicals. Reprinted with permission.)

to administer. This has made gathering data in disease and healthy control subjects fairly easy and has facilitated studies that have shown EIM's ability to differentiate patients with ALS from healthy patients (Rutkove et al., 2002, 2008; Esper et al., 2006; Chin et al., 2008; Tarulli et al., 2009). It also makes feasible a multicenter trial using multiple investigators. Next, EIM is able to clearly document disease progression (Tarulli et al., 2009). It correlates well with other accepted outcome measures but declines more rapidly (Ahad & Rutkove, 2010; Rutkove et al., 2007, 2012, 2013). Rutkove et al. (2007), using a linear EIM measure called the $\theta_{z\text{-}max}$ megascore, showed that this measure declined in patients with ALS by 21% during the study, whereas manual muscle testing declined by only 9.75%. In another study, Rutkove and colleagues (2012) showed that the rate of decline in 50 patients with ALS over 6 months (Fig. 7–5) was similar between ALS Functional Rating Scale, handheld

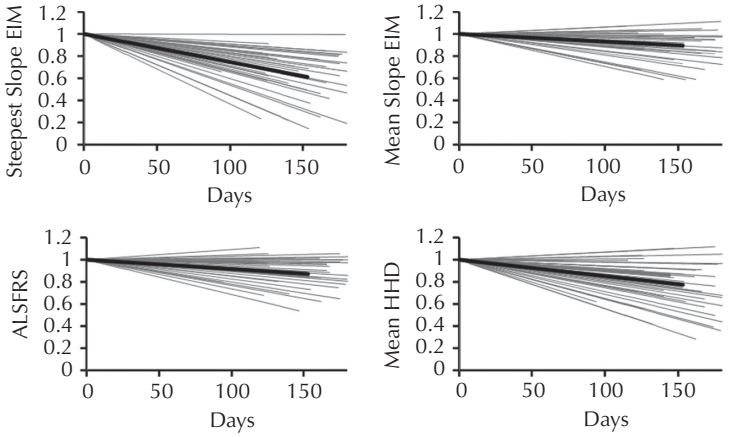

Figure 7–5. Comparison of steepest slope electrical impedance myography (EIM), mean slope EIM, ALS Functional Rating Scale (ALSFRS), and handheld dynamometry (HHD) outcome measures over 6 months in 50 patients demonstrating the fastest rate of change in the steepest slope EIM variable. (From Rutkove et al., 2012.)

dynamometry, and an EIM parameter called steepest slope EIM. Another desirable feature of EIM is that it correlates with prognosis making it potentially useful in clinical trials to stratify patients so that treatment arms are balanced with regard to patients with more or less aggressive disease (Rutkove et al., 2012; Wang LL, et al., 2011). One study looked at MUNE and EIM in SOD1 G93A rats and found that EIM was highly correlated with survival and correlated well with MUNE (Wang LL, 2011). Studies in human patients have confirmed this. Clinical trial simulations with these data have shown that EIM has significant power as a measure of disease progression to significantly reduce the number of patients required in a clinical trial compared with other traditional outcome measures (Rutkove et al., 2007). Finally, EIM has very high test-retest reliability with only 4.2% variability between tests and good intraclass correlation coefficients as high as 0.971 (Felice, 1998; Rutkove et al., 2006).

Despite these desirable characteristics, there are still many questions about EIM that must be answered before it can be reliably used in ALS studies. It is still not clear what measure is ideal. Although θ_{50} has been favored most recently, it may be that other variables or combinations of variables may be more suited as ALS outcome measures. $\theta_{z\text{-}max}$, θ_{avg}, and steepest slope EIM have all been shown to have useful functions as ALS biomarkers. Whether the near-current or far-current montage is superior for clinical trials is also uncertain. Although the far-current approach is less apt to be influenced by excess subcutaneous tissue, it is much less convenient and more difficult to standardize than the near-current approach. The near-current approach has become favored more recently because it allows for a compact handheld EIM device. Another question is whether the linear single-frequency EIM is superior to the multifrequency EIM. It is likely that the latter has superior potential but the number of variables, from electrode placement to angle of orientation to frequency range, requires much more study. A final criticism of EIM is that, intuitively, it does not address ALS pathophysiology. It may correlate with the most terminal effect of the disease (i.e., atrophy, muscle fibrosis, and muscle fiber loss), but the effect of reinnervation, variable progression in different muscles, and more upstream pathophysiology are not easily accounted for by EIM. It also does not address the effect of upper motor neuron dysfunction in ALS or how it may affect muscle anisotropy through phenomena, such as spasticity or immobility. The great promise of EIM warrants further study that is currently underway.

TMS

TMS is a noninvasive method of assessing the functional integrity of the motor cortex and its projections. In the study of ALS, the technique allows for the study of the upper motor neurons. Most other physiologic biomarkers only focus on the lower motor neuron so TMS is of great interest. It involves using electromagnetic induction to induce weak electric currents in the brain and therefore excite a network of neurons in the motor cortex. A variety of measurements can be elucidated downstream from this stimulus to assess the integrity of the motor system from cortex to muscle (Vusic et al., 2013). TMS has the potential to reflect an upper motor neuron dysfunction in patients with ALS that is not yet clinically detectable, a desirable trait for a biomarker.

The motor threshold (MT) is a term for the minimum stimulus intensity required to elicit a small motor evoked potential (MEP) in a target muscle. MT is a marker for the density of corticomotoneuronal projections onto the spinal motor neuron. Longitudinal studies have documented a reduction of MTs early in the disease course of patients with ALS, increasing to the point of cortical inexcitability with disease progression (Mills & Nithi, 1997).

MEP amplitude reflects the density of corticomotoneuronal projections onto motor neurons, and when expressed as a percentage of the maximum CMAP on electrical peripheral nerve stimulation, it provides insight into the percentage of the motor neuron pool activated in the MEP. Increases in MEP amplitude have been reported in ALS, most prominently early in the course of the disease (Vucic & Kieman, 2006, 2007; Vucic, Nicholson, & Kieman, 2008), a feature that makes this measurement a potential candidate for a biomarker.

Central motor conduction time (CMCT) is another TMS parameter that represents the time from stimulation of the motor cortex to

the arrival of the signal at the spinal motor neuron (Kobayashi & Pascual-Leone, 2003). It is calculated by first magnetically stimulating the motor cortex and recording the latency to the MEP. Then the cervical root is magnetically stimulated and the latency of its motor evoked response is recorded. Subtracting the latter from the former yields the CMCT. An alternative method uses the F-wave response instead of the cervical root magnetic stimulation to indirectly measure the peripheral conduction time. CMCT is typically prolonged in ALS, thought to represent axonal degeneration of the fastest conducting corticomotoneuronal fibers and increased desynchronization of their signals secondary to axon loss (Kohara, Kaji, & Kojima, 1996; Eisen, Entezari-Taher, & Stewart, 1996; Komissarow, Rollnik, & Bogdanova, 2004). However, because of a variety of technical, physiologic, and pathologic factors, normative data range widely, limiting the use of this measurement (Mills, 2004).

Cortical silent period refers to the interruption of voluntary electromyography activity in a target muscle induced by TMS of the contralateral motor cortex. The cortical silent period duration is measured from the onset of the MEP response to resumption of voluntary electromyography activity and increases with stimulus intensity. Reduction of cortical silent period duration is not seen in other neuromuscular disorders and may be specific for ALS (Vusic & Kiernan, 2008; Vusic, Cheah, & Yiannikas, 2010a, 2011; Vusic, Nicholson, & Kiernan, 2010b).

TMS studies have demonstrated cortical and corticospinal dysfunction in ALS. Furthermore, these studies have shown that cortical hyperexcitability is an early feature of the disease in patients with SALS and it precedes clinical onset of disease in patients with FALS (Vusic et al., 2013). Whereas some longitudinal studies have reported a significant reduction in MEP amplitude, MT, and CMCT (Floyd et al., 2009), others have failed to demonstrate this (Mills, 2003). Use of TMS as a biomarker is marred somewhat by these inconsistent data in documenting significant longitudinal changes in TMS parameters, arguing against the ability of TMS to act as a biomarker of disease progression in ALS. Further study is needed to standardize TMS approaches, identify the ideal parameter to monitor, and delineate the change in these parameters in ALS and its various subtypes before TMS can serve as a legitimate biomarker in ALS studies.

IMAGING BIOMARKERS

Because of its widespread availability and noninvasive nature, magnetic resonance imaging (MRI) has the potential to become a valuable tool for biomarker discovery in ALS. The various magnetic resonance techniques that have been studied in ALS include conventional MRI, magnetic resonance spectroscopy (MRS), diffusion tensor imaging (DTI), assessment of regional atrophy with voxel-based morphometry, and functional MRI (fMRI).

Conventional MRI

Overall, conventional MRI has shown little promise in ALS biomarker research but deserves mention because of its role in stimulating further investigation into the role of imaging in ALS. Conventional MRI studies have revealed that T2 hyperintensity in the corticospinal tract can be pronounced in ALS. However, this finding has rather low sensitivity (<40%) and limited specificity (<70%; Iwasaki et al., 1991). It is seen more often in patients with ALS that are younger and have rapid disease progression. T2 hyperintensity of the corticospinal tract has not proved to be a sensitive or specific enough measure to be a useful biomarker for ALS.

Regional brain atrophy has also been investigated. One study reported that 50% of the patients with ALS studied had superior parietal atrophy (Peretti-Viton et al., 1999). However, cerebral atrophy has not been shown to be consistently detectable on standard sequence MRI of patients with typical ALS.

MRS

MRS is a useful, noninvasive strategy for in vivo measurement of proton-based tissue metabolites and thus has the potential to reveal information about the metabolites with specific relevance to the pathogenesis of ALS (Turner

et al., 2011). Metabolites, such as myoinositol, glutamate, and γ-aminobutyric acid, all have particular relevance but have not been thoroughly investigated because of their relatively low concentrations. Most MRS studies to date have used the ratio of *N*-acetylaspartate (NAA) to choline (Cho) or creatine (Cr) as a nonspecific marker of neuronal damage.

MRS studies have shown that patients with ALS exhibit decreased ratios of NAA/Cr and NAA/Cho in the primary motor cortex (Pioro et al., 1994; Pohl et al., 2001). These changes have also been observed in the brainstem of patients with prominent upper motor neuron or bulbar signs (Pioro et al., 1999). From a diagnostic standpoint, MRS is able to distinguish ALS from disease mimics, such as spinal muscular atrophy or progressive muscular atrophy.

MRS may be a useful biomarker in clinical trials. NAA/Cr and NAA/Cho ratios have been correlated with disability and disease progression. Using whole-brain analysis, Stagg and coworkers recently identified decreased levels of NAA in patients with ALS compared with control subjects throughout the cerebral corticospinal tract. Furthermore, there was a relationship between corticospinal tract NAA levels and degree of clinical disability (Stagg, Knight, & Talbot, 2013). In a longitudinal study, Pohl et al. (2001) found that the NAA/Cho ratio in patients with ALS dropped by 9.1% per month. Three patients in their study demonstrated a change in NAA/Cho only at the follow-up study. This correlated well with the lack of clinical upper motor neuron signs at the first visit and development of them by the follow up visit. Wang et al. showed that NAA/Cr correlated with disease duration in patients with ALS (Wang S, et al., 2006). Finally, a small study showed increased NAA/Cr in the motor cortex of patients with ALS after only a short course of treatment with riluzole (Kalra et al., 1998, 2006).

The ideal MRS marker has yet to be defined. NAA/Cr, NAA/Cho, and Cr/Cho have been use primarily because of the high concentrations of the constituent metabolites but more specific markers need to be studied and will likely be more suited to biomarker research in the future. In addition, practical considerations of MRS technology also limit its use in ALS biomarker research or in multicenter clinical trials. It is currently highly operator-dependent and the number of centers that have the expertise for these studies is limited (Bowser, Turner, & Shefner, 2011).

DTI

DTI is a method of mapping neuronal pathways by detection of the movement of water molecules. Water movement within intact white matter tracts tends to be highly directional and is termed anisotropic. Therefore, the findings of reduced fractional anisotropy (FA) and increased mean diffusivity are potential biomarkers for loss of neuronal integrity. Decreased FA and increased mean diffusivity in the corticospinal tract of patients with ALS was reported by Ellis and coworkers (1999). They found, more specifically, that decreases in FA might be an early indicator of disease, whereas mean diffusivity changes tended to be a measure of neuronal loss and therefore disease chronicity.

Tractography, a technique of mapping interconnectivity among neuronal pathways, was used in one study to segment the corticobulbar tract. By using DTI with this technique, investigators were able to distinguish those patients with ALS with bulbar-onset disease from those with limb-onset disease (Aoki et al., 2005). DTI has also been used to distinguish patients with upper motor neuron–predominant disease from those with lower motor neuron disease (Roccatagliata et al., 2009). In addition, DTI has been shown to be a useful technique for monitoring disease progression in ALS because investigators found that decreased FA in the white matter of the right precentral gyrus was associated with progression of the disease (Ciccarelli et al., 2006).

DTI was used to study patients with a highly penetrant dominant mutation in the SOD1 gene. Presymptomatic individuals who were known to have this mutation had decreased FA in the posterior limb of the internal capsule, suggesting this finding may be one of the earliest detectable changes in those at risk for ALS (Ng et al., 2008).

DTI is noninvasive and able to detect changes early in the course of the ALS. The degree of these changes correlates with disease progression, making it highly attractive for use as a biomarker in ALS. However, further longitudinal studies are needed at this point.

Voxel-based Morphometry

The technique of voxel-based morphometry uses standard sequence MRI and involves software quantification of gray and white matter volumes to study regional differences. This technique has been demonstrated as effective in detecting the extramotor, largely frontotemporal cerebral changes that are seen in some patients with ALS with memory deficits or frontotemporal dementia (Abrahams et al., 2005; Chang et al., 2005; Grosskreutz et al., 2006). However, the use of voxel-based morphometry as a more robust biomarker remains uncertain because of its inconsistent findings of motor cortical atrophy in ALS, lack of longitudinal natural history data in patients with ALS, and its apparent lack of changes in patients with ALS without cognitive involvement.

fMRI

fMRI is an imaging technique that measures regional changes in the blood–oxygenation level dependent responses in brain tissues. fMRI testing on patients with ALS has revealed expanded areas of cortical activation during motor tasks (Lule, Ludolph, & Kassubek, 2009) suggesting that there is a compensatory response to upper motor neuron loss. One study showed that this juxtacortical activation increased in patients with ALS over time (Lule et al., 2007). In addition, resting state fMRI studies in patients with ALS suggests that reduced interhemispheric functional connectivity between motor cortices is an early feature of the clinical disease (Jelsone-Swain et al., 2010). This finding is consistent with corpus callosum involvement, a finding also corroborated with DTI (Filippini et al., 2010). Recently, using fMRI, Cosottini et al. (2012) identified increased functional activation in frontoparietal circuits in patients with ALS, despite associated frontoparietal atrophy, suggesting an overrecruitment of existing sensory-motor networks, perhaps rendered hyperexcitable by the loss of inhibitory interneurons.

All of the MRI techniques described here are accessible, noninvasive, and free of radiation, making them highly practical potential biomarkers. Conceptually, the sheer number of possible imaging measures and combinations of these measures will make it difficult to identify optimal imaging biomarkers. Practical challenges to MRI in patients with ALS arise due to the fact that these patients often have difficulty in lying flat, usually because of bulbar or respiratory dysfunction. In addition, the bulk of MRI studies in ALS have focused on MRI of the brain, emphasizing the upper motor neuron component of the disease pathology. MRI will become even more useful in ALS with the development of high-resolution spinal cord imaging. Comparisons between proposed imaging markers, correlation with traditional clinical markers, and longitudinal studies need to be performed for this promising modality to become a mainstream biomarker.

CONCLUSIONS AND FUTURE DIRECTIONS

There have been tremendous advances in the discovery of biomarkers for ALS in the past decade. Biologic (genetic, protein, metabolites), imaging, and physiologic markers have been shown to identify ALS and track disease progression. However, none of these biomarkers have been validated for clinical use. Future studies that combine these technologies will likely generate a signature for ALS with the highest level of sensitivity and specificity and facilitate a more personalized medicine approach toward therapy. Future use of motor neurons derived from patient-derived iPS cells offers a new source to discover biomarkers for ALS, because proteins or metabolites could be examined in motor neurons derived from sporadic or familial patients and these compared with motor neurons derived from iPS cells collected from other neurologic diseases or healthy control subjects. In the near future, studies regarding the epigenetics of ALS will also provide new key information regarding susceptibility and prognosis within individuals.

Regardless of the approach used to discover candidate biomarkers for ALS, there are a large number of hurdles remaining before any of these biomarkers demonstrate clinical use (Bowser & Lacomis, 2009). Most of the proposed ALS biomarkers require further verification studies performed by multiple laboratories on separate patient cohorts. The actual assay or method used to detect the biomarker must

be validated and standard operating procedures generated that are strictly followed in all subsequent studies. For antibody-based technologies, each of the antibodies must be carefully characterized and sufficient volumes of antibody generated for downstream validation studies. Large prospective validation studies are required that enroll patients at multiple sites to determine the overall sensitivity and specificity for the biomarker. Appropriate disease mimics must be incorporated in all these studies. Finally, early discussions with regulatory agencies will assist in developing the prospective validation studies required to determine the clinical use for the biomarker. Although these hurdles are quite challenging, generation of consortia including academics, biotechnology and pharmaceutical companies, interested disease-associated foundations, and governmental agencies will greatly aid in progressing these candidate ALS biomarkers through the development pipeline.

Longitudinal studies to examine changes in biomarkers (biochemical or imaging) within individual patients are greatly needed to further explore the heterogeneity of disease progression within patients with ALS. This involves careful collection of samples and linkage to clinical information. Included are studies to collect blood and CSF from presymptomatic patients that harbor mutations in a gene that may ultimately lead to ALS. These studies will provide invaluable samples to identify the earliest biomarkers of disease onset. The sharing of large datasets containing clinical, genetic, biologic, and imaging information will further facilitate biomarker development for ALS and likely require developing public and private partnerships as seen for other neurodegenerative disorders.

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Chapter 8

Imaging in Motor Neuron Disease

Martin R. Turner

BACKGROUND AND NEW POINTS

Neuroimaging studies retain pivotal importance in the differential diagnosis of motor neuron disease (MND), in particular the identification of cervical or lumbar spinal lesions that might account for the signs detected on clinical examination. Among the range of imaging modalities, magnetic resonance imaging (MRI) has been the most used in this regard.

MRI signal processing has advanced greatly however, with a focus on detecting tissue alterations within the corticospinal tracts (CST), but also their wider structural and functional cerebral connections in MND. Quantitation of structural imaging abnormalities is one goal. Functional changes, including tissue metabolic imaging with MR spectroscopy (MRS); blood-oxygen dependent functional MRI (fMRI) during motor, cognitive and task-free paradigms; activation and ligand-based positron emission tomography (PET), are all techniques applicable to detecting altered patterns of neuronal activity. Given the noninvasive nature of MR scanning and the clear extension of MND pathology to multiple areas of the cerebral cortex, it offers leading potential as a biomarker source for MND.

INTRODUCTION

The ability to visualize the structure of the brain without the need for craniotomy has transformed the understanding of cerebral neuroscience and practice of clinical neurology more than any other technologic development of the last 50 years. Prior to this, morbid anatomy was the only window into the structural pathology associated with neurologic diseases. For MND this meant that only the end-stage

of the neurodegenerative process was observable for over a century, although these were nonetheless key observations. Neuroimaging has been able to validate and explore these processes further, functionally and structurally and now, crucially, throughout the symptomatic evolution of the disease.

Although the visible manifestation of most cases of MND is the muscle wasting arising as a result of the degeneration of peripheral lower motor neurons (LMNs), the early pioneers recognized MND as a disease involving cerebral and spinal pathology. Reports from the turn of the 20th century noted extension of the degenerated lateral CSTs to the motor cortex (Mott, 1895), and in the decades soon after also recognized the presence of dementia in some cases. There is now an acknowledged clinical (Phukan et al., 2012), histopathologic (Neumann et al., 2006), and genetic (DeJesus-Hernandez et al., 2011; Renton et al., 2011) overlap between MND and a large proportion of cases of frontotemporal dementia (FTD), so that the study of the brain in MND has become a core part of research. With the extension of advanced MRI techniques to the spinal cord, neuroimaging is now unique in its potential to explore the entirety of the degenerative process in MND i.e., at the systems-level (reviewed in Turner et al., 2012).

TYPES OF NEUROIMAGING

"Computed tomography" is a generic term referring to the concept of acquiring data from tissues in planes (slices), which can then be reconstructed to form a recognizable three-dimensional structure. It is the method of generating contrast between different tissues and pathology that varies between the different types of imaging (Table 8–1).

In classical CT scanning, contrast is generated by the variable absorption of X-rays by tissues or injected contrast medium. Single-photon emission tomography (SPECT) and PET involve the detection of gamma radiation emitted from injected radiotracers, using a detector array mounted around the head. PET detects time-coincident gamma rays with exactly 180 degrees of separation, which occur as a result of a positron (emitted from the tracer) annihilating with an electron, and ensures significantly higher spatial resolution compared with SPECT (Fig. 8–1). Radiotracers are injected peripherally and either travel within the blood, acting as surrogates for cerebral blood flow and neuronal activity (activation PET), or bind transiently to specific receptors (ligand PET). The latter usually requires the provision of a local cyclotron (and radiochemistry expertise) to produce the specific radioligands, which often have a very short half-life.

MRI generates contrast between tissues according to their differing nuclear (typically proton) resonance and response to radiofrequency stimulation. The magnetic field of the main bore of the scanner is continuously applied, and aligns the magnetic "spin" of the body's protons. Brief radiofrequency pulses, of variable form, alter the axis of this spin (precession), which generates current within a receiving coil around the head. The variable decay of this excited precession back to the steady state is involved in generating different contrasts between tissues. Magnetic field gradients applied in orthogonal planes around the tissue of interest alter the spin properties of nuclei differentially according to their location, allowing the spatial reconstruction of a three-dimensional image from multiple two-dimensional slices.

In its most common application the MRI signal broadly reflects the variable water content of tissues. T1- and T2-weighted sequences refer to different measurements of the relaxation of tissue protons after radiofrequency pulses have been applied. T1-weighting generates excellent grey and white matter contrast, whereas T2-weighting is most sensitive to tissue water content. Although the appearance of the image to the naked eye forms the core of clinical neuroradiology, advanced MRI techniques in the research setting are based on additional complex mathematical and statistical processing of the numerical data contained within the individual "cubes" of tissue (typically 1–2 mm^3), termed voxels. Examples of such applications are those sensitive to tissue volume and density (voxel-based morphometry [VBM]), to the movement of water within tissues (diffusion tensor imaging [DTI], and its extension, diffusion tensor tractography), and to individual tissue metabolites (MRS).

The differing paramagnetic properties of deoxygenated and oxygenated blood can be exploited to generate contrast according to cerebral blood flow, and termed fMRI. This blood oxygenation level–dependent (BOLD) contrast can be measured in response to a specific activity of the subject in the scanner

Table 8–1 **Types of Neuroimaging, Nature of the Tissue Contrast, and Generic Advantages and Disadvantages**

Neuroimaging Modality	Subtype	Contrast Substrate	Advantage	Disadvantage
CT	Structural	X-ray absorption of tissues	Ubiquitous	Limited spatial resolution; ionizing radiation
	Vascular, perfusion	X-ray absorption of injected contrast medium	Ubiquitous; three-dimensional vascular reconstruction	Invasive; ionizing radiation
SPECT		Gamma radiation emitted by tracer isotope	Ubiquitous	Limited spatial resolution; invasive; ionizing radiation
PET	Activation	180-degree coincident gamma radiation from the annihilation of emitted positrons with electrons	High spatial resolution; long half-life of ^{18}F-fluorodeoxyglucose	Invasive; ionizing radiation
	Ligand		High spatial resolution; specific receptor targeting	Few centers with local cyclotron and expertise; limited ligands; invasive; ionizing radiation
MRI	Structural	Nuclear magnetic resonance (typically proton) reflecting tissue water content, or movement within white matter tracts (DTI)	Ubiquitous; high spatial resolution	Lack of more limited availability (for research) and limited standardization of advanced techniques such as DTI
	Functional	Paramagnetic properties of deoxygenated versus oxygenated blood (BOLD)	Noninvasive; high spatial resolution	Limited temporal resolution; lack of harmonization across institutions
	Metabolic (MRS)	Nuclear magnetic resonance (typically proton) of tissue metabolites	Tissue metabolite measurement	Limited metabolite resolution; lack of standardized analysis; typically single region-of-interest based, with whole-brain techniques still in evolution

CT = computed tomography; SPECT = single-photon emission tomography; PET = positron emission tomography; MRI = magnetic resonance imaging; DTI = diffusion tensor imaging; BOLD = blood oxygenation level-dependent; MRS = magnetic resonance spectroscopy.

(task-based), or analysis of the regional coherence of spontaneous BOLD fluctuations in the so-called resting-state can be undertaken.

Many centers now routinely use high-field 3-T MRI in the clinical and research setting, but it is possible to acquire some high-quality advanced MRI scans at 1.5 T. The ultra high-field 7 T (or higher) systems offer particular benefit to fMRI, through increased signal-to-noise, and also MRS through the improved separation of tissue metabolite peaks. Bigger is not always better in a simplistic sense, however, and very high field strengths bring additional challenges, in particular magnetic field inhomogeneity.

NEUROIMAGING FOR DIAGNOSTIC EXCLUSION

MND is still fundamentally a clinical diagnosis and the primary role of routine neuroimaging is in the exclusion of mimic disorders (Filippi et al., 2010). The sine qua non of MND is

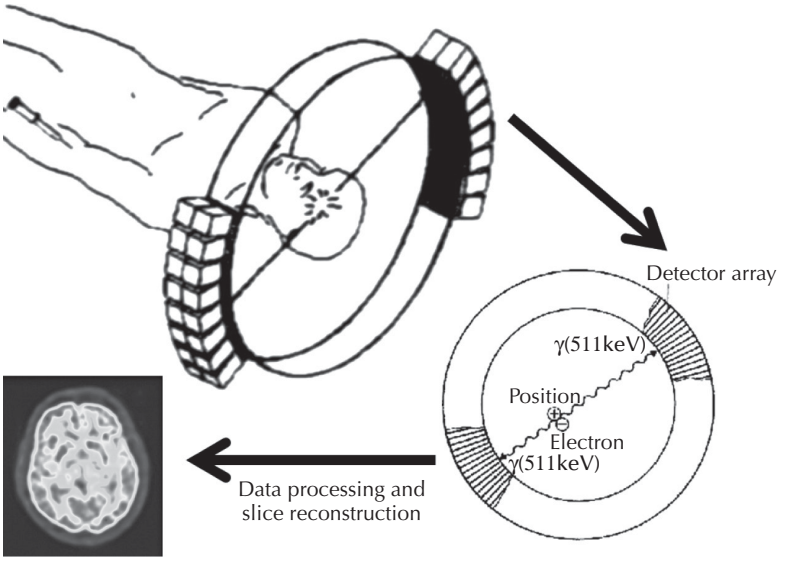

Figure 8–1. Steps in the acquisition of a positron emission tomography scan of the brain. A ring of detectors around the head detects coincident gamma rays with 180-degree separation caused by positron annihilation, which may then be localized and reconstructed as a 3D image that can be overlaid on a standard MRI scan.

progressive motor weakness. In its commonest amyotrophic lateral sclerosis (ALS) manifestation (>85% of cases), there is a clinically detectable combination of loss of LMNs of the spinal cord anterior horns and brainstem nuclei, with loss of UMNs of the CST and primary motor cortex (PMC). The individual with mixed signs and progressive weakness presents little diagnostic difficulty, but when there is a plausible mimic MRI has a unique role in its exclusion. Examples include cervical myeloradiculopathy (Fig. 8–2), lumbar radiculopathy, and the very rare entities of syringomyelia and syringobulbia.

Initial studies of MRI in ALS noted T2-weighted hyperintensity of the CSTs (Goodin, Rowley, & Olney, 1988). It was quickly realized that this appearance, although appealing in its localization, lacked sensitivity and specificity for ALS (Fig. 8–3). Other inconsistent observations in MND cases from routinely acquired sequences have included T1-hyperintensities in the PMC and upper CST

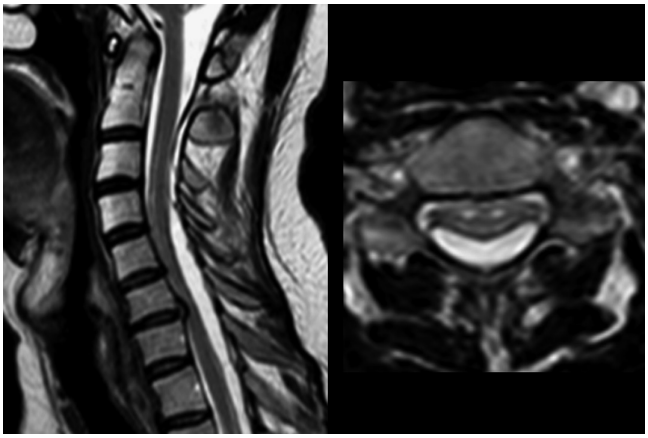

Figure 8–2. MRI has an important role in the detection of pathology that may mimic MND. Sagittal (left) and transverse (right) views through the cervical cord of a patient who presented clinically with a "flail arm"-like MND syndrome. In fact this was caused by anterior compression of the cord by a dural fluid expansion, with signal change visible as "snake eyes" in the anterior horns. (With kind permission of Professor Kevin Talbot, University of Oxford, UK.)

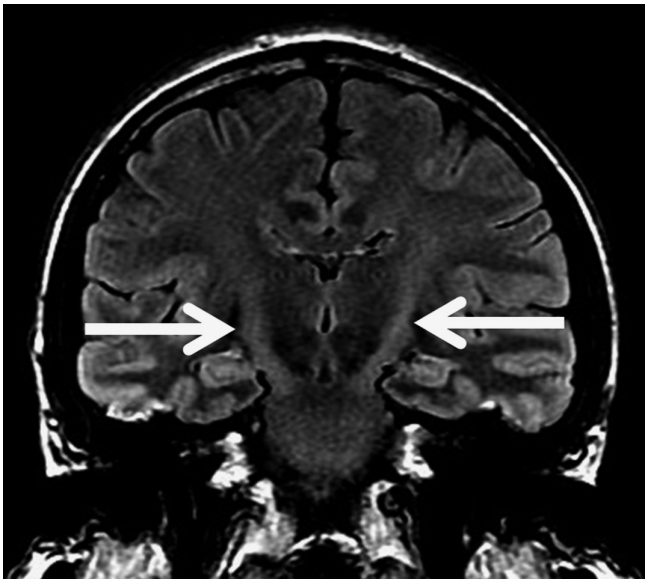

Figure 8–3. Hyperintensity of the corticospinal tracts in MND was one of the first observations made on MRI, but it is neither sensitive nor specific enough to be a biomarker. This coronal MRI (tracts marked with arrows) was undertaken in an apparently healthy individual.

(Waragai, 1997), and atrophy of the corpus callosum (CC; Yamauchi et al., 1995). Frank atrophy of the PMC in ALS is surprisingly unusual even in advanced cases postmortem, but is a more common finding in established cases of the UMN-only variant of primary lateral sclerosis (PLS) (Fig. 8–4; Pringle et al., 1992). The application of quantitative techniques, originally developed for more sophisticated analysis of volumetric changes, to standard clinical MRI

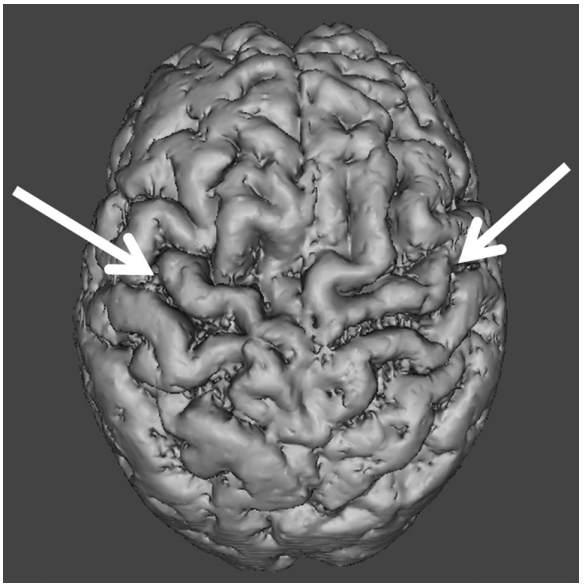

Figure 8–4. Visible atrophy of the pre-central gyri (arrows) on a 3D-rendered MRI scan of a PLS patient shown from above. This level of macroscopic atrophy is often absent in cases of typical ALS-type MND, but though it may be detected using voxel- and surface-based morphometry in such cases.

sequences offers renewed hope for biomarkers that could be acquired from the routine work-up of patients with MND. So-called "proton density" MRI signal increase in the PMC, CC, and CSTs showed some promise in this regard (Ding et al., 2011).

NEUROIMAGING AS A BIOMARKER

By the time a case of typical ALS reaches a specialist, the diagnosis often takes minutes to make clinically. The lack of an objective biomarker for MND is only one reason for the consistent average diagnostic delay from first symptom of 1 year (Mitchell et al., 2010), with postponement in seeking medical attention, and inappropriate specialist referral from primary care likely to be other important sources of delay. Fundamentally, with 90% of MND cases apparently sporadic, it is the lack of understanding of the at-risk population that is the major obstacle to early intervention.

The clinical heterogeneity of MND (e.g., UMN versus LMN-predominant, limb- versus bulbar-onset, and variable rates of progression) may be an important contributing factor in the failure of all but one therapeutic trial (namely Riluzole). Biomarkers allowing prognostic stratification of patients would have clear value. Furthermore, those sensitive to disease progression might allow faster "no-go" decisions in clinical trials, which currently rely on death or tracheostomy as end-points (Turner, Kiernan, Leigh, & Talbot, 2009). The diagnosis of ALS crucially relies on the demonstration of simultaneous UMN and LMN features. Electromyography is currently only 60% sensitive, and its core value is in demonstrating LMN signs in clinically unaffected areas. MRI is uniquely able to study both the UMN lesion, which can be clinically silent in ALS (Swash, 2012), and also the inherent extramotor cerebral pathology functionally and structurally. For a ubiquitous, noninvasive technology that does not involve ionizing radiation, this makes MRI very appealing for MND biomarker discovery (reviewed in Turner et al., 2012) and understanding pathogenesis more widely (Table 8–2). The major challenge is that the neuroimaging biomarker candidates to date have been derived from group-level analysis, whereas electromyography, for all its limitations, can be applied to the single patient (Fig. 8–5).

CORE CEREBRAL MOTOR PATHOLOGY

Neuroimaging has identified a "core" cerebral motor pathology across a range of MND clinical subtypes, albeit at group-level analysis at present. The findings closely mirror those of historic postmortem analysis (Smith, 1960; Brownell, Oppenheimer, & Hughes, 1970), and represent a new era of in vivo neuropathology. The common motor pathology involves the PMCs, their interhemispheric CC fibers (Fig. 8–6), the rostral and descending CSTs, particularly the region of the posterior limb of the internal capsule (PLIC).

Table 8–2 **Contribution To Date of Different Types of Neuroimaging to the Understanding of MND Pathology and the Mechanisms of Pathogenesis**

Neuroimaging Modality	Subtype	Motor Pathology	Extramotor Pathology	Mechanisms
SPECT			+	
PET	Activation	+	++	+
	Ligand	+	++	+++
VBM		+	++	+
DTI		+++	++	++
fMRI	Task-based	+	++	++
	Resting-state	+	++	++
MRS		++	+	+

SPECT = single-photon emission tomography; PET = positron emission tomography; VBM = voxel-based morphometry; DTI = diffusion tensor imaging; fMRI = functional magnetic resonance imaging; MRS = magnetic resonance spectroscopy.

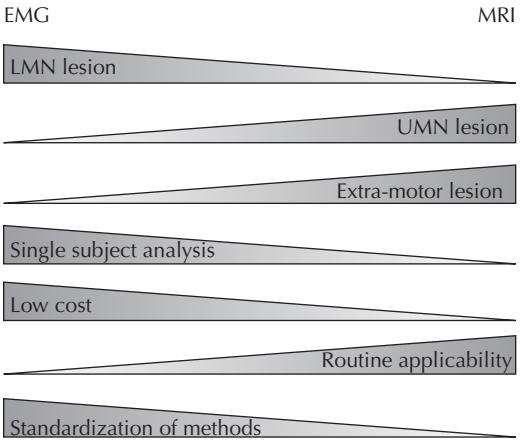

Figure 8–5. Schematics showing the relative strengths of electromyography versus MRI in the future biomarker-driven diagnostic work-up of MND patients.

Voxel- and Surface-based Morphometry

VBM refers to the automated technique of segmenting grey and white matter tissues within individual scans and, across groups of patients, comparing morphology at the voxel level, typically with those from healthy age-matched control subjects. Meta-analysis of the numerous grey matter studies carried out in patients with MND confirmed the PMC (precentral gyrus) as the common region of change (Chen & Ma, 2010). The apparent dominance of the right hemisphere PMC remains unclear, and has been a feature of other white matter studies (Ciccarelli et al., 2009; Zhang et al., 2011), without obvious clinical correlates. The natural asymmetry of the human CST might have relevance (Nathan, Smith, & Deacon, 1990).

Surface-based morphometry is a related technique that is able to consider the segmented grey matter as a continuous surface whose thickness and sulcal geometric morphology can be assessed. In the studies in ALS, involvement of the PMC in the form of cortical thinning has been confirmed (Roccatagliata, Bonzano, Mancardi, Canepa, & Caponnetto, 2009; Verstraete et al., 2012). The link between VBM, surface-based morphometry, and clinical measures has been disappointingly inconsistent, and currently the sensitivity of both techniques in cross-sectional and longitudinal study (Agosta et al., 2009; Senda et al., 2011) is limited.

DTI

Postmortem study of the brain recognized widespread involvement of the white matter tracts in ALS (Smith, 1960). Even among the 10–15% of patients with clinically LMN-only forms of MND (progressive muscular atrophy), there is detectable involvement of the CST postmortem (Ince et al., 2003). A "dying back" process

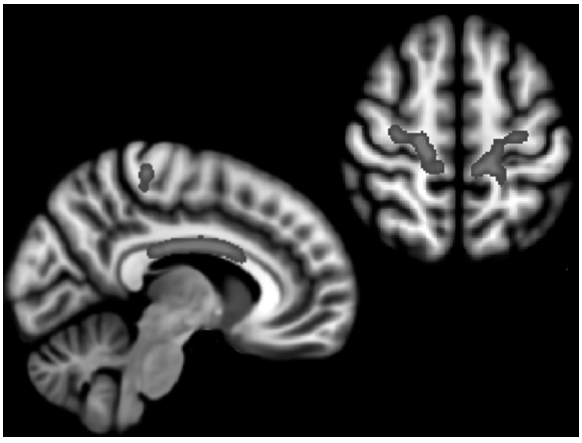

Figure 8–6. The core MRI white matter signature of MND. Areas of increased radial diffusivity in MND patients compared to healthy controls, superimposed on generic MRI images (sagittal and transverse). The core signature involves the white matter in the region of the primary motor cortices and interhemispheric motor fibres of the body of the corpus callosum.

beginning at the neuromuscular junction is still proffered. Conversely, the very small minority with clinically UMN-only and very slowly progressive syndrome of PLS have minimal loss of spinal anterior horn cells histologically, but much more marked atrophy of the PMC, supporting an alternative top-down, "dying forward" process that is also more aligned to the clear links between MND and FTD.

DTI is an advanced MRI application whereby the random movement of water within neuronal tracts can be assessed. Water in structurally intact neuronal tracts has a high level of directionality, which is correspondingly reduced in areas of damage. DTI assigns values for three orthogonal vectors within in each voxel, allowing the calculation of two principal composite measures, the fractional anisotropy ([FA] reduced in damaged tracts) and mean diffusivity (increased in damaged tracts). Analysis of individual directional diffusivities can provide information on the underlying mechanisms of white matter damage. Myelin breakdown is thought to be associated with increased perpendicular diffusivity (radial diffusivity), and axonal damage perhaps reflected in diffusivity changes parallel (axial diffusivity) to the principle tract orientation. Tractography is the technique whereby the principle direction of diffusion within a voxel can be used to reconstruct the path of neuronal tract visually and explore connectivity in a quantitative manner.

The earliest applications of DTI demonstrated clear involvement of the CSTs (Ellis et al., 1999). Some studies reported the most significant changes subcortically (Ciccarelli et al., 2009; Sage et al., 2007), but meta-analysis highlighted the PLIC as a consistent area of change (Li et al., 2012), perhaps reflecting its content of descending fibers from motor and premotor regions forming most of the CST (Zarei et al., 2007). FA values in this PLIC region have been shown to have potential value in predicting progression rate (Fig. 8–7; Menke et al., 2012).

Perhaps as a result of the more widespread use of high-field 3-T magnets, the CC has emerged as a consistently involved tract across a range of ALS cases (Filippini et al., 2010), most strikingly in those with PLS (Ciccarelli et al., 2009; Iwata et al., 2011). DTI has demonstrated that those with progressive muscular atrophy also have evidence of cerebral white matter involvement, including the CC (van der Graaff, et al., 2011). Although CC involvement is not specific, a predilection for involvement of the interhemispheric motor cortical fibers (middle-posterior in sagittal view) in MND has been noted (Muller, Unrath, Huppertz, Ludolph, & Kassubek, 2002), supported by the observation of a loss of fMRI connectivity across this pathway (Jelsone-Swain et al., 2010).

There has been inconsistency in the correlation between cerebral CST involvement and clinical disability (e.g., the ALS Functional Rating Score), although a strikingly tight relationship was reported within the upper cervical spinal cord (Valsasina et al., 2007). DTI has also been surprisingly variable in its ability to detect change longitudinally (Zhang et al., 2011; Sage et al., 2007; van der Graaff et al., 2011; Blain et al., 2007). This may represent a "floor effect" of advanced pathology by the time symptoms emerge and individuals become available for scanning. Two cross-sectional studies have additionally reported that patients with the longest disease duration appeared, paradoxically, to have higher FA values (Filippini et al., 2010; Iwata

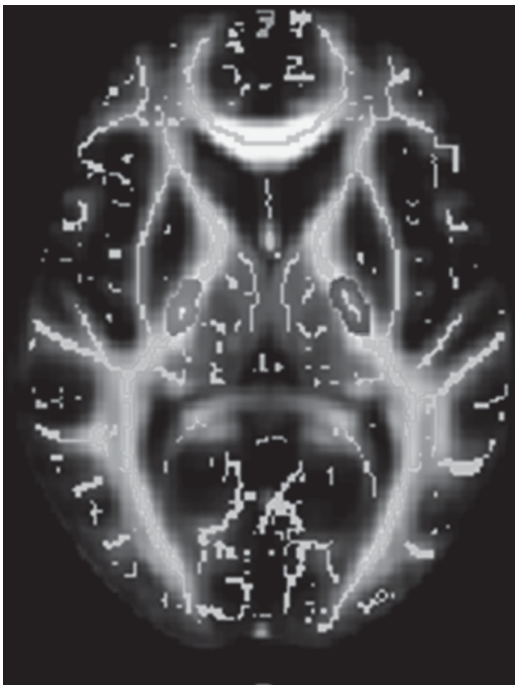

Figure 8–7. The posterior limbs of the internal capsules are a very consistent signal of diffusion tensor imaging in MND patients compared to healthy controls. They are marked on the generic white matter skeleton (transverse view) and abnormal diffusion measures may have prognostic value.

et al., 2011), suggesting a possible resistance to damage or a higher baseline integrity. FA in the CST has been shown to have independent prognostic value (Agosta et al., 2010b). A particularly interesting finding in relation to phenotype has been the relative sparing (in DTI terms) of CST involvement in a group of consistently slowly progressive familial ALS patients homozygous for the superoxide dismutase (*SOD1*) "D90A" mutation, compared with sporadic ALS patients matched for disability and clinical UMN involvement (Stanton et al., 2009; Blain et al., 2011).

Finally, high levels of physical disability and orthopnea frequently preclude MRI in the later stages of ALS. The continued, albeit slow, diffusion of water molecules within neuronal tracts after death, offers the possibility of applying DTI to the postmortem brain (Miller et al., 2011), allowing visualization of pathology at end-stage, and comparison with traditional histologic analysis to improve in vivo MRI applications such as DTI (Fig. 8–8).

Spectroscopy

MRS is an application whereby tissue metabolites can be quantified. Traditionally, this is sensitive to those proton-containing molecules with easily identified peaks in the resulting spectrograph. *N*-Acetylaspartate (NAA) is the exemplar, localized primarily in neurons so that its concentration in the brain as measured by MRS is related to neuronal density, metabolism, and functional status. The creatine (Cr) peak consists of Cr and phosphocreatine, and indicates the status of cellular energetics. The choline (Cho) peak has combined contributions from cell membrane components glycerophosphocholine and phosphocholine, and free Cho. It provides information on the membrane structural integrity, synthesis, and degradation (Fig. 8–9).

Until recently, MRS has been limited to relatively small manually placed "voxels-of-interest," rather than a whole-brain assessment. In MND, reduced NAA (typically expressed as a ratio with Cr) is a consistent finding within the PMC, but only in patients with clinical UMN signs (Pioro, Antel, Cashman, & Arnold, 1994). MRS changes have also been reported in the brainstem (Cwik, Hanstock, Allen, & Martin, 1998). Importantly, progressive reduction was seen in the PMC in longitudinal study (Pohl et al., 2001), and in one study even small increases in response to riluzole therapy (Kalra, Tai, Genge, & Arnold, 2006).

The excitatory neurotransmitter glutamate (Glgu, or Glx in combination with glutamine) has obvious appeal for the study of MND, linking directly with excitotoxicity as a pathogenic mechanism (Rothstein, 2009). Increases in Glu:Cr and Glx:Cr have been demonstrated within the

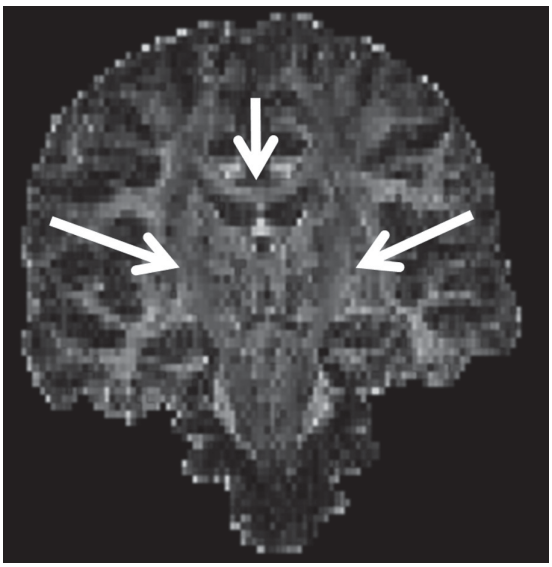

Figure 8–8. Postmortem diffusion tensor MRI. The descending corticospinal tracts and corpus callosum are arrowed in coronal section. These data have the potential to be directly compared with histological samples in order to understand the tissue correlates for MRI changes. (With kind permission of Dr Karla Miller, University of Oxford, UK.)

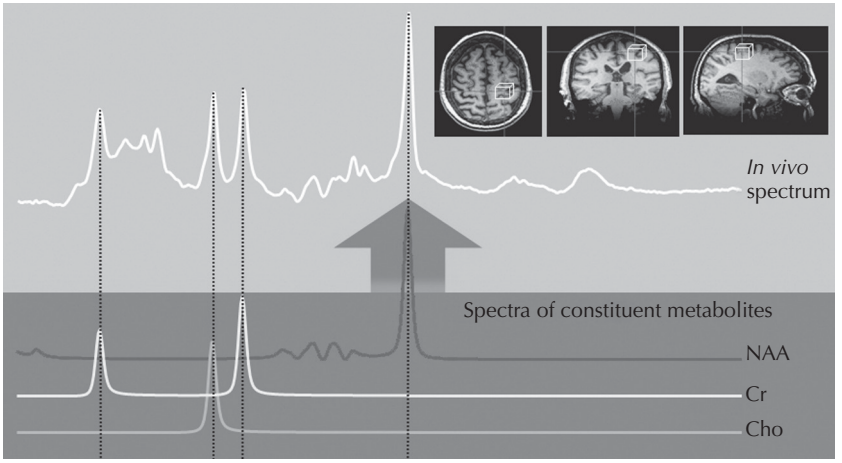

Figure 8–9. Magnetic resonance spectroscopy is a method of quantifying metabolites within brain tissue. A spectrum can be derived from a voxel of tissue placed prior to the acquisition of data (wire box shown on MRI images). Three robust peaks can be identified corresponding to N-acetylasparate, Creatine and Choline whichin varying ratios act as surrogates for different types of pathology. (Adapted with kind permission of Dr Govind Varan, University of Miami, USA.)

PMC and PLIC regions (Han & Ma, 2010). Reductions in levels of the inhibitory neurotransmitter γ-amino butyric acid (GABA) in the PMC (Foerster et al., 2012) may indicate a more important role for loss of inhibitory cortical influence in MND pathogenesis (discussed later).

So far, MRS has not capitalized on its clear sensitivity to motor pathology in MND. This probably reflects a lack of automation and standardization, over and above the more generic limitation of group-level-only analysis. The emergence of whole-brain NAA measurement, and confirmation of reduction along the entire length of the cerebral CST (Stagg et al., 2013), offers renewed hope for MRS as a biomarker source in MND (Fig. 8–10).

EXTRAMOTOR CEREBRAL PATHOLOGY

Case reports of MND involving cognitive impairment are found in the earliest 20th century scientific literature. Even when postmortem studies confirmed widespread cerebral white matter tract involvement, it was generally accepted that MND characteristically spared the higher cognitive as well as oculomotor and sphincter functions. More detailed neuropsychological assessment began to identify a common theme of frontal lobe dysexecutive impairments (Gallassi et al., 1985). A pathologic and genetic overlap between ALS and some forms of FTD is now an established concept, and a dichotomy of phenotype is recognized within members of the same pedigree carrying pathological hexanucleotide expansions in *C9orf72* (Majounie et al., 2012). The MND-related cerebral pathologic network extends widely, but concentrated within the premotor and dorsolateral prefrontal cortices, extending into the frontal and temporal lobes (Douaud, Filippini, Knight, Talbot, & Turner, 2011).

Radionuclide Imaging

Radionuclide studies of blood flow, as a surrogate for regional cerebral metabolism, were pivotal in cementing the view of MND as a widespread multisystem cerebral disorder. SPECT studies demonstrated loss of frontotemporal tracer uptake in cases with dementia (Neary et al., 1990; Talbot et al., 1995). PET studies using ^{18}F-fluorodeoxyglucose confirmed widespread and progressive reductions linked to neuropsychological deficits of a frontal lobe nature in patients, particularly verbal fluency (Ludolph et al., 1992). Alternative tracers used to measure reductions in regional cerebral blood flow were linked to abnormalities of verbal fluency, even in those patients without overt dementia (Kew et al., 1993a).

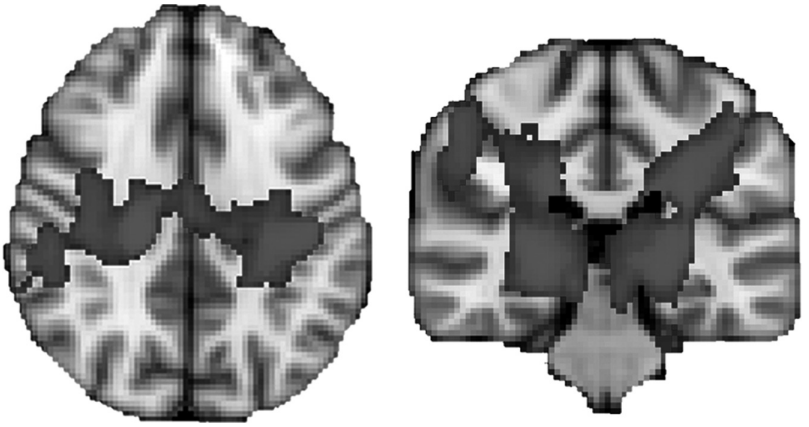

Figure 8–10. Whole-brain magnetic resonance spectroscopy. This emerging application allows assessment of tissue metabolites across the whole brain, without the need to place a voxel-of-interest a priori. The transverse and sagittal images show areas of reduced N-acetylaspartate in the region of the corticospinal tracts in MND patients versus healthy controls.

VBM

Comparative VBM studies in patients with ALS and ALS-FTD clearly demonstrated the involvement of premotor, frontal, and temporal cortex in relation to cognitive impairment (Fig. 8–11; Chang et al., 2005; Abrahams et al., 2005; Grossman et al., 2008; Tsujimoto et al., 2011). Involvement of frontal regions was found in a grey matter study comparing rapid- versus slow-progressing patients (Agosta et al., 2009), and separately a significant correlation, using the emerging technique of surface-based morphometry, between temporal lobe cortical thinning and rapid disease progression (Verstraete et al., 2012). Both reflect the clinical observation that early cognitive impairment is a poor prognostic factor (Elamin et al., 2011).

DTI

DTI studies have demonstrated changes (decreased FA or increased mean diffusivity)

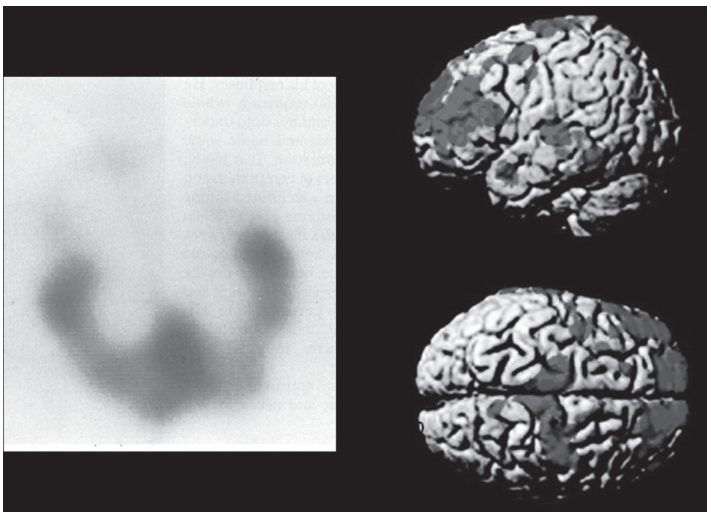

Figure 8–11. The overlap of FTD and MND. Early SPECT studies showed reduced frontotemporal uptake of radiotracer in MND patients with cognitive impairment (left image, adapted with permission from Neary et al., 1990), and areas of reduced grey matter could be shown in similar patients using voxel-based morphometry (adapted with permission from Chang et al., 2005).

in extramotor regions in MND, and with seemingly greater sensitivity than VBM (Canu, 2011). Regions include prefrontal and temporal lobes (Ciccarelli et al., 2009; Canu, 2011; Sage et al., 2007, 2009). Using diffusion tensor tractography, decreased FA (Sato, 2010) and increased axial diffusivity (Agosta et al., 2010a) were demonstrated in the uncinate fasciculus. Performance in cognitive tasks, particularly those assessing attention and executive functions, has been correlated with DTI changes in multiple extramotor regions (Sarro et al., 2011).

Spectroscopy

Decreased NAA:Cho in the thalamus and basal ganglia (Sharma, Saigal, Maudsley, & Govind, 2011), midcingulate cortex (Sudharshan, 2011), and the frontal and parietal lobes (Rule et al., 2004) have been noted in MRS studies in ALS, but have been limited by the need for a priori placement of regions-of-interest.

fMRI

fMRI is unique in its ability to study cerebral activity noninvasively with high spatial resolution. BOLD analysis assumes that alteration in neuronal activity leads to a reduction in local blood oxygenation, and in turn to an increase in cerebral blood flow. A change in the proportion of oxygenated blood forms the image contrast, due to differences in the paramagnetic properties of oxygenated and deoxygenated blood. Studies in patients with MND during cognitive tasks have revealed widespread, particularly frontotemporal activation changes related to word retrieval (Abrahams et al., 2004) and other tests of executive function (Goldstein et al., 2011).

MECHANISTIC IMAGING

In addition to simply mapping regional motor and extramotor pathology in vivo, PET and MRI are capable of revealing important clues to pathologic mechanisms in MND (Fig. 8–12).

Loss of Cortical Inhibitory Influence

A "boundary shift" in ^{18}F-fluorodeoxyglucose PET activation involving the adjacent "face region" of the motor cortex in response to an

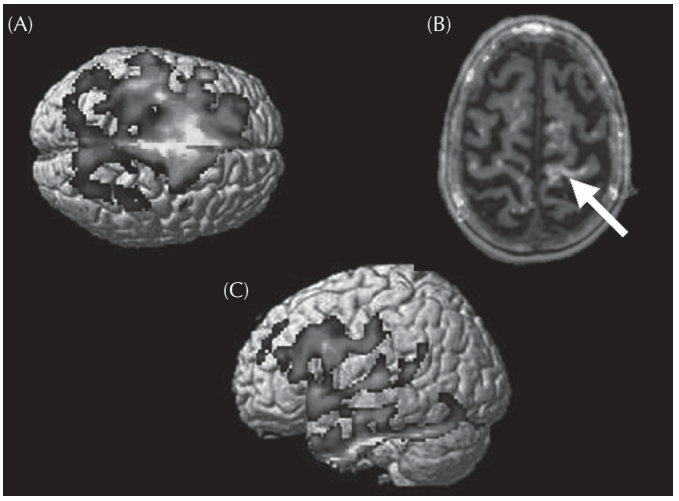

Figure 8–12. Ligand PET studies can reveal mechanisms in MND. Areas of reduced binding of flumazenil over the motor and association cortices (shown on generic 3D brain from above, A) support neurophysiological evidence of increased cortical excitability in ALS. Focal uptake of the microglial ligand PK11195 (shown on axial MRI image, B) was seen in the left motor cortex (arrow) of an MND patient with right hemiparesis. Markedly reduced frontotemporal binding of the 5-HT1A receptor ligand WAY100635 was noted in non-depressed MND patients (shown on generic 3D brain seen from left).

upper-limb joystick task was postulated to represent a loss of inhibitory local circuits (Kew et al., 1993b). Such studies have been reproduced using BOLD fMRI, carefully gated to active motor tasks. Across several studies (Konrad et al., 2002; Tessitore et al., 2006; Han & Ma, 2006; Stanton et al., 2007), patients with MND demonstrated a widened recruitment of premotor and other cortical regions linked to higher motor processing (reviewed in Turner et al., 2012). Increased activity in the contralateral sensorimotor cortex was observed with greater physical impairment (Mohammadi, Kollewe, Samii, Dengler, & Munte, 2011).

The interpretation of these changes remains an issue of debate. An altered boundary of activation has been noted in response to stroke (Weiller, Chollet, Friston, Wise, & Frackowiak, 1992), lending strong support to the intuitive notion that this is a compensatory response to neuronal loss. The response in MND is presumably futile, compared with stroke, due to an overwhelming cascade of degeneration.

However, a wider range of evidence, including histopathology and neurophysiology, demonstrates a potential role for loss of cortical (and spinal) inhibitory interneuronal influences in the pathogenesis of MND (Turner & Kiernan, 2012), which might also underlie these functional activation changes. PET studies with the GABA-A receptor ligand flumazenil demonstrated widespread cortical loss of binding in ALS (Lloyd, Richardson, Brooks, Al Chalabi, & Leigh, 2000). Flumazenil binding in a group of homozygous "D90A" *SOD1* familial ALS patients of uniformly slow progression, appeared notably preserved in motor and premotor regions compared with sporadic ALS patients (Turner et al., 2005a). GABA levels have also been shown to be reduced in the PMC using MRS (Foerster et al., 2012).

Resting-state fMRI detects regional correlation between the low-frequency spontaneous fluctuations across the resting brain. These form functionally distinct networks (Smith et al., 2009), and the technique has emerging biomarker potential across a range of cerebral disorders. Initial studies in MND reported reduced activity in the somatosensory and default-mode networks (Mohammadi et al., 2009), and interhemispherically between the PMCs (Jelsone-Swain et al., 2010). Subsequently, regions of increased functional connectivity were identified, including somatosensory areas (Agosta et al., 2011), and with higher rates of functional connectivity in those with faster disease progression (Verstraete et al., 2010).

Using a tractography-defined ALS-specific cortical network, increased functional connectivity was found over a large area, significantly overlapping areas of structural damage, and those regions with reduced flumazenil (GABAergic) receptor binding seen in a separate study (Douaud et al., 2011). Those with the highest rates of disease progression were found to have the greatest functional connectivity. This challenges the view that increased cerebral activation boundaries are simply secondary to structural damage, but might instead reflect a pathogenic loss of local inhibitory circuits, with implications for future therapeutic development.

Neuroinflammation

A non–cell-autonomous pathologic cascade of motor neuronal cell death is a long-established concept, and multiple neuroinflammatory mechanisms have been implicated (Philips & Robberecht, 2011). Whether such activity is a primary or secondary pathologic response, or indeed beneficial (e.g., in limiting spread), is debated. Much of the evidence has come from transgenic mouse models of ALS. Studies with the PET ligand PK11195, which binds to receptors only expressed by activated microglia, provided the first in vivo human evidence for widespread cerebral neuroinflammatory activity in ALS (Turner et al., 2004). Changes were seen in the PMC and descending CSTs including the pons, and also the dorsolateral prefrontal cortices and thalamus, the latter possibly reflecting its wider cortical connectivity.

Serotonergic Involvement

Studies with the serotonin 5-HT1A receptor PET ligand WAY100635 in nondepressed patients with ALS revealed large reductions in binding (Turner et al., 2005b). The frontotemporal localization was strikingly similar to those seen in FTD (Lanctot et al., 2007; Bowen et al., 2008). Serotonergic pathways interact in several ways with potentially pathogenic pathways in ALS (Sandyk, 2006), but the full significance of these findings is yet to emerge.

FUTURE DIRECTIONS

Aspirations in research include a fuller understanding of regional spread of symptoms, the very earliest changes in pathogenesis, and the applicability of neuroimaging to animal models of ALS.

Imaging in Animal Models

Ultra high-field MRI has been applied to the study of animal models of ALS. T2-hyperintensities within the brainstem nuclei (Fig. 8–13), MRS alterations in NAA, glutamate and GABA levels, and DTI changes have all been demonstrated in studies of transgenic rodent models (Evans, Modo, Talbot, Sibson, & Turner, 2012). An emerging application is the use of MRI-visible injectable magnetic particles of iron oxide to which can be bound antibodies to specific targets of interest, including vascular endothelium (Evans et al., 2014).

Spinal Cord

Quantitative spinal cord imaging is challenging. The small dimensions make tissue segmentation highly susceptible to partial volume effects. This is complicated by physiologic motion distortions and magnetic field inhomogeneities posterior to the vertebral bodies. Nonetheless, advanced MRI is becoming applicable to the spinal cord lesion in MND, with the potential to capture LMN and UMN pathology in relation to the spread of symptoms (Bede et al., 2012).

Presymptomatic Pathology

The timing of the first pathologic changes in MND is not certain. Data in those at high genetic risk of Alzheimer disease, and the identification of premotor symptoms, such as anosmia and rapid eye movement sleep behavior disorder in Parkinson disease, suggest changes occur long before the onset of symptoms.

Only 5–10% of patients report a family history of MND or the related condition FTD. Of these, approximately 60% are either carriers of dominant pathogenic mutations of *SOD1*, or the intronic hexanucleotide repeat expansion associated with *C9orf72*. Both seem to be highly penetrant. Presymptomatic carriers offer a unique possibility to study the very earliest changes in cortical function in MND. Such changes might then be useful in characterizing the much larger population at risk for apparently sporadic disease, and help to reduce

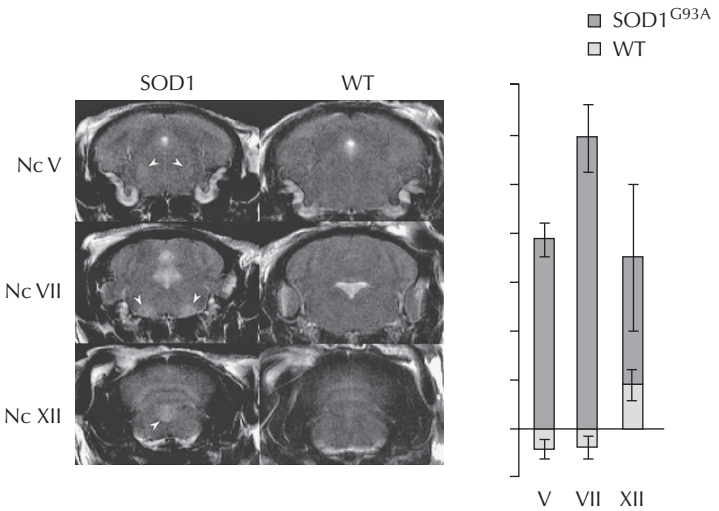

Figure 8–13. MRI hyperintensity in the superoxide dismutase mouse model of MND. These are seen in the brainstem nuclei V, VII and XII, and are detectable pre-symptomatically, with potential as a biomarker of therapeutic intervention. (With kind permission of Dr Matthew Evans, University of Oxford.)

the diagnostic delay in those developing initial symptoms, in turn allowing earlier administration of future candidate drugs.

A DTI study in presymptomatic *SOD1* mutations carriers revealed reductions in FA detectable in the PLIC regions (Ng et al., 2008), and an MRS study demonstrated a metabolite profile in presymptomatic individuals that was more similar to that seen in affected patients rather than healthy control subjects (Carew et al., 2011). Both results support the view that changes may be detectable long before symptoms emerge, and offer the first hope for eventual primary prevention.

Network-based Approaches

The human brain can be modelled as a network in which regions are interlinked by white matter connections according to functions (Sporns, 2011). Network-based analyses suggest that the brain has a mathematical "small-world" topology (van den Heuvel, Stam, Boersma, & Hulshoff Pol, 2008). Highly connected brain hubs seem to have a strong tendency to be interconnected, with a central "premier" collection of hubs perhaps having the greatest impact on brain functioning (van den Heuvel & Sporns, 2011). Whole-brain DTI study with network analysis demonstrated a subnetwork of impaired connectivity overlapping the normal motor pathways in MND, supporting the hypothesis of propagation of disease along structural connections (Fig. 8–14; Verstraete et al., 2011). This type of network-based analysis offers potential to understand MND as a systems-level degeneration, possibly to stage the disease process, and to understand clinical subtypes (e.g., for cognitive impairment, regional spread, and prognosis).

CONCLUSION

Neuroimaging has established itself as a relatively easily applicable technique in MND research, at a time when the extent of the overlap with FTD is fully emerging. It has demonstrated its potential to reveal important mechanisms of pathogenesis, generate much needed biomarkers, and moved permanently beyond its clinical role in excluding structural mimic disorders. The biomarker aspiration is likely to involve a multimodal approach, integrating structural and functional candidates from both brain and spinal cord to create a "signature" applicable to the range of phenotypes, and which might be used to more sensitively monitor efficacy of future therapeutic agents. A major shift in the understanding of neurodegenerative disorders at the systems-level looks set to be led by involve novel applications of MRI in conjunction with sophisticated mathematical network modelling.

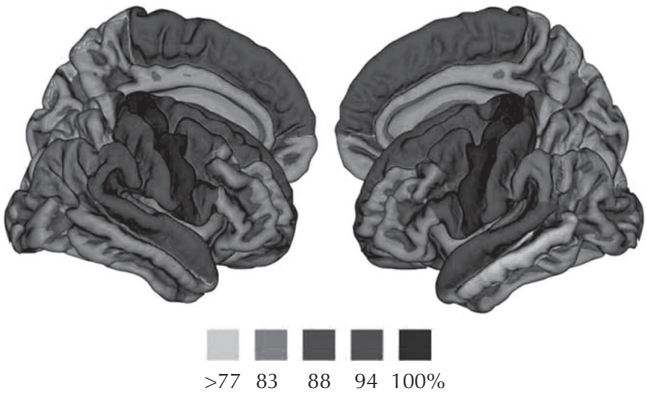

Figure 8–14. The prominent frontotemporal structural connections of the primary motor cortices in relation to the rest of the brain (shown on 3D generic brain hemispheres from the right and left sides). This supports models of degeneration defined by neuronal connections. (Adapted with permission from Verstraete et al., 2011.)

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Chapter 9

Diagnostic Criteria for Motor Neuron Disease

Mark B. Bromberg

BACKGROUND AND NEW POINTS

The diagnosis of the various forms of motor neuron disease (MND) rests on clinical features because there are no specific tests or biomarkers to make an unequivocal diagnosis. Formal diagnostic criteria for amyotrophic lateral sclerosis (ALS) were put forward in 1994 as the El Escorial Criteria under the auspices of the World Federation of Neurology. These criteria were for research purposes, but are also used in the clinic for diagnosis. Primary lateral sclerosis (PLS) and progressive muscular atrophy (PMA) were not included.

The El Escorial Criteria have been revisited and revisions published in 2000 and 2009, and another review is scheduled for 2014. Criteria for PLS have been reviewed several times based on experience from clinical series resulting in general guidelines. Clinical descriptions for PMA have been reviewed but formal criteria have not been considered.

INTRODUCTION

The diagnosis of the various forms of MND is based on clinical features, because there are no biomarkers at this time. Formulation of the El Escorial Criteria was a major step in standardizing the diagnosis of ALS. Although intended for research purposes, they are generally used in the clinic as a basis for making the diagnosis (Bromberg, Schenkenberg, & Brownell, 2011). Criteria for PMA and PLS were not included in the El Escorial Criteria, and their diagnosis is more challenging because of a broad differential diagnosis for PMA and the need for the passage of several years to ensure that PLS does not advance to ALS.

TIME TO DIAGNOSIS

The time from symptom onset to diagnosis for ALS is 12–19 months, which represents a significant proportion of the duration of the disease (median, 2–4 years). The time to diagnosis of PLS is longer, with a 4-year minimum

to ensure that progression to ALS has not occurred (Gordon et al., 2006). Efforts to reduce the time have been made and are challenging. Data from 640 patients diagnosed in one ALS/MND clinic in the United Kingdom over 20 years showed a median time to diagnosis of 0.95 years with little change over 20 years (Mitchell et al., 2010). This represented a diagnosis at 50% of disease duration. Factors include rate of progression, with earlier diagnosis among patients with rapid progression, and initial referral to nonneurology providers. A study of 202 patients with ALS from Japan found an increase in the mean time from symptom onset to diagnosis among patients with limb onset (15.2 months) compared with bulbar onset (9.2 months), but noted possible referral differences between countries (Kano et al., 2013).

ALS

The World Federation of Neurology felt the need to formalize diagnostic criteria for ALS to aid in uniform enrollment of patients with ALS in clinical trials.

El Escorial Criteria

The El Escorial Criteria were set forth at a meeting held in El Escorial, Spain in 1990 and the initial set of criteria was published in 1994 (World Federation of Neurology Research Group on Neuromuscular Diseases, 1994). The diagnosis is based on assessment of clinical signs of upper motor neuron (UMN) degeneration (pathologic spread of reflexes, clonus, pathologic reflexes, spasticity, and pseudobulbar findings) and lower motor neuron (LMN) degeneration (weakness, atrophy, and fasciculation potentials), which can be aided by electrodiagnostic signs, and progression of signs within a region or to other regions. Regions of the neuroaxis are bulbar, cervical, thoracic, and lumbosacral, and no distinction is made as to involvement of sides of the body within a region or need for bilateral involvement within a region. Alternative causes of degeneration are to be excluded. This leads to four levels of diagnostic certainty (Fig. 9–1): (1) definite ALS, (2) probable ALS, (3) possible ALS, and (4) suspected ALS.

The role of electrophysiologic studies, in addition to excluding alternative pathology and diagnoses, is to confirm LMN degeneration in clinically affected regions and document subclinical evidence of involvement in clinically normal regions. Electrophysiologic evidence for LMN degeneration has the same significance as clinical evidence for degeneration and can elevate diagnostic certainty from one level to a higher level. Electrophysiologic data are most secure when abnormalities are found in two muscles of different nerve or root innervations in two or more regions. Electrophysiologic data are in three categories: (1) required, (2) supportive-probable, and (3) supportive-possible. Electrophysiologic support is based on attempts to document both active and chronic denervation.

Airlie House Criteria

The El Escorial Criteria were reviewed in 1998 at the Airlie House in Warrington, Virginia and a revised set was published in 2000 as the El Escorial-Revised or the Airlie House Criteria (Brooks, Miller, Swash, & Munsat, 2000). The requirements for levels of certainty for definite ALS and probable ALS remain unchanged, but the word "clinical"

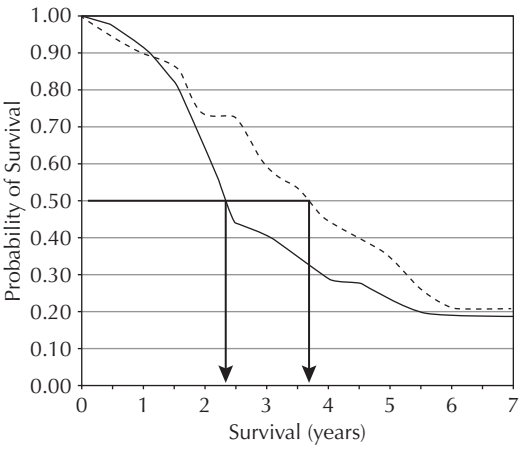

Figure 9–1. Kaplan-Meir survival curves for El Escorial Criteria definite ALS (solid line) compared with suspected ALS (dashed line) based on the initial diagnosis. Median survival not statistically significantly different for definite ALS 27 months and for suspected ALS 40 months. (Data estimated, simplified, and redrawn from Traynor et al., 2000.)

was added to distinguish additional features that were added. An added level, clinically probable ALS–laboratory-supported, allows signs of LMN degeneration to be defined by electrophysiologic (electromyogram [EMG]) criteria (Table 9–1). This addition was based in part on experience with a large number of patients with ALS who were believed to have an accurate diagnosis ALS based on less reliance on clinical evidence for LMN degeneration and more reliance on EMG findings (Ross et al., 1998). Clinical signs of LMN involvement (atrophy and fasciculation potentials) were not used because they were believed to be subjective: in their place are electrophysiologic findings of fibrillation potentials. The new laboratory-supported criterion requires evidence for LMN involvement in two limbs and UMN involvement in one region. In the Airlie House Criteria the level of "clinically suspected ALS" was deleted because it was believed to be of insufficient diagnostic certainty for inclusion of subjects in research protocols.

Awaji Criteria

A third review of the El Escorial Criteria was undertaken in 2006 on Awaji Island, Japan and a revision was published in 2007 as the Awaji Criteria (de Carvalho et al., 2008). The goal was to identify patients with ALS earlier in their course to enhance their management, because data from an Irish study indicate that up to 10% of patients die before reaching the level of probable ALS (Traynor et al., 2000). It was pointed out that in the El Escorial Criteria clinical electrophysiologic evidence for LMN degeneration could not be combined in a similar limb (however, this may represent a restricted interpretation of the El Escorial Criteria [discussed later]). Furthermore, it was pointed out that definite electrophysiologic criteria require evidence for both active denervation (positive waves and fibrillation potentials) and chronic denervation (motor units with reduced recruitment, and high amplitude and long duration). Revisions to the El Escorial Criteria and Airlie House Criteria incorporated in the Awaji Criteria include allowing clinical and electrophysiologic evidence for LMN degeneration to be combined within a limb (but electrophysiologic confirmation of LMN degeneration in clinically weak muscles was believed to be important); and allowing fasciculation potentials (preferably of complex morphology) in a muscle to serve as evidence for active LMN degeneration when positive

Table 9–1 **World Federation of Neurology Criteria for the Clinical Diagnosis of ALS: El Escorial Criteria and Subsequent Airlie House Criteria (El Escorial–Revised) Revision**

El Escorial Criteria			
Definite ALS	UMN: bulbar region + ≥2 spinal regions + LMN: bulbar + ≥2 spinal	or	UMN: 3 regions + LMN: 3 regions
Probable ALS	UMN: ≥2 regions (1 region above LMN region) + LMN: ≥2 regions		
Possible ALS	UMN: 1 region + LMN: 1 region	or or	UMN: ≥2 regions LMN: rostral UMN region
Suspected ALS	LMN: ≥2 regions		
Airlie House Criteria (El Escorial Criteria–Revised)			
Clinically Probable–Laboratory Supported	UMN: ≥1 region + LMN: ≥1 region	or	UMN: ≥1 region + LMN-EMG: 2 regions

In addition to the bulbar region, there are three spinal regions: cervical, thoracic, and lumbosacral.
Modified from World Federation of Neurology Research Group on Neuromuscular Diseases (1994) and Brooks et al. (2000).

waves and fibrillation potentials are absent and signs of chronic denervation are present.

Pending El Escorial Review

In keeping with the need to update criteria with new information, a review and revision is planned for 2014 with the plan to include features of frontotemporal lobe dysfunction.

WORLD FEDERATION OF NEUROLOGY CRITERIA PERFORMANCE REVIEWS

The goal of the World Federation of Neurology is to define patients for enrollment in clinic trials and other research. The success or utility of the criteria and their revisions can be reviewed in terms of the percentage of patients fulfilling the levels of diagnostic certainty at time of diagnosis and the time period from symptom onset to the diagnosis. However, there is a degree of circularity to the efforts to include more patients with a high likelihood of truly having ALS by refining (lowering) the diagnostic criteria. The circularity is that the disease "exists," but because it has individual variability in presentation and rate of progression it cannot be easily defined at onset. Comparisons of numbers of diagnoses of definite and probable ALS among the various criteria revisions are from retrospective reviews of patient data, and lowering or liberalizing one or more individual criterion always results in inclusion of more patients. In turn, this raises the possibility of including patients without ALS.

The requirement of finding abnormalities in two muscles of different nerve or root innervation in two or more regions has been investigated using receiver operating characteristics (Makki & Benatar, 2007). Based on applying the electrophysiologic category of definite LMN degeneration (Table 9–2) to 35 consecutive patients evaluated for and given the diagnosis of ALS (20 definite, 12 probable, 3 possible) the best combination of sensitivity and specificity for ALS and not an alternative diagnosis (radiculopathy, PLS, neuropathy, benign fasciculation potentials) was achieved by requiring definite abnormalities in two regions based on finding abnormalities in two muscles in cervical and lumbosacral regions and in one muscle in cranial and thoracic regions. Of note, a uniform muscle sampling protocol was not followed and it is not clear which muscles in a region were most sensitive to denervation in ALS, except for cranial innervated muscles where evidence of denervation was highly specific.

Comparisons among the three electrophysiologic criteria (El Escorial Criteria, Airlie House Criteria, Awaji Criteria) for the level of ALS certainty have been made. The various comparisons are complicated to interpret because most represent retrospective reviews of patient data, and for electrophysiologic data, comparisons frequently involve investigation of different numbers of muscles among patients and among centers. Furthermore, the comparisons showing elevation of diagnostic certainty between the criteria are viewed from different perspectives. Overall, with each set of revisions there are more patients classified at the higher levels of certainty, about a 10% increase with the Awaji Criteria. A number of comparisons focus on the Awaji Criteria showing an increase in sensitivity (more patients reaching definite ALS) with little loss of specificity (alternative diagnoses) (Carvalho & Swash, 2009; Douglass, Kandler, Shaw, & McDermott, 2010; Boekestein, Kleine, Hageman, Schelhaas, & Zwarts, 2010; Chen et al., 2010). Some of the underlying issues in these comparisons have been critically commented upon (Benatar & Tandan, 2011).

Of note, one comparison of 388 patients by El Escorial Criteria and Airlie House Criteria showed similar Kaplan-Meier survival curves between the four El Escorial criteria levels of diagnostic certainty (Table 9–1), suggesting that all levels of diagnostic certainty are assessing the same disease process (Fig. 9–1; Traynor et al., 2000).

Another aspect in the diagnostic process is accuracy of interpretation of the same clinical data by different clinicians and the resultant level of assigned diagnostic certainty. Diagnostic reproducibility based on review of clinical records of 65 consecutive patients suspected of having MND between two experienced neurologists for both the El Escorial Criteria and Airlie House Criteria has been investigated. Congruency was generally high, but it is noted that fewer patients were diagnosed with definite ALS by the Airlie House Criteria (Forbes, Colville, & Swingler, 2001).

Table 9–2 **Electrodiagnostic Criteria for LMN Involvement With Evolution of Changes With Revisions to the El Escorial Criteria (Airlie House and Awaji Criteria)**

El Escorial Criteria: Definite LMN-EMG Degeneration
- Reduced recruitment (firing rates >10 Hz)
- Large amplitude, long-duration motor units
- Fibrillation potentials

El Escorial Criteria: Probable LMN-EMG Degeneration (≥1 features)
- Reduced recruitment or large motor units, fibrillation potentials, unstable motor units
  Or
- Reduced motor unit estimates and increased macroEMG potentials

El Escorial Criteria: Possible LMN Degeneration (≥1 features)
- Reduced recruitment, large motor units, fibrillation potentials or unstable motor units
  Or
- Polyphasic motor units or increased fiber density
  Or
- Low amplitude motor unit potentials if disease progression >5 years or muscle atrophy

Airlie House Criteria: Definite LMN Degeneration
- Fibrillation and positive wave potentials
- Large motor units, with increased duration, with polyphasia
- Unstable motor units
- Fasciculation potentials (particularly if long duration and polyphasic) helpful

Awaji Criteria:
- Large amplitude, increased duration, usually with polyphasia
- Reduced recruitment with firing rates >10 Hz (rates may be <10 Hz if prominent UMN degeneration)
- Unstable motor units observed with a 500 Hz low frequency filter
- Fasciculation potentials (preferably with complex morphology) = fibrillation or positive wave potentials

Modified from World Federation of Neurology Research Group on Neuromuscular Diseases (1994) and Brooks et al. (2000).

PLS

The question of PLS as a clinical entity distinct from ALS has been challenged from the earliest descriptions. Most efforts to define the clinical features of PLS are based on reviews of relatively small numbers of patients (8–35), and the primary concerns are an UMN onset of ALS and differentiation from spastic paraparesis (Rowland, 2005). The first modern attempt at a set of diagnostic criteria was by Pringle et al in 1992 (Box 9.1; Pringle et al., 1992). The features emphasized the continued absence of denervation by EMG studies 3 years from symptom onset (although increased insertional activity and occasional fibrillation potentials were not considered sufficient to change the diagnosis).

From a review of 24 patients with an initial diagnosis of PLS followed for greater than 4 years from symptom onset, 54% eventually showed signs of denervation, leading to the concept of UMN-dominant ALS. Some of these patients had a progression of LMN signs fulfilling criteria for ALS over 60–137 months from onset (Gordon et al., 2006). As a consequence, a 4-year follow-up period was recommended as a practical duration to exclude most, but not all, patients who will develop ALS (Singer, Statland, Wolfe, & Barohn, 2007).

Several tests that may not be routinely used or available have been assessed as aids in the diagnosis of PLS, but they cannot differentiate PLS from ALS (Le Forestier et al., 2001). Muscle biopsies to look for evidence of denervation (angular fibers and fiber-type grouping) provide similar information as EMG studies. In one study of nine patients with predominant UMN features needle EMG showed chronic denervation (large and unstable motor units) but not active denervation changes (positive waves and fibrillation potentials), but did show scattered angular fibers and mild fiber-type grouping (Soraru et al., 2008). Transcranial magnetic stimulation shows at

Box 9.1 Clinical Features Supportive of the Diagnosis of PLS

Pringle Criteria

- Insidious onset of spastic paresis, usually beginning in the legs but occasionally in bulbar of arms
- Adult onset, usually fifth decade or later
- Absence of family history
- Gradually progressive course (not step-like)
- Duration greater than 3 years
- Clinical findings limited to corticospinal dysfunction
- Symmetric distribution, ultimately leading to severe spastic spinobulbar paresis

Singer Criteria

- Presence of UMN findings: spasticity, pathologic reflexes, weakness in corticospinal distribution
- Adult onset: second decade or later
- Duration greater than 4 years
- Involvement of legs, legs and arms, limbs and bulbar regions
- Progressive course
- Bladder urgency due to UMN dysfunction may be present

Modified from Pringle et al. (1992) and Singer et al. (2005).

least some abnormalities of central conduction in essentially all patients with PLS, ranging from increased central motor conduction time in the legs (may be asymmetric in degree) to absent responses (Le Forestier et al., 2001; Zhai, Pagan, Statland, Butman, & Floeter, 2003). Magnetic resonance imaging, especially diffusion tensor tractography, and magnetic resonance spectroscopy can detect UMN involvement, but cannot differentiate between ALS and PLS (Evans et al., 1993).

The Pringle criteria excluded familial forms. However, several families have been described with PLS (Dupre, Valdmanis, Bouchard, & Rouleau, 2007). A more common hereditary (genetic) issue in the diagnosis of PLS is confusion with hereditary spastic paraparesis, which may present in a familial or sporadic pattern. Experience from a large Dutch cohort of patients with adult-onset sporadic UMN involvement found marked overlap in clinical presentation and distribution between PLS and hereditary spastic paraparesis with the exception that patients with rostral extension to arm and bulbar spasticity and asymmetric limb spasticity were more likely to have PLS, and genetic testing is necessary to separate the two disorders (Brugman et al., 2009).

PMA

PMA as a clinical entity distinct from ALS has also been questioned from the earliest descriptions (Visser, de Jong, & de Visser, 2008). PMA is defined as sporadic adult-onset LMN degeneration evident from strength testing or needle EMG, but no formal criteria have been offered. In the El Escorial Criteria, suspected ALS included PMA, but this category was removed in the Airlie House Criteria. In follow-up studies of greater than 4 years, about 10–20% of patients with PMA develop clinical signs of UMN degeneration and the diagnosis is changed to ALS (Van den Berg-Vos et al., 2009; Kim et al., 2009). At postmortem examination, however, about 50% of patients with PMA (without clinical UMN signs) have pathologic loss of UMNs (Ince et al., 2003). Patients with PMA have longer survival than those with ALS (Kim et al., 2009).

There is also clinical uncertainty in distinguishing between PMA and adult-onset spinal muscular atrophy (SMA; SMA type IV with and without chromosome 5 mutations). One clinical feature described for adult-onset SMA is symmetric proximal or less commonly distal weakness that progresses very slowly and does not include significant respiratory involvement (Visser et al., 2008). Patients considered to have SMA type IV seem to be rarely tested for mutations in the survival motor neuron gene. In a series of 21 patients with very slowly progressive proximal leg weakness with onset in adult years 4 had homozygous absent of survival motor neuron 1 genes (Phukan et al., 2012).

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Chapter 10

Differential Diagnosis of Motor Neuron Disease

Mark B. Bromberg

BACKGROUND AND NEW POINTS

The diagnosis of all forms of motor neuron disease (MND) is based on clinical features with no biomarker to confirm the diagnosis. Given the poor prognosis of MND, amyotrophic lateral sclerosis (ALS) and progressive muscular atrophy (PMA) in particular, there is concern for disorders that mimic these and that might be treatable.

Serum tests commonly considered to detect a mimic disorder include a large number of basic metabolic tests that are not linked to an alternative diagnosis. The number of disorders with similar clinical features is very small, and even fewer are treatable disorders.

DIFFERENTIAL DIAGNOSIS OF ALS

It can be argued that the symptoms and signs of ALS and PMA are unique, and no other disorder truly mimics them. In the setting of a fatal disease there is concern for a false-positive diagnosis and consideration of an alternative disease, especially one that is treatable. There are disorders that have some overlapping clinical features, especially early in the clinical course, but careful consideration of the features is usually sufficient to exclude them (Table 10–1; Turner & Talbot, 2013). The opposite concern, a false-negative diagnosis of an alternative disease, is less common, because with the passage of time the forms of MND declare themselves.

KENNEDY DISEASE

Kennedy disease, or spinal bulbar muscular atrophy, is an X-linked neurodegenerative disorder of both motor and sensory nerves associated with an expansion of cytosine-adenosine-guanosine (CAG) repeat in the androgen receptor gene (Finsterer, 2010). Similarities with ALS include age range of symptom onset from 30–60 years,

Table 10–1 **Features That if Present Help Support ALS and PMA From Other Disorders**

Supportive Feature	Clinical Findings
Supratentorial localization	Emotional lability Features of frontotemporal lobe dysfunction
Corticospinal tract localization	Pathologic or asymmetric tendon reflexes Extensor plantar responses Snout response
Diffuse distribution of LMN involvement	Diffuse spontaneous and contraction fasciculation potentials with large amplitude twitches Diffuse fasciculations; active and chronic denervation on EMG study
Progression of weakness	Focal site of onset Steady progression Respiratory involvement

EMG, electromyogram; LMN, lower motor neuron.

bulbar weakness (dysarthria and dysphagia), limb weakness that may present asymmetrically, muscle cramps, diffuse denervation on needle electromyography (EMG) study, and elevated serum creatine kinase values.

Features that distinguish Kennedy disease from ALS are sensory disturbance with numbness in 50%, reduced or absent tendon reflexes, prominent lip weakness (Fig. 10–1), perioral muscle contraction fasciculation potentials, very slow progression (decades) with rare death from respiratory failure, gynecomastia in a large portion (Fig. 10–2), and no pseudobulbar affect or frontotemporal lobe syndrome symptoms. Electrodiagnostic features that differ from ALS are reduced or absent sensory nerve responses. A genetic test for an expanded CAG repeat number is diagnostic in equivocal cases.

MULTIFOCAL MOTOR NEUROPATHY

Multifocal motor neuropathy is a mononeuropathy multiplex that affects motor nerves with focal conduction block at discrete sites away from

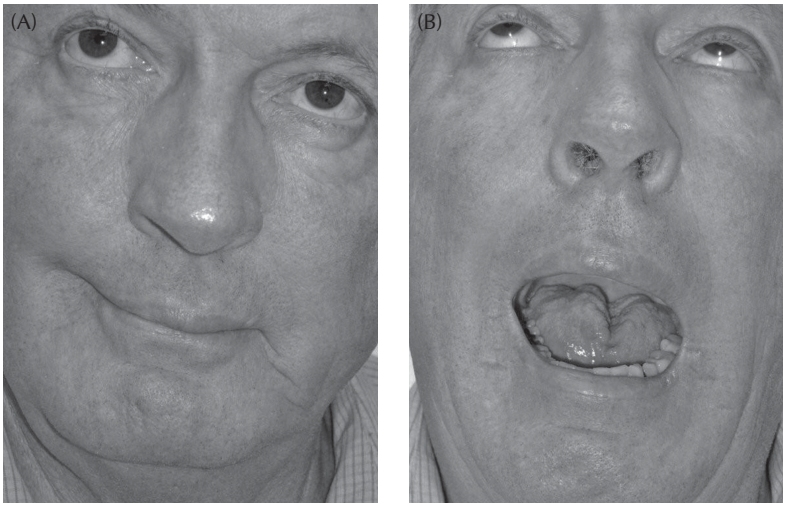

Figure 10–1. Cranial nerve features of Kennedy disease. (A) Asymmetric lip weakness. (B) Marked tongue atrophy. (With permission from patient.)

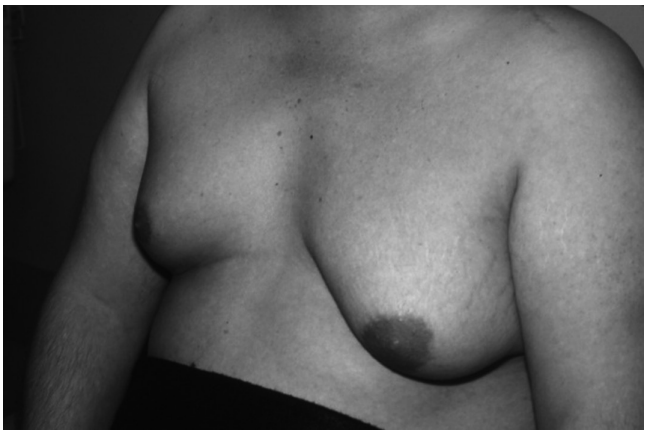

Figure 10–2. Gynecomastia in patient with Kennedy disease. (With permission from patient.)

common sites of entrapment (Renton et al., 2011). This disorder is treatable with intravenous immune globulin (Slee, Selvan & Donaghy, 2007). Similarities with ALS include age range from 20–70 years with mean age 40 years, asymmetric and predominantly distal weakness, muscle atrophy, and progression of weakness.

Clinical features that distinguish multifocal motor neuropathy from ALS are predominance and early weakness of finger and wrist extension (Fig. 10–3), muscle atrophy that is mild in proportion to the degree of weakness of the affected muscle group, reduced or absent tendon reflexes (although pathologically brisk reflexes are occasionally observed), the absence of bulbar involvement, and no pseudobulbar affect or frontotemporal lobe syndrome symptoms. The prototypic electrodiagnostic feature of multifocal motor neuropathy is focal conduction block (Fig. 10–4), but block cannot always be demonstrated either because it is at proximal sites not easily tested or there exist forms of the clinical disease without conduction block. The degree of EMG findings is mild and the distribution restricted compared with ALS.

ALS clinics report varying percentages of patients referred for ALS who ultimately have the diagnosis of multifocal motor neuropathy

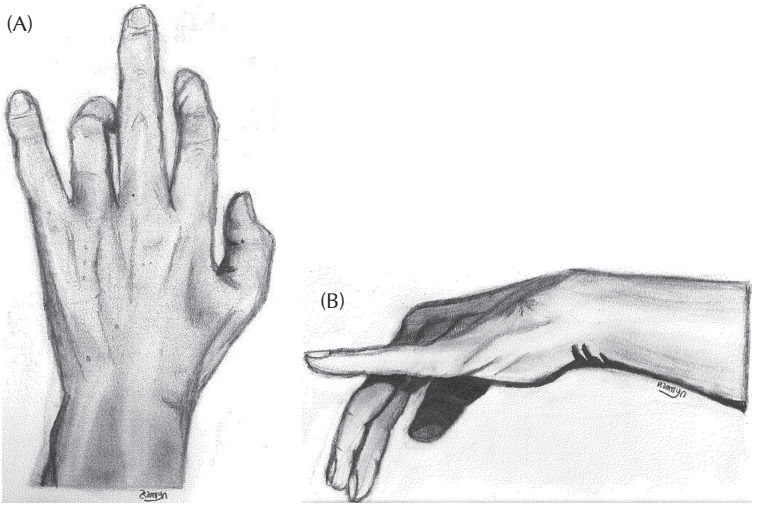

Figure 10–3. Drawing of characteristic distribution of asymmetric finger (A) and wrist extensor weakness (B) in multifocal motor neuropathy with conduction block.

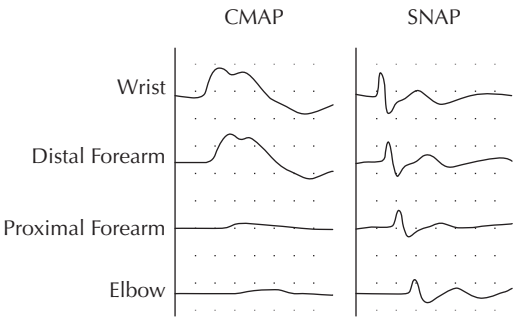

Figure 10–4. Characteristic nerve conduction waveforms showing focal motor conduction block away from common sites of entrapment (between distal and proximal forearm) with marked loss of compound muscle action potential (CMAP) amplitude and area, but with preservation of sensory potentials (SNAP).

with conduction block. A review of 89 patients from one center with the initial diagnosis of PMA (thus excluding ALS) indicates that 8% had multifocal motor neuropathy, but diagnostic support in the form of a response to treatment was not presented (DeJesus-Hernandez et al., 2011).

INCLUSION BODY MYOSITIS

Inclusion body myositis is a degenerative disorder of muscle that has elements of inflammation on muscle biopsy but does not respond to immune-modulating drugs (Hilton-Jones et al., 2010). There is a prototypic clinical pattern of muscle atrophy and weakness that is usually distinct from ALS, but similarities include age range from 45–80 years, asymmetric muscle atrophy, and frequent elevation of serum creatine kinase.

Differences from ALS are characteristic pattern of atrophy and weakness of forearm flexor muscles (weakness of distal digital flexion) and quadriceps muscles, normal tendon reflexes, and insidious onset of weakness and very slow progression (Fig. 10–5). Despite being a myopathy, needle EMG includes high-amplitude complex motor unit potentials with reduced recruitment, but upon careful observation also includes low-amplitude, complex, and rapidly recruited potentials. The pathologic diagnosis from muscle biopsy is a myopathic pattern of variation in muscle fiber size and inclusion bodies with rimmed vacuoles (Fig. 10–6). A review from one ALS center revealed that among 70 patients with a pathologic diagnosis of inclusion body myositis 13% had an original diagnosis of MND, and most had some degree of finger flexion weakness (Dabby et al., 2001). Two cases initially diagnosed as ALS eventually progressed to have clinical and pathologic features of inclusion body myositis upon follow-up examination (Schellenberg, Johnston, Kalra, Resch, & Johnson, 2010).

SPONDYLOTIC RADICULOPATHY AND MYELORADICULOPATHY

Cervical disk disease is reported in more than 50% of middle-aged people, but only 10–15% has symptoms for which surgery is a consideration, and most of these present with limb pain. In the setting of possible ALS, cervical and thoracic myelopathy could account for upper motor neuron (UMN) and lower motor neuron (LMN) symptoms and signs, whereas with possible ALS or PMA, cervical and lumbosacral spondylotic radiculopathies could account

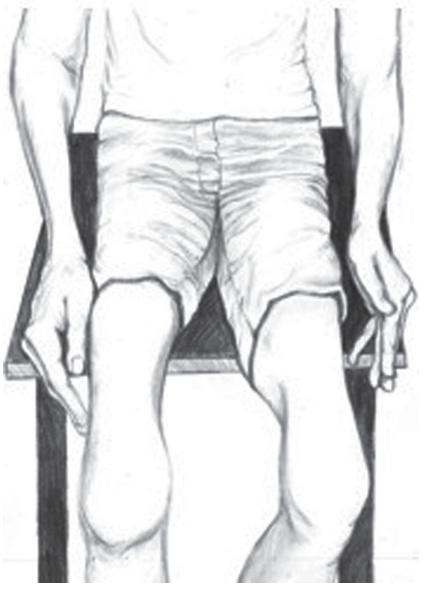

Figure 10–5. Drawing of prototypic pattern of muscle atrophy with inclusion body myositis. Forearm flexor muscle atrophy in asymmetric pattern preventing adequate grip due to weak finger flexion.

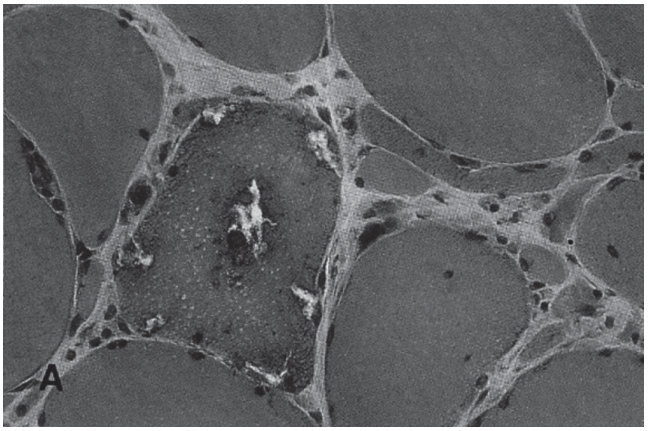

Figure 10–6. Muscle biopsy in cross-section showing variation in fiber diameter and inclusions (trichrome stain).

for LMN symptoms and signs. Imaging studies in one group of 63 patients raised the question of a cervical myeloradiculopathy in 48%, lumbar spondylosis in 13%, and other imaging abnormalities in 12.6% (Yamada, Furukawa, & Hirohata, 2003). In one center's review of 1,131 patients with ALS, 47 (4.2%) had one spinal decompression surgical procedure and 8 had a second procedure before the diagnosis of ALS was firmly established (Yoshor, Klugh III, Appel, & Haverkamp, 2005). Among the 47 patients, 29% had a myelopathy and 56% had cervical surgery and 4% thoracic surgery, whereas 21% had a unilateral foot drop and 42% had lumbosacral surgery. For 86% there was no improvement from the surgery, for 9% there was equivocal improvement, and for 4% there was objective improvement. However, for all patients there was overall progression and survival statistics were no different between the 1,084 who did not have surgery and the 47 who did have surgery.

MONONEUROPATHIES

Atrophy and weakness of muscles in the lateral hand occurs early in the course of ALS, and is called the splint hand syndrome (Menon, Kiernan, Yiannikas, Stroud, & Vucic, 2013). This includes median innervated thenar eminence muscles, and an initial diagnosis of a median nerve mononeuropathy, with or with out symptoms of carpal tunnel syndrome, is frequently considered. In a study of 554 patients with ALS 10 (2%) were initially diagnosed with a median neuropathy at the wrist and had a surgical release (Kollewe et al., 2011). Ulnar nerve decompression or transposition was less frequent. The 2% incidence of median neuropathy at the wrist/carpal tunnel syndrome among patients with ALS was similar to that in the general population. The issue of a peroneal (fibular) mononeuropathy to explain a progressive foot drop is rarely raised.

MYASTHENIA GRAVIS

Bulbar myasthenia gravis frequently causes flaccid-type dysarthria and dysphagia with some similarities to bulbar-onset ALS. However, bulbar myasthenia gravis remains localized, or includes ocular symptoms, and is not associated with atrophic limb weakness or signs of neurogenic denervation on EMG study. In one study of 49 patients with bulbar-onset ALS, 22% had acetylcholine receptor antibody testing prior to referral to an ALS center (Turner et al., 2010). Furthermore, 43% were initially referred to an otolaryngology clinic and 42% to a stroke clinic. Elevated acetylcholine receptor antibody titers are occasionally reported in patients with ALS and no signs of myasthenia gravis (Mehanna, Patton Jr., Phan, & Harati, 2012).

HEREDITARY SPASTIC PARESIS

Clinical features of hereditary spastic paresis and primary lateral sclerosis (PLS) overlap,

especially when there is no apparent family history of spastic paresis (Fink, 2001). A review of patients genotyped for one of several forms of hereditary spastic paresis (*SPG4* or *SPG7*) permitted comparisons between patients with PLS or with predominant UMN findings (Brugman et al., 2009). Features previously thought associated with PLS and not hereditary spastic paresis, such as older age of onset, bulbar involvement, minor needle EMG changes, urinary urgency, and mild dorsal column involvement, were also observed in hereditary spastic paresis. The only feature unique to hereditary spastic paresis was cerebellar signs (signs not described). It was concluded that genetic testing for hereditary spastic paresis is the only reliable method to distinguish between the two disorders.

LYME DISEASE

Chronic infection with *Borrelia burgdorferi* (Lyme disease) has been raised as mimicking symptoms and signs of ALS. A chart review of 414 patients with ALS indicated four with positive Western blot testing, a prevalence of 0.97% compared with 5.8% who had only serologic evidence for past exposure (Qureshi, Bedlack, & Cudkowicz, 2009). The prevalence rate was comparable with the occurrence of Lyme disease in the endemic area. Two of the four patients had antibiotic treatment with no effect on their ALS symptoms.

VIRAL DISEASES

Poliomyelitis and West Nile viruses are the most common viral-mediated LMN disorders (Leis & Stokic, 2012). Flaccid weakness for both has acute onset, distinguishing it from PMA or ALS at onset. The possibility of viral persistence and recrudescence has been raised for polio as a predisposing factor for the later development of ALS or PMA Distinguishing between post-polio progressive muscular atrophy is occasionally raised (Shimada, Lange & Hays, 1999).

RETROVIRAL DISEASES

A viral factor has been considered in the etiology of ALS. An MND-like syndrome has been reported in individuals with human immunodeficiency virus (Verma & Berger, 2006). In most examples the clinical features are consistent with El Escorial Criteria probable or possible ALS. However, the age of onset of MND-like symptoms was younger (mean ~35 years vs. ~55 years) and the rate of progression frequently much more rapid than for classic ALS. Some individuals responded to antiretroviral therapy, and the age of onset among those that did not respond to treatment was in the sixth decade raising the possibility of coincidence of two unrelated disorders.

Human T-lymphotropic virus types I and II are associated with a myelopathy/tropical spastic paraparesis but there have been several case reports of an ALS-like syndrome (Silva et al., 2005). The course has been slower and pathologic findings different than classic ALS.

HEAVY METAL TOXICITY

Lead, mercury, and selenium have been questionably linked to ALS, and early cases described by Aran in 1850 included two patients who were exposed to lead. A review of 10 case-controlled studies investigating a possible relationship between lead exposure and ALS found no consistent evidence among the studies for a relationship (Callaghan, Feldman, Gruis, & Feldman, 2011). It is possible that genetic susceptibility to lead binding may be a factor (Kamel et al., 2003). Of interest, one study found a longer 5-year survival among patients with ALS with an exposure to lead (Kamel et al., 2008). No evidence for mercury or selenium exposure was found (Callaghan et al., 2011).

CANCER AND PARANEOPLASTIC SYNDROMES

The linkage between ALS and cancer has been studied epidemiologically using National Cancer Institute data that included 2,700,000 incident cancer cases (Freedman et al., 2013). The risk of death due to ALS among cancer survivors was not increased overall, but there were positive associations between ALS death and melanoma. A register-based study from Sweden

did not confirm an association of ALS with melanoma or another form of cancer (Fang et al., 2013). Paraneoplastic antibodies have not been linked to classic ALS (Sharp & Vernino, 2012).

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Chapter 11

Motor Neuron Disease Progression

Mark B. Bromberg

BACKGROUND AND NEW POINTS

All forms of motor neuron disease (MND) are progressive, and amyotrophic lateral sclerosis (ALS) and progressive muscular atrophy (PMA) are more rapid than primary lateral sclerosis (PLS). Measurements of progression are important as endpoint measures for drug and intervention trials in ALS, and also for patient prognosis for all forms of MND. Primary endpoint measures for ALS trials initially focused on survival, and later on functional scales that incorporate the heterogeneous patterns of involvement and progression among patients.

Recent efforts to chart progression include prognosis based on region of onset, El Escorial Criteria at time of diagnosis, and type of MND. Clinimetric scales have generally evolved away from strength measures and toward global functional scales or electrophysiologic tests that assess lower motor neuron (LMN) loss. New techniques have been introduced that include brain magnetic resonance imaging (MRI) for upper motor neuron (UMN) loss and muscle imaging for LMN loss, ultrasound imaging of muscles, and electrical impedance myography for LMN loss. Clinical staging schemes for ALS progression have been introduced.

INTRODUCTION

An invariant feature of all forms of MND is inexorable progression. Clinical progression can be assessed from a number of perspectives: (1) time from symptom onset to diagnosis; (2) time and pattern of progression from the initial site of weakness to other regions; (3) time to progress from one type of MND to another (from progressive bulbar palsy [PBP] to ALS, from PMA to ALS, from PLS to ALS, and within the El Escorial Criteria from one degree of certainty to another); (4) global rate of progression (functional scales); and (5) time from symptom onset to death. Most

measures are used for research, and in particular, for clinical trials, and although scales are frequently used to monitor progression in the clinic they are uncommonly used for prognostication. Efforts are being made for scales that stage ALS.

CLINICAL PROGRESSION

ALS

Symptoms in ALS start focally, and combining three studies, 19–35% of patients experience symptoms beginning in the bulbar region, 58–74% in the limbs, and 7% diffusely (Traynor et al., 2000; Chio et al., 2002; Zoccolella et al., 2006). Among patients with bulbar onset, 75–88% starts with dysarthria. Among those with limb onset, 39–58% starts in the legs, 30–42% in the arms, and the remainder starts in both. Asymmetry in limb onset is common, and among 153 patients diagnosed with definite or probable ALS by El Escorial Criteria, 80% experienced asymmetry with onset in the arms and 76% with onset in the legs (Korner et al., 2011). ALS progression and comparisons of regions of involvement from diagnosis to last follow-up indicate a 19% increase in involvement of the bulbar region, 16% increase in the cervical region, 22% increase in the thoracic region, and a 15% increase in the lumbosacral region. At death, most patients had involvement of all regions.

Degree of involvement and progression has been examined in terms of the El Escorial Criteria. In a study of 383 patients, at time of diagnosis, 34% met criteria for definite ALS, 23% for probable, 36% for possible, and 8% for suspected ALS (Traynor et al., 2000). At last follow-up (median, 15.0 months; range, 0.4–68.9 months) 67% met criteria for definite ALS, 19% for probable, 12% for possible, and 2% for suspected ALS. Of note, the greatest changes were from the possible ALS category upward and in the definite category.

Time from symptom onset to diagnosis has also been examined. In a study that included 201 patients from six countries, the median time from symptom onset to diagnosis was 14 months (range, 12–17 months; Chio, 2000). In a study of 383 patients from Ireland, the median time was 8.0 months (range, 1.0–79.7 months). A study of 1,359 US veterans (greater than 97% male) revealed that the mean time to diagnosis (including evaluations within and outside of Veterans Affairs Medical Centers) was 11 months (first quartile = 6; third quartile = 20; Khishchenko et al., 2010). Of note, the Veterans study included records from 1957 and showed a trend over time for a shortening of the diagnostic process. General factors influencing the interval to diagnosis are the time to see a consultant (60% see a general practitioner first) and delay in seeing a neurologist. However, when a neurologist was the first consultant the median time remained long at 17.3 months (Chio, 2000). Patient-specific factors are site of initial symptoms, and those with bulbar symptoms were diagnosed in 14 months, whereas those with leg onset were diagnosed in 20.8 months. It is noteworthy that patients who experienced fasciculation potentials were diagnosed a mean of 16.9 months compared with those without fasciculation potentials at 19 months. Older patients experienced a longer delay. Patients not meeting El Escorial Criteria, presumable mostly with PMA, also experienced a longer delay (Chio, 2000; Traynor et al., 2000).

Mortality data combining seven population-based studies (1,740 patients) indicate a median survival of ~29 months, with very little variability among studies (Traynor et al., 2000). When survival is plotted by El Escorial classification at time of diagnosis, survival among the groups is similar. Data from two studies show survival time of 23–27 months for those with definite ALS, 27–34 months with probable ALS, 30–35 months for possible, and 40–58 months for suspected ALS (Traynor et al., 2000; Chio et al., 2002; Fig. 11–1).

Clinical prognostic risk factors in ALS vary among studies, likely in part due to different types of databases and sample sizes (Chio et al., 2009). Patient age at time of diagnosis is a factor, with shorter survival (median, 1.7 years) after age 80 years (Forbes, Colville, & Swingler, 2004). Gender does not have an affect on survival (Chio et al., 2009). Bulbar onset is a negative factor at any age (Chio et al., 2002). Patients with language-dominant frontotemporal features have a shorter median survival than those with behavior-dominant features (Coon, Sorenson, Whitwell, Knopman, & Josephs, 2011).

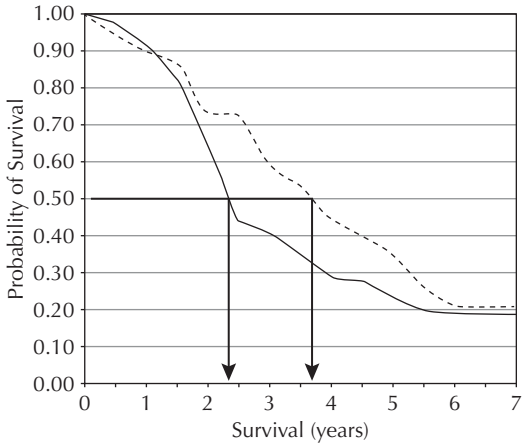

Figure 11–1. Kaplan-Meir survival curves for El Escorial Criteria definite ALS (solid line) compared with suspected ALS (dashed line) based on the initial diagnosis. Median survival not statistically significantly different for definite ALS 27 months and for suspected ALS 40 months. (Data estimated, simplified, and redrawn from Traynor et al., 2000.)

There are several clinically unique MND phenotypes that have longer survival; those with marked UMN involvement and those with marked LMN involvement affecting primarily the arms or the legs (flail arm or leg syndrome). In a study of 749 patients who fulfilled El Escorial Criteria for definite, probable, or laboratory-supported probable ALS, 163 (22%) had a predominance of UMN symptoms and signs (UMN-dominant), and as a group had significantly longer survival than patients with classic ALS (56 months vs. 33 months; Sabatelli et al., 2011). In another study of 1,620 patients fulfilling Airlie House Criteria for definite, probable, and possible ALS, 16.5% had flail arms and 6.3% had flail legs associated with significantly longer survival (61–71 months) compared with classic ALS (31–35 months; Wijesekera et al., 2009). However, when patients present with predominant UMN or focal LMN involvement a high percentage follow a more typical ALS-type pattern of involvement and rate of progression.

PBP

Most patients presenting with PBP progress to ALS (Karam, Scelsa, & Macgowan, 2010). Among 15 patients with PBP, defined as isolated dysarthria, dysphagia, or both, with or without UMN signs, and no signs of limb muscle denervation upon needle electromyography at initial evaluation, 13 progressed to El Escorial definite ALS with a median time course of 12 months (range, 6–27 months). Of the two patients who did not progress, one died due to other medical conditions and the other died due to aspiration pneumonia. The median survival for the 15 patients was 35 months (range, 24–116 months).

PMA

The initial diagnosis of PMA represents ~7% of patients with MND. In a study of 91 patients with PMA compared with 871 patients with ALS, those with PMA were older and more likely to be male. PMA seems to cause diagnostic uncertainly because the time from symptom onset to diagnosis was longer for PMA, with a mean of 20.8 months, compared with 17.7 months for ALS.

Progression from PMA to ALS (development of UMN signs) occurred in 22% of patients with PMA over a median time of 8.5 months (range, 0.5–61.3 months), and most developed UMN signs within 24 months (Kim et al., 2009). In another study of 37 patients with PMA, 35% developed UMN signs, including two who developed pseudobulbar affect as the only sign of UMN involvement (Visser et al., 2007). The development of UMN signs and change to ALS did not affect survival compared with those who remained with the diagnosis of PMA. In a comparison with 871 patients with ALS, 91 patients with PMA experienced longer survival, up to 80 months, after which the rates were the same for both diseases (Kim et al., 2009). Among 10 patients with PMA who did not display UMN signs and were followed for 12 months, all showed progression of LMN loss but to varying rates, and the pattern of changes was similar to patients with ALS (de Carvalho, Scotto, & Swash, 2007).

PLS

PLS is rare, at less than 3% of patients with MND (Singer, Statland, Wolfe, & Barohn,

2007). Onset is between 45 and 54 years of age with initial spasticity of legs or less common bulbar dysfunction. Progression is slow and there are periods of stability. Survival statistics are better than those with ALS, but difficult to quantify due to the longer survival and fewer deaths: mean duration from nine studies is 10.2 years (range, 3–53 years; Singer et al., 2007). It is significant that signs of LMN loss on electromyography or muscle biopsy develop in 40–70% of patients with an initial diagnosis of PLS, and development of LMN signs occurs ~3 years into the course, but as long as 10 years from symptom onset (Le Forestier et al., 2001; Singer et al., 2005; Gordon et al., 2006; D'Amico, Pasmantier, Lee, Weimer, & Mitsumoto, 2013).

Based on the concept that PLS represents a spectrum from pure UMN loss to varying degrees of LMN loss, a clinical entity of UMN-dominant ALS has been put forward, and such patients seem to have a clinical course between patients with pure PLS and ALS (Gordon et al., 2006).

CLINIMETRIC SCALES

Progression can be quantified with clinimetric scales that numerically rate progression. Clinimetric scales can be indexes, rating scales, and other metrics that measure symptoms, physical signs, laboratory signs, or other distinct clinical phenomena. Clinimetric scales developed for ALS include measurements of strength, composite scales that are combinations of strength and function (which assess levels of functions of daily activities), functional scales, and electrophysiologic metrics (Table 11–1).

One characteristic of MND is heterogeneous site of initial weakness or dysfunction with progression to other sites. Accordingly, most clinimetric scales cover the spectrum of likely involvement leading to a total score, acknowledging normal values for some regions or functions early on that might have mild or subclinical involvement. Subscores from specific regions of involvement can be analyzed separately. Respiratory function and electrodiagnostic metrics have been used as single-item assessments.

Composite Scales

The Norris scale was introduced in 1974 and includes combined assessments of activities of daily living, physical activities involving basic degrees of strength, tendon reflexes, plantar responses, labile affect, spasticity, and fatigue. Scores range from 100 (normal) to 0 (lowest).

The Appel scale was devised in 1987 and includes combined assessments of bulbar function, forced vital capacity, qualitative measurements of arm and leg strength, quantitative measurements of grip and pinch strength, and physical activities of arm and leg function (Appel, Stewart, Smith, & Appel, 1987). The range in scores is 30 for a normal individual and 164 for someone who is maximally disabled.

The Tufts Quantitative Neuromuscular Examination (TQNE) was developed in 1988 with 28 items that include measurements of pulmonary function, oropharyngeal function

Table 11–1 **List of Scales Used to Estimate the Rate of Progression of ALS**

Clinimetric Scales	Assessed Modalities
Norris	Multiple modes
Appel	Multiple modes
TQNE	Multiple modes
MMT	Summed manual muscle strength
ALSFRS-R	Summed functions
Electrophysiologic Scales	**Assessed Modalities**
MUNE	Estimate of surviving motor units
MUNIX	Estimate of surviving motor units
Neurophysiologic index	Combination of multiple electrophysiologic measures

TQNE, Tufts Quantitative Neuromuscular Examination; MMT, manual muscle testing; ALSFRS-R, ALS Functional Rating Scale-Revised; MUNE, motor unit number estimation; MUNIX, motor unit index.

(pronunciation and timed activities), timed activities (hand function and walking), and measurements of maximal voluntary isometric contraction strength from a large number of muscles with conversion of scores to "megascores." Megascores are normalized to scores expected in normal individuals based on age and gender and expressed as *z* scores. TQNE testing required physical therapy expertise and expensive equipment stationed in a designated area. Recently, a portable system based on TQNE has been developed (Andres et al., 2012).

Summed isometric manual muscle testing, either qualitatively based on Medical Research Counsel ratings or quantitatively using handheld dynamometry, has been used as a technically simpler and less expensive alternative method to TQNE (Great Lakes ALS Study Group, 2003).

Motor Unit Number Estimation

Motor unit number estimation (MUNE) is an electrophysiologic method to estimate the number of motor neurons innervating a muscle or group of muscles, most commonly applied to hand muscles innervated by the median or ulnar nerves (Bromberg, 2007). MUNE is unique because it is unaffected by collateral reinnervation, which has the effect of blunting the fall in the compound muscle action potential (CMAP) with progressive axonal loss. Thus, MUNE can provide estimates of the number of surviving LMNs. There are several MUNE techniques, most of which yield roughly equivalent values, but there are operational issues that make direct comparisons between normal subjects and subjects with ALS difficult and rarely performed (Shefner, 2001; Bromberg, 2007).

MUNE in ALS has been used mostly as a secondary endpoint in drug trials (Shefner, Cudkowicz, Zhang, Schoenfeld, & Jillapalli, 2004). There are issues with the statistical technique (Shefner, Cudkowicz, Zhang, Schoenfeld, & Jillapalli, 2007). A MUNE modification combining two techniques, the incremental and multipoint techniques, has been proposed for clinical trials but has not yet been used (Shefner et al., 2011).

Motor Unit Number Index

Motor unit number index represents a mathematic modeling of the interference pattern with respect to the power of the CMAP, and is sensitive to change over time among patients with ALS (Neuwirth, Nandedkar, Stalberg, & Weber, 2010). It is simple to perform and multiple muscles can be assessed and summed into a combined score that includes heterogeneous rates of LMN loss within a patient (Bromberg, 2013).

CMAP Scan

The amplitude or area of the CMAP is a measure of the number of motor axons innervating a muscle, but not a quantitative measure in ALS. In health individuals, plots of CMAP response with increasing electrical activation of a motor nerve results in a smooth sigmoid curve, but in patients with ALS the curve is lower in amplitude and interrupted by steps due to the enlarge motor units. Estimates of motor neuron loss can be made with this technique (Maathuis, Drenthen, van Doorn, Visser, & Blok, 2013).

Neurophysiologic Index

The neurophysiologic index is a composite scale of several neurophysiologic metrics that includes the maximal CMAP amplitude divided by the distal latency multiplied by the F-wave frequency to 20 shocks (Swash & de Carvalho, 2004). The neurophysiologic index is straightforward to apply and shows change in ALS over time (de Carvalho, Scotto, Lopes, & Swash, 2005).

Electrical Impedance Myography

Electrical impedance of muscle is a noninvasive technique that assesses the changes in composition and architecture, including atrophy and reinnervation, edema, and changes in endomysial connective tissue and fat (Rutkove, 2009). The changes are not specific for an underlying process, but impedance values

are different in ALS muscle compared with control muscle. In a multicenter trial of 60 patients with ALS followed for 1 year, impedance values changed and the rate of change in the most rapidly progressive muscles was faster than other clinical measures (Rutkove et al., 2012).

Muscle Ultrasound

Ultrasound is a noninvasive means to assess echogenic properties and thickness of individual muscles and detect the presence of fasciculation potentials. A 6-month longitudinal study of six muscle groups in 31 patients with ALS showed changes in echogenic properties and thickness, but the changes varied among patients (Arts, Overeem, Pillen, Schelhaas, & Zwarts, 2011). Changes in ultrasound values over time were not correlated with clinical measures of strength and the ALS Functional Rating Scale-Revised (ALSFRS-R) scores.

MRI

Muscle MRI is a noninvasive means to assess brain and muscle, and a variety of analysis techniques are being developed and applied to determine onset of corticospinal tract changes in the brain and loss of muscle thickness. Diffuse tensor imaging (DTI) is one technique and fractional anisotrophy (FA) is a quantitative metric (Turner et al., 2011). Efforts are underway to use MRI as a biomarker of UMN loss to aid in the diagnosis, but general clinical usefulness has not been achieved. Another imaging technique under exploration is functional MRI to assess interhemispheric connections that are lost with the disease process.

Given the variety of techniques used, results with DTI and FA, and variations among individual patients with ALS, there is variability among MRI studies. A meta-analysis has been conducted yielding 11 suitable studies that include comparisons between patients with ALS and control subjects (Foerster et al., 2013). The analysis concluded that at this time DTI with FA has relatively low diagnostic accuracy as an independent marker of corticospinal tract loss compared with healthy control subjects.

MRI has been used to chart changes due to muscle atrophy over time. A small study of normal subjects and four patients with ALS were imaged five times (tongue, first dorsal interosseous, thenar, anterior tibialis muscles) at 3-month intervals (Jenkins et al., 2013). Muscle volume did not change in normal subjects, but did change among ALS subjects, with differences in volume among ALS subjects. Over time, there were trends for volume loss, but not significant for some muscles (thenar, anterior tibialis), and no change for other muscles (first dorsal interosseous, tongue). Thus, the technique was not sensitive to subclinical changes.

ALSFRS

The ALSFRS (Cedarbaum & Stambler, 1997), and recently a revised version (ALSFRS-R; Cedarbaum et al., 1999), is the most commonly used measures of ALS progression. The ALSFRS-R was derived from the older ALS Severity Scale, but with a reduced list of possible functional ratings for five domains of function: (1) bulbar, (2) upper extremity, fine motor control, (3) lower extremity, gross motor control, (4) gait, and (5) respiration (expanded upon in the revised scale). For each domain, there is a 4 (normal function) to 0 (no function) scale leading to a maximal score (normal function) of 44. An advantage of the ALSFRS-R scale is that it is based on assessments of functions that can be made by the patient (the original intent) or by a caregiver or nurse (Miano, Stoddard, Davis, & Bromberg, 2004), and can be administered over the telephone (Kaufmann et al., 2007) or retrospectively from clinic notes (Lechtzin et al., 2009). Interestingly, a small number of patients with ALS who are profoundly weak, but alive, have a score 0 on the ALSFRS-R scale. An extended scale (ALSFRS-EX) has three additional items that focus on ability to (1) manipulate devices with fingers, (2) communicate by facial expression, and (3) get around inside the house (Wicks, Massagli, Wolf, & Heywood, 2009).

A clinically meaningful change in ALSFRS-R score has been determined by a survey of ALS care providers (Castrillo-Viguera, Grasso, Simpson, Shefner, & Cudkowicz, 2010). The consensus was that a 20–25% change in the

slope of the ALSFRS-R over time would be clinically meaningful.

Efforts have been made to remove confounding factors from the ALSFRS-R, such as the positive effects of interventions (reducing sialorrhea by medications) and patient refusal for certain interventions, and this has resulted in an abbreviated scale retaining six questions, ALSFRS-6 (Box 11.1; Kasarkis, Kryscio, Mendiondo, Manamley, & Moore, 2012). The ALSFRS-6 was correlated with the full scale and conveyed the same prognostic significance for survival.

The ALSFRS-R scale has been questioned for a single score to accurately reflect different dimensions or functions (bulbar, motor, and respiratory; Franchignoni, Mora, Giordano, Volanti, & Chio, 2013). There are different clinical meanings to metrics from the three dimensions, and possible different linguistic, cultural, or technical meanings, and thus the ALSFRS-R should be considered as a profile of scores from the three domains and not as a single or global score.

QUANTITATING PROGRESSION

ALS is an inexorably progressive disease and all scales of function or strength fall with time. Of note, there are no specific scales for PLS and PMA, but the ALSFRS-R is suitable for PMA (de Carvalho et al., 2007). Most quantitative scales are used in research, and the major issues relate to ease and expense, reproducibility, and correlations among scales. The ALSFRS-R is frequently used in clinical care.

For most strength- and function-based scales (Appel, TQNE, ALSFRS-R), rates of progression for an individual are linear but there is a 20- to 30-fold difference in rates among patients. Because the ALSFRS-R scale is the most commonly used scale, it has received the most attention. The Appel and ALSFRS scales are highly correlated, but there may be differences between scales within portions of the time course (early, mid, and late portions) with the notion that the Appel scale with its greater number of measures detects subtle changes (Voustianiouk et al., 2008). Rates of progression are generally linear, although a degree of nonlinearity occurs early and late (Gordon et al., 2010).

Neurophysiologic scales have been used as a measure of LMN loss, and all show changes over time. A recurring question is which scale is most sensitive for change: most comparisons of individual neurophysiologic scales are with the ALSFRS-R but few are comparisons among neurophysiologic scales.

MUNE and the neurophysiologic index show rates of change that are more sensitive than strength- and function-based scales. MUNE has been applied to determine the rate of LMN loss, and when the statistical technique has been applied to a large number of subjects (100–163) followed in clinical drug trials over 6–12 months, estimate values from intrinsic hand muscles show a steady decline over time (Shefner et al., 2004, 2007). Motor unit number index also shows changes in ALS over time and more rapidly than the ALSFRS-R scale (Neuwirth et al., 2010). The neurophysiologic index is also sensitive to change over time (de Carvalho et al., 2005).

Box 11.1 ALSFRS-6 Subset From the ALSFRS-R

Question 1: Speech
Question 4: Handwriting
Question 6: Dressing and hygiene
Question 7: Turning in bed
Question 8: Walking
Question 10 Dyspnea

From Kasarkis et al. (2012).

Electrical impedance myography changes more rapidly than handheld dynamometry measures of strength or the ALSFRS-R scale (Rutkove et al., 2012).

ALS STAGING

Efforts have been made to establish stages of ALS as a measure of progression and for analysis of changes related to time from one stage to another and to disabilities and associated costs at each stage. One effort is based on progressive impairment in new body regions: first, second, and third body regions and need for nutritional and respiratory interventions (Table 11–2; Roche et al., 2012). Another is based on sequential loss of independence in functional regions: loss of ambulation, loss of speech, loss of swallowing, marked respiratory impairment (Table 11–2; Chio, Hammond, Mora, Bonito, & Filippini, 2013).

PREDICTION OF PROGRESSION AND SURVIVAL

Efforts have been made to predict patient survival based on data on rates of change from clinical and clinimetric studies. For example, from a study of 150 patients with estimates based on site of disease onset and rate of spread to other regions, there are lower survival rates for (1) patients with early bulbar involvement, (2) when two regions are simultaneously affected, and (3) when the second region is affected within 3 months of the first (Fujimura-Kiyono et al., 2011). Another study based on retrospective data from 713 patients with ALS in an ALS registry used multivariate analysis to identify predictive variables easily gathered at the time of diagnosis (Scotton et al., 2012). The prognostic categories were "good," "moderate," "average," and "poor" survival, and based on 95% confidence limits. The predictive variables were age at symptom onset

Table 11–2 **Two Staging Outlines for ALS**

A: Stage	Clinical Milestone	Percent of Disease Course
Stage 1	Involvement of first region = symptom onset	0%
Stage 2A	Diagnosis	33%
Stage 2B	Involvement of second region	38%
Stage 3	Involvement of third region	61%
Stage 4A	Need for gastrostomy	77%
Stage 4B	Need for respiratory support = noninvasive ventilation	80%
B: Stage	**Clinical Milestone**	**Percent of Disease Course**
Stage 0	Functional involvement but no loss independence any domain	0%
Stage 1	Ambulation:	33%
⇓	Normal to impaired = 0	38%
⇓	Loss or no purposeful leg movement = 1	61%
Stage 2	Swallowing:	77%
⇓	Normal to impaired = 0	80%
⇓	Needs supplemental tube feeding = 1	
Stage 3	Communication:	
⇓	Normal to impaired = 0	
⇓	Speech combined with nonvocal communication and unable to write = 1	
Stage 4	Dyspnea:	
⇓	None to mild = 0	
⇓	At rest or continuous noninvasive ventilation at night = 1	
Stage 5	Death	

A: Based on progression of dysfunction from one body region to another, with average percent time spent in each stage based on 100% equals total survival time. Modified from Roche et al. (2012). B: Based on loss function (score increasing from 0 to 1) of clinical milestones derived from portions of the ALSFRS-R scale. Modified from Chio et al. (2013).

(older worse), delay in diagnosis from symptom onset (shorter time worse), El Escorial Criteria category at first examination (definite category worse than others), and whether taking riluzole (worse taking riluzole).

The ALSFRS-R scale has been used to predict survival, based on the score at the initial clinic visit, and shows a shorter survival for patients were have scores below the median score (Kaufmann et al., 2005). The respiratory subscore was the strongest predictor.

To circumvent factors that influence when a patient is first assessed, linear estimates of the rate of disease progression have been explored as a method to estimate survival (Armon et al., 2000). Linear estimates are based on the slope of a line determined by two points over time: one point is known, and is temporally the second of the two and represents measured datum, whereas the other point is estimated. Where the calculated slope intersects the "zero" point defines the time of survival. Modeling has been performed with forced vital capacity and single-item quantitative measures of strength.

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Chapter 12

Epidemiology of Amyotrophic Lateral Sclerosis

Adriano Chiò and Giancarlo Logroscino

BACKGROUND AND NEW POINTS

The epidemiology of amyotrophic lateral sclerosis (ALS) has provided insight into many aspects of the disease, including geographic distributions, possible causative features, costs, and social factors. Epidemiology can also describe prognostic factors. Recent epidemiologic efforts are directed to prognostic factors, frontotemporal dementia (FTD), and the distribution of genetic forms.

INTRODUCTION

ALS is a relatively rare disease, but with a strong impact on the life of patients, their families, and the society as a whole. It has been estimated that its annual cost is more than $1 billion in the United States and more than €2 billion in Europe, including direct medical and nonmedical costs and loss of income, more than any other neuromuscular disease (Gustavsson et al., 2011; Larkindale et al., 2013), However, its social, psychological, and personal costs largely extend over financial costs, including the life of the patients' family members (Chiò, 2010). Understanding the epidemiology of a disease such as ALS is therefore relevant for several reasons. First, it helps to define the magnitude of the phenomenon, making it possible for policy makers to allocate resources for health care and social services. Second, it allows health professionals to wisely plan patients' care over the different phases of the disease. Third, it helps patients and their families to plan their future life. Finally, it represent an invaluable resource for designing future research, including meaningful clinical trials.

This chapter focuses on the descriptive epidemiology and prognostic factors of ALS. Besides classic epidemiology, two emerging issues are particularly considered: the epidemiology of

the ALS-FTD spectrum, which has seen a major growth of interest after the identification of cognitive changes in 20–50% of patients with ALS (Phukan et al., 2012); and genetic epidemiology, which is rapidly developing after the discovery of several genes related to ALS and its oligogenic nature (van Blitterswijk et al., 2012; Renton, Chiò, & Traynor, 2014).

DESCRIPTIVE EPIDEMIOLOGY

The last decade has seen an extraordinary increase in methodologically well conducted studies on the descriptive epidemiology of ALS.

Methodologic Considerations

Epidemiologic studies can be classified into two broad categories: retrospective studies, based on the identification of ALS cases from referral (tertiary) centers and current dataset (e.g., hospital discharge archives and certificates of death); and prospective studies, based on an active search of cases over time, using all available concurring sources and a direct validation of diagnoses. The retrospective design has several pitfalls: (1) it is less accurate with regard to certainty of disease diagnosis, because it is usually based on the retrospective revision of clinical data; (2) there is a risk of missing specific subsets of patients not captured by the study design; and (3) it does not allow collection of demographic and clinical information using a standardized methodology.

Prospective population-based registers using multiple sources of information to ensure complete case ascertainment within a defined geographic area have been published. These studies represent an advancement of the classical epidemiologic studies and are considered the best available methodologic design for the study of rare disorders. They have the advantage of allowing the application of uniform and definite diagnostic criteria and permit the assessment of patients in a standardized manner. Most importantly, the completeness of case ascertainment permits the analysis of the full clinical spectrum of the ALS population, in particular the old or very old cases, as demonstrated by the older mean age of onset observed in the registry cohorts compared with that of both retrospective epidemiologic series and referral series. The drawbacks of epidemiologic registries are the costs of a prospective follow-up and the complexity in organization and coordination.

Incidence

ALS incidence has been extensively studied in the last decades in developed countries, but few data are available on other countries (Cronin, Hardiman, & Traynor, 2007; Chiò et al., 2013). Most published studies refer to European populations. Overall, the median crude incidence of ALS in Europe was estimated to be 2.08 (interquartile range [IQR], 1.47–2.43), with a wide range, from 0.5 per 100,000 population in Belgrade, Serbia (Alcaz et al., 1996) to 3.6 per 100,000 in Faroe Islands (Joensen, 2012). European studies based on prospective ALS registers, which use a similar methodology of case ascertainment and the same diagnostic criteria for ALS, report higher and more homogenous incidence rates (Logroscino et al., 2010). The relatively few data available on North America indicate a median crude incidence rate of 1.75 per 100,000 population (IQR, 1.73–1.78), similar to the estimated incidence in Japan (1.97; IQR, 1.70–2.23; Kihira et al., 2005; Chiò et al., 2013). Overall, it can be estimated that every year ~18,000 subjects develop ALS in Europe and 6,000 in the United States and Canada (Table 12–1). Studies directly comparing the incidence of nonwhite and white populations in the United States indicate a lower incidence among African-American and Hispanic populations than whites (McGuire, Longstreth Jr, Koepsell, & van Belle, 1996), with the exception of the survey on Harris County, Texas, where crude incidence rates were similar in whites, African-Americans, and Hispanics but only in men (Annegers, Appel, Lee, & Perkins, 1991). There are virtually no epidemiologic papers from other ethnic groups (Cronin et al., 2007; Marin et al., 2012). Clinical papers on African or African-American populations indicate that the age at onset is lower and that there is possibly an increase of women in early onset ALS (<45 years) compared with whites (Kazamel et al., 2013).

Interestingly, besides methodologic consideration, ALS incidence rates seem to be significantly correlated to the median age (Chiò et al., 2013) or the life expectancy (Byrne, Jordan,

Table 12–1 **ALS Case Estimates for Incidence and Prevalence in the Total Population Using Descriptive Statistics From Included Studies by Geographic Areas**

	Incidence (I; per 100,000)			Prevalence (P; per 100,000)		
	IQ-25	**Median**	**IQ-75**	**IQ-25**	**Median**	**IQ-75**
Europe						
Statistics based on search results						
All European studies (*n* = 24-I/13-P)	1.47	2.08	2.43	4.06	5.40	7.89
Prospective (*n* = 12-I/7-P)	2.15	2.39	2.68	6.25	7.89	7.98
Retrospective (*n* = 12-I/6-P)	1.22	1.52	2.04	3.92	4.04	4.70
Estimated cases in Europe, *n*						
All European studies (*n* = 24-I/13-P)	10,852	15,355	17,938	29,971	39,863	58,244
Prospective (*n* = 12-I/7-P)	15,871	17,643	19,784	46,137	58,244	58,908
Retrospective (*n* = 12-I/6-P)	9,006	11,221	15,059	28,937	29,823	34,695
Total European population (2010): 738,199,000*						
North America						
Statistics based on search results						
All North American studies (*n* = 3-I/2-P)	1.75	1.80	2.02			
US studies (*n* = 2-I/2-P)	1.73	1.75	1.78	3.15	3.40	3.65
Canadian study (*n* = 1-I/0-P)		2.24				
Estimated cases in North America, *n*						
All North American studies (*n* = 3-I/2-P)	6,027	6,199	6,957			
US studies (*n* = 2-I/2-P)	5,370	5,432	5,525	9,777	10,553	11,329
Canadian study (*n* = 1-I/0-P)		762				
Total US and Canada population (2010): 344,401,000; US population (2010): 310,384,000; Canada population (2010): 34,017,000*						
China and Japan						
Statistics based on search results						
All Asian studies (*n* = 5-I/4-P)	0.40	0.60	1.43	1.44	2.34	5.13
Chinese studies (*n* = 2-I/2-P)	0.38	0.46	0.53	1.48	2.01	2.54
Japanese studies (*n* = 2-I/1-P)	1.70	1.97	2.23		11.3	
Estimated cases in Asia, *n*						
All Asian studies (*n* = 5-I/4-P)	6,167	9,251	22,048	22,203	36,079	79,097
Chinese studies (*n* = 2-I/2-P)	5,097	6,170	7,109	19,852	26,961	34,070
Japanese studies (*n* = 2-I/1-P)	2,151	2,493	2,822		14,299	
Total China, Japan, and Iran population (2010): 1,541,845,000; China population (2010): 1,341,335,000; Japan population (2010): 126,536,000; Iran population (2010): 73,974,000*						

* http://esa.un.org/unpd/wpp/unpp/panel_indicators.htm
From Chiò et al. (2013).

Elamin, & Hardiman, 2013) of the underlying population. This indicates that with the increasing of the median age of a population, an increase of the incidence of ALS can be expected.

It is now generally accepted that ALS is an age-related disease (i.e., its incidence shows a peak in the eight decade of life in males and females with a subsequent decrease in older ages). All studies but one (Rochester) (Yoshida et al., 1988) agree on this concept (Logroscino et al., 2010). The implication of this concept is that ALS pathogenetic process is not related to the "aging" process (the old concept of "abiotrophy"), as possibly in Alzheimer disease (AD), but rather to pathogenetic noxae acting at certain ages. Some of these noxae are now clear,

in particular the genetic ones, whereas others are still under scrutiny (i.e., the environmental ones).

Prevalence

Prevalence of a disease is a relevant measure of the burden of the disease in a given population, and therefore it is particularly valuable for healthcare providers, government agencies, and insurers to establish the cost of a disease and allocation of resources. Despite its importance, prevalence rate has been more rarely estimated that incidence, and, more importantly, most estimations are biased by the short period of observation of cases, with a risk of underestimation of the real figures. On the basis of the European prospective studies, the crude point prevalence rate of ALS has been estimated to be 7.9 per 100,000 population (IQR, 6.3–8.0), giving an estimated number of patients with ALS of ~60,000 (Chiò et al., 2013; Table 12–1). According to North American studies, the prevalence rate is 3.4 per 100,000 (IQR, 3.2–3.7) with an estimated number of 10,000 ALS cases. Prevalence rate may change over time if more effective disease-modifying therapies become available, but also with the improvement and diffusion of the use of supportive therapies (i.e., enteral nutrition, noninvasive ventilation, invasive ventilation), which could increase the survival of patients with ALS.

Mortality

Because ALS is a fatal disorder, ALS mortality rate estimated using death certificates should be similar with incidence rates.

Death certificates could be a valuable source of information of ALS trends over time across different countries, because they are almost universally coded using the International Classification of Diseases of the World Health Organization. However, the coding changes over time (the last being the adoption of the 10th Revision), and there are no studies assessing the effect of the change of codes on the accuracy of single disease reports. Moreover, the accuracy of death certificates for ALS varies among countries, and possibly also over time, partly because of the variable indication of ALS as the underlying or contributing cause of death (Stickler, Royer, & Hardin, 2012). Overall, false-negative rates range between 10% and 20% (Yeo, Lynch, & Hardiman, 2010; Stickler et al., 2012), and there are virtually no data about false-positive rates of death certificates (i.e., the erroneous reporting of the code corresponding to ALS in patients who died from other disorders). In the United States, positive predictive value has been estimated to be as low as 65% (Stickler et al., 2012). A paper assessing 29 studies reporting ALS mortality rates found that only three compiled criteria for a high-quality methodology and showed good accuracy with regard to incidence rate (Marin, Couratier et al., 2011).

Most studies on ALS mortality, differently from those on ALS incidence, indicate an increase of mortality rates over time, likely reflecting an improvement of the accuracy of death certificates. However, a recent study on ALS mortality in the United States from 1999 to 2009 showed a steady rate of the disease, with a possible increase in those aged 20–49 and a decrease for persons 65 years of age and older (Mehal, Holman, Schonberger, & Sejvar, 2013). Another paper assessing ALS mortality in the 1995–2004 period in Japan found that mortality rates did not modify, while reporting a decrease for those older than 70 years (Doi et al., 2010).

Two studies using long-term mortality data assessed with an age-period cohort model in France and Denmark indicate a substantial increase of mortality rates in succeeding birth cohorts from 1880 to 1920 with a subsequent plateau (Gordon et al., 2011; Seals, Hansen, Gredal, & Weisskopf, 2013). However, these findings were not confirmed in a study on the Switzerland population (Ajdacic-Gross, Schmid, Tschopp, & Gutzwiller, 2012). If true, this finding may be interpreted as the effect of an environmental cause of ALS that became more common in the early decades of the 20th century and later decreased.

ALS TIME TRENDS

Disease time trends are important for two reasons: to improve health resource planning, especially in countries with a national health system; and to generate hypotheses on disease etiology. The identification of time

trends and the presence of different trends in different groups of subjects may help to identify areas for intervention for subjects in categories with higher risk of disease, or identification of prevention and modifiable risk factors.

Recent data have shown a change in trends in neurodegenerative diseases the last few decades and how change in risk factors may be responsible for these findings. A recent example comes from dementia studies. A recent community survey in three states conducted at the Mayo Clinic showed a decline of AD incidence in the last 20 years (Rocca et al., 2011). Similarly, in the Rotterdam Study the incidence of dementia in 2005 was lower compared with the incidence in 1990. A better control for vascular risk factors and vascular diseases has been hypothesized as responsible for the decline in AD incidence (Schrijvers et al., 2012).

Time trends in rare diseases as ALS are more difficult to study compared with common disease as AD because of the lack of data collected in the same source population for a sufficient time period (at least one decade).

ALS prevalence and incidence is generally higher in more recent studies compared with studies conducted before the 1990s. In a recent meta-analysis a clear trend between rate of prevalence and incidence and year of study publication has been shown. In particular, the incidence and prevalence rates were higher for studies conducted after the publication of the El Escorial criteria in 1994 (Chiò et al., 2013). Definition of diagnostic criteria and improvement of case-search strategy are the main contributors of this apparent rise in ALS frequency in more recent studies.

There are, however, only a few studies conducted in the same population with the same methodology over a long time period and all are population-based studies conducted in Europe: Piemonte and Valle d'Aosta ALS register shows a stable incidence around 2.9 per 100,000 in a 10-year period from 1995 through 2004, using capture-recapture method (Chiò, Mora et al., 2009). The Irish registry show a stable incidence between 1995 and 2004 around 2 per 100,000 (O'Toole et al., 2008), whereas the Scottish registry shows around 2.4 per 100,000 between 1989 and 1998 (Forbes, Colville, Parratt, & Swingler, 2007). The only data available outside Europe are from the Epidemiological Rochester Project in Minnesota where a stable but much lower incidence (1.7 per 100,000) was found over a period of almost 80 years between 1925 and 1998 (Chiò et al., 2013).

Mortality studies compared with registries and other types of prospective incident studies have the advantage to investigate longer periods of time and are therefore well suited to investigate time trends. The main disadvantages are the lack of control of the quality of diagnosis and the absence of clinical information about individual subjects.

In a study conducted in Norway death certificates in the period 1961–1994 for ALS were collected from the office of Central Statistics (Seljeseth, Vollset, & Tysnes, 2000). The annual mortality of ALS almost doubled during the study period, going from 1.4 to 2.5 per 100,000 inhabitants at the end of the study. The increased mortality was present in the population older than 60 years of age, with a peak in mortality in the age group 80–84 years. Another mortality study conducted in the United States showed an increase in overall mortality rates from 1.2 to 1.8 per 100,000, representing an almost 50% increase during the 30-year period (Noonan, White, Thurman, & Wong, 2005). In both studies most of the increase is among women (between 40 and 60%). Based on the results of the mortality studies the increase in ALS mortality is mainly age related. The increase of death among the population older than 70 in the Norwegian study is about 275% but the increase of that elderly segment of the population is about 70%. Therefore, other factors beyond the demographic changes may play a role: an increase in resources and ability in identifying ALS cases with a more significant diagnostic improvement among the elderly.

In conclusion, population-based studies show a stable frequency of incident ALS (Fig. 12–1), whereas mortality studies show an increase of cases mainly attributable to aging and an increase of subjects at risk among the elderly.

GENETIC EPIDEMIOLOGY

The rapid discovery of novel genes related to ALS in the last few years has largely modified the clinical approach to patients but also

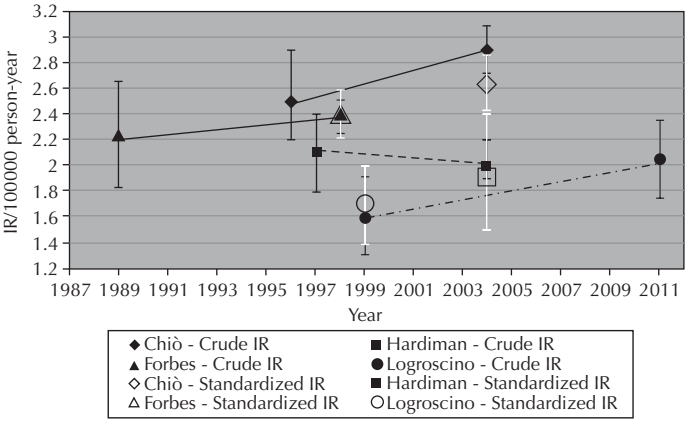

Figure 12–1. Variation of amyotrophic lateral sclerosis incidence over time in population-based study (time trend).

influenced the perception of ALS epidemiology. Information is accumulating concerning the ethnic and national differences in the distribution of major ALS genes mutations and of minor genes influencing ALS age at onset and clinical course.

However, only a few papers have systematically assessed ALS genetics in population-based series. In the first of such papers, based on the Piemonte and Valle d'Aosta ALS register in Italy, patients diagnosed between 2007 and 2011 were included. A total of 51 out of 475 patients carried a genetic mutation, *C9ORF72* being the most frequent mutation. Besides, in one-third of patients with a positive family history for ALS or FTD, no genetic mutation was found. Subsequently, other population-based series have been published from Ireland and the Netherlands (Kenna et al., 2013; van Blitterswijk et al., 2011; Table 12–2). Interestingly, genetic findings were rather different: for example, no cases with *SOD1* mutations were identified in Ireland, whereas *SOD1* mutations are quite rare in the Netherlands. Genetic isolates with peculiar gene frequency are also emerging. A large series from Finland demonstrated that about two-thirds of patients with ALS are characterized by two single genetic mutations, homozygote p.D90A *SOD1* and *C9ORF72* (Laakssovirta et al., 2011). Similarly, in Sardinia, about two-thirds of familial ALS and 15% of sporadic ALS carry either the p.A383T missense mutation of the *TARDBP* gene or the GGGGCC hexanucleotide repeat expansion in the first intron of *C9ORF72* gene (Chiò et al., 2010; Chiò, Borghero et al., 2012; Sabatelli et al., 2012); patients with the p.A382T mutation carry a common haplotype, indicating a common founder (Chiò et al., 2010). This population, different from patients with different ancestry carrying the same mutation, is also characterized by a high frequency of comorbid FTD.

Some genetic mutations are characterized by a particular elevated frequency in restricted geographic areas. For example, *VAPB* mutations are exceptionally frequent in Brazil and

Table 12–2 **Genetic Epidemiology of ALS: Summary of Published Studies**

	# of Cases (fALS+sALS)	*SOD1*	*TARDBP*	*FUS*	*C9ORF72*	Other Mutations
Ireland	444	0	0.5%	0.5%	8.8%	4.4%[a]
Italy	475	2.1%	1.5%	0.2%	6.7%	0.2%[b]
Netherlands	1,289	0.2%	1.0%	0.7%	9.5%	0.6%[c]
Sardinia[d]	372	0.9%	23.6%	0	12.8%	0

fALS = familial ALS; sALS = sporadic ALS.
[a]SETX, 2.5%; ALS2, 1.6%; OPTN, 0.2%; VCP, 0.2%. [b]OPTN, 0.2%; no cases with ANG mutations were detected.
[c]ANG, 0.6%. [d]Unpublished data.

very rarely described elsewhere, while recessively inherited mutations of the *Alsin* gene (ALS2) are mostly found in populations with Arab/Maghreb ancestry (Renton et al., 2014).

Genetic epidemiology in ALS is still in its beginnings. Future research is needed to better understand the population-specific genetic characteristics of ALS and to improve genetic counselling.

COGNITIVE CHANGES AND MOTOR NEURON DEGENERATION IN POPULATION-BASED STUDIES

One of the most recent challenges in the study of neurodegenerative diseases is the identification of transition phenotypes that include nontypical clinical features of the disease. A good example is Parkinson disease; in the last 10 years a large part of the research has been developed in the area of nonmotor symptoms. Recently, new research diagnostic criteria have been proposed to include nonmotor symptoms in the identification of Parkinson disease cases (Berg et al., 2013). Nonmotor symptoms that may precede motor signs by many decades include cognitive and behavioral changes, and gastrointestinal, autonomic, and sleep disorders. Similarly, ALS has classically been described as a pure motor neuron disease. In the last decade several studies have shown that in ALS a set of nonmotor symptoms, especially in the area of cognition and behavior, are present. Although there are several studies on cognitive decline in patients with ALS in tertiary centers (Ringholz et al., 2005; Gibbons, Richardson, Neary, & Snowden, 2008) there are only a few studies conducted in population-based settings, all from the Ireland registry, a registry operating since 1995 and part of the EURALS collaboration. In a prospective population-based study of cognitive function, 160 incident Irish patients with ALS and 110 matched control subjects were visited at home and underwent an extensive neuropsychological testing (Phukan et al., 2012). All subjects were incident cases and tested within 1 year from diagnosis. About 50% of patients with ALS presented cognitive impairment; 15% were classified as demented according to the Neary criteria (Neary, Snowden, Northen, & Goulding, 1988). Patients were classified using modified cognitive impairment classification criteria (Strong et al., 2009). About 21% presented executive dysfunction (40% of patients who had no evidence of dementia) and another 14% presented nonexecutive cognitive impairment (mainly language but also memory). Interestingly, about 15% presented multidomain executive impairment.

In the same population-based study both FTD dementia and executive dysfunction were negative prognostic indicators for survivorship (Elamin et al., 2011). Only a portion of patients with ALS present cognitive impairment and 40% of patients with ALS are cognitively intact. Therefore, cognitive impairment in ALS is not a universal feature, and its manifestations are mainly executive. However, cognitive impairment may be more heterogeneous than previously recognized, including more posterior involvement. Further studies need to be conducted examining visuospatial abilities.

Comorbid FTD is a known negative prognostic indicator in ALS. From the Irish population-based studies, a new piece of information is added for prognosis. In patients with ALS without dementia, executive dysfunction, but not impairment in other cognitive domains, is an additional negative prognostic indicator.

The recent discovery of *C9ORF76* gene (Renton et al., 2011; DeJesus-Hernandez et al., 2011) has determined a new risk factor to be assessed when examining cognition in patients with ALS, considering the role of *C9ORF76* in FTD. Patients with ALS carrying the C9orf72 repeat expansion have different cognitive decline compared with subjects without C9orf72 repeat expansion (Byrne et al., 2012). Subjects enrolled in the Irish registry with complete assessment of cognitive and behavioral function, without *SOD1, TARDP*, or *FUS* pathogenic variant, were searched for C9orf repeat expansion and 21 (11%) showed the expansion with 86% % showing a family history for ALS or FTD. Comorbid FTD was more prevalent in subjects carrying the repeat (50% vs. 12%) Of the 21 patients only two did not have either behavioral or cognitive impairment. According to the results from the Irish registry there are two main groups of ALS: patients without cognitive impairment and no family history of ALS and FTD (true sporadic); and patients with predominant executive functions and behavioral changes. A large proportion of

the second group carries the repeat expansion. This has important implications for prognosis and therefore for the stratification of subjects enrolled in clinical trials.

FTD: NEW END OF THE ALS SPECTRUM?

The recent identification of *C9ORF72* as a gene responsible for a large number of sporadic and familial cases of FTD and ALS has underlined FTD is the most common of a group of clinical syndromes associated with degeneration localized to the prefrontal and anterior temporal lobes and non-AD type pathology, which has been called frontotemporal lobar degeneration (Neary et al., 2005; Rabinovici & Miller, 2010). The behavioral variant (bvFTD) seems to account for nearly 60% of cases of FTD, whereas 40% of cases are language variants (Johnson et al., 2005). In most clinical and epidemiologic studies the disease typically presents in the sixth decade, although the age of onset can vary widely from the third to the ninth decade (Neary et al., 2005; Johnson et al., 2005). Although FTD is generally considered a presenile dementia, more recent studies show that individuals older than age 65 years account for a large number of cases (20–25% of all cases) and probably this segment is more numerous (Neary et al., 2005). Gislason and colleagues (2003) found a surprisingly high (3%) prevalence of frontal lobar syndrome mainly bvFTD after using a screening method based on behavioral symptoms in subjects 85 years and older. A recent clinicopathologic study found similar results, showing that elderly (>65 years) patients with FTD represented 3.2% of all elderly patients with dementia referred for autopsy (Baborie et al., 2012).

The challenges of descriptive epidemiologic studies for FTD are similar to epidemiologic studies of other rare conditions. Because frequency of the disease is low and the number of persons at risk in the reference population is large, performing a classic door-to-door survey of FTD directly in the general population would be very difficult, expensive, and inefficient. Several research groups in the United States, Europe, and Japan have used surveillance methods to estimate prevalence or incidence of FTD, based on the availability of neurologic and psychiatric expertise in the community (Knopman & Roberts, 2011). Within the last decade prevalence and incidence data have been collected from several population studies.

All the prevalence studies but one were conducted in Europe or North America, selecting samples of people that are mostly white. These studies used mainly administrative data with medical record linkage and coding; few relied on disease registries or FTD case finding (Gilberti et al., 2012; Garre-Olmo et al., 2010; Borroni et al., 2010; Bernardi et al., 2012; Feldman et al., 2003). Prevalence estimates of FTD varies in a wide range from 2.7 per 100,000 in Zuid-Holland (with a peak of 9.4 per 100,000 in the 60- to 69-year age group; Rosso et al., 2003) to 31 per 100,000 in Vallecamonica (Gilberti et al., 2012). This wide variation can be likely explained by possible differences in the age distribution of phenotypes of FTD in different regions, differences in sampling or ascertainment methods, and also by the presence of different regional distributions of genetic risk factors for FTD (Ratnavalli et al., 2002). The study conducted in the Cambridgeshire area (Ratnavalli et al., 2002), reported prevalence estimates of 15.1 per 100,000 inhabitants in the age range 45–64 years for FTD and AD; almost one-third of patients with FTD (29%) had a positive family history, a figure similar to that reported (38%) in a nationwide survey of familiar FTD conducted in the Netherlands (Stevens et al., 1998). The Italian studies reported the highest prevalence estimates. In an isolated community in the Calabria region in Southern Italy, Bernardi et al. (2012) sampled a relatively isolated community of subjects older than 50 and found that 60% of subjects with dementia were FTD. The unusually high FTD prevalence in this community with an extraordinary low prevalence of AD among the elderly may be explained by the clustering of FTD genes in this isolated community. The Brescia County study (Borroni et al., 2010), analyzed data from 8 years of surveillance based on a consolidate network among participant centers, reporting a cumulative prevalence of 17.6 cases out of 100,000 inhabitants that significantly increases at the ages 66–75 (78 per 100,000) and older than 75 years (54 per 100,000). The Vallecamonica Study also reported a high 10-year cumulative prevalence (Gilberti et al., 2012). Knopman and

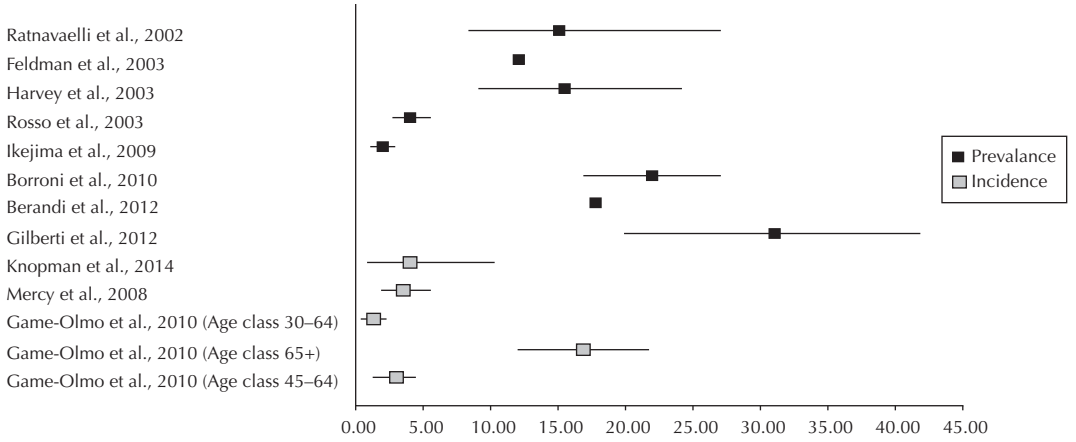

Figure 12–2. Incidence and prevalence of frontotemporal dementia in population-based studies (*n* per 100,000).

Roberts (2011), based on large neurophatologic (Rascovsky et al., 2011; Brunnstrom et al., 2009) and clinical series (Knopman & Roberts, 2011), estimated that the true point prevalence of frontotemporal lobar degeneration is 15–22 per 100,000. It is possible that some of the very high estimates of some prevalence study do not reflect neuropathologic FTD and therefore represent at least partially an overdiagnosis of the disease (Knopman & Roberts, 2011). There are sparse data on the incidence of FTD. Nevertheless, the incidence estimates for FTD were clustered in a tight range from 2.7 per 100,000 inhabitants (45–64 years) in the Girona Study (Garre-Olmo et al., 2010), 3.5 per 100,000 inhabitants (45–64 years) in the Cambridgeshire Study (Mercy et al., 2008), to 4.1 per 100,000 (40–69 years) in the Rochester Study (Knopman, Petersen, Edland, Cha, & Rocca, 2004). Gender distribution in FTD seems to vary by clinical syndrome, with most studies reporting a male preponderance in bvFTD, and several studies describing a male predominance in semantic dementia and a female predominance in primary progressive aphasia (Neary et al., 2005).

Autopsy studies based on consecutive, unselected cases have demonstrated that FTD accounts for roughly 5% of all pathologic diagnoses in patients with dementia. The epidemiologic data suggest that FTD is a common cause of early onset (age <65 years) dementia and is likely to be an underestimated cause of dementia in older individuals. In this age group many cases of FTD are labeled as AD.

The data on ALS and FTD (Fig. 12–2) show that both are rare conditions according to the definition of National Institutes of Health (NIH) (prevalence) but patients with symptoms and signs of both motor and cognitive signs are probably underestimated. Selective assessment of cognitive functions in FTD and memory clinics and motor signs in motor neuron clinics are inadequate for the identification of mixed phenotypes, and future epidemiologic data should focus on these mixed phenotypes.

ALS PROGNOSTIC FACTORS

ALS prognosis has been extensively studied in the last decade, with the detection of several prognostic factors. In a review published in 2009 (Chiò, Logroscino et al., 2009), median survival time of patients with ALS has been found to be between 20 and 48 months from onset of motor symptoms, with slightly lower values from population-based studies.

Contrasting data have been published concerning the modification of ALS outcome in the last decade. According to clinic-based studies, ALS significantly increased over the last decades (Testa, Lovati, Ferrarini, Salmoiraghi, & Filippini, 2004). Conversely, two population-based studies published so far found an unexpected decline of survival. In a Scottish study a 2.4-month decline was found in patients diagnosed in 1989–1993 compared with those diagnosed in 1994–1998 (Forbes,

Colville, Cran, & Swingler, 2004), whereas in a study from the Wakayama Prefecture in Japan assessing the period 1966–2005, patients diagnosed after 1990 had a significantly shorter survival than those diagnosed before that date (Kihira et al., 2008). However, this apparent decline in survival could be a statistical artefact due to confounding variables, such as the increase of the median age of ALS cases over time.

Generally, recognized prognostic factors are age and bulbar onset. Also, the progression rate of symptoms, calculated using the ALS Functional Rating Scale-Revised (ALSFRS-R; Kimura et al., 2006; Kolleve et al., 2008), as well as the involvement of respiratory function (Chiò et al., 2002; Czaplinsli, Yen, & Appel, 2006) are strong and independent predictors of the future course of the disease. Data are emerging that ALS clinical phenotype at diagnosis, classified as classic, bulbar, flail arm, flail leg, and prevalent upper motor neuron (Chiò et al., 2011) and spreading patterns of symptoms (Fujimura-Kiyono et al., 2011) are also predictive of ALS outcome.

Besides these classically recognized prognostic factors, some other factors are emerging. First, cognitive status strongly influences the course of the disease, not only in the presence of full-blown FTD (Olney et al., 2005; Phukan et al., 2012), but also when involvement of executive and nonexecutive functions not meeting the criteria for FTD is present (Phukan et al., 2012). It is not clear how cognitive impairment influence prognosis in ALS. It has been found that both FTD and a dysexecutive syndrome negatively influence the use of noninvasive ventilation and enteral nutrition and are independent prognostic factors for ALS (Chiò, Ilardi et al., 2012). Also, behavioral changes have a negative impact on ALS survival (Hu et al., 2013). The timing of onset of cognitive and motor impairment influence the following course of ALS: in a study comparing patients who developed both ALS and FTD, those who had cognitive symptoms before motor symptoms had a more prolonged survival than those who had motor symptoms before or simultaneously with cognitive impairment (Hu et al., 2009).

Second, an impaired nutritional status reduces survival. Body mass index has been proposed has a strong predictor of ALS prognosis (Desport et al., 1999; Marin, Desport et al., 2011). However, it has been found that the relationship between body mass index and ALS survival is not linear, with the best prognosis in subjects with body mass index range between 30 and 35 (Paganoni, Deng, Jaffa, Cudkowicz, & Wills, 2011), and that the rate of reduction of body mass index or weight is related to ALS prognosis more than the body mass index at diagnosis (Jawaid et al., 2010; Marin, Desport et al., 2011; Shimizu et al., 2012).

ALS prognosis is also influenced by specific genetic mutations. Patients carrying *FUS* mutations have a shorter survival, associated with a younger age at onset than any other gene mutation (Millecamps et al., 2012; Chiò, Borghero, et al., 2012). Among *SOD1* mutations, p.A4V is generally related to a poor prognosis, whereas p.D90A in heterozygosis is associated with a very slow clinical course, often associated with atypical symptoms (Andersen, 2006). In apparently sporadic patients with ALS, a genetic locus has been related to a better ALS prognosis (*UNC13A*) in two independent studies (Diekstra et al., 2012; Chiò et al., 2013), whereas all other identified loci or genes have not been confirmed (Chen, Sayana, Zhang, & Le, 2013). Patients carrying pathogenetic mutations of the *FUS* gene have a younger age at onset and a worse prognosis, compared with nonmutated patients or patients carrying other genetic mutations (Millecamps et al., 2010). Also, patients with *C9ORF72* mutations have worse prognosis than nonmutated patients (Chiò, Borghero et al., 2012; Sabatelli et al., 2012; Millecamps et al., 2012).

Prognosis is also modified by the use of noninvasive ventilation and invasive ventilation via tracheostomy (Bourke et al., 2006; Marchese, Lo Coco, & Lo Coco, 2008). Conversely, there are no clear data that enteral nutrition modifies the course of the disease in term of survival. According to some studies, patients who undergo percutaneous enteral gastrostomy have a better survival than those who did not receive or refuse it (Mazzini et al., 1995; Spataro, Ficano, Piccoli, & La Bella, 2011). However, a recent paper that used marginal structural models where the mortality hazards and ALSFRS-R slopes between percutaneous enteral gastrostomy treated and nontreated patients, after adjusting by indication, reported a significantly increased slope of ALSFRS-R score and an increased mortality hazard in those who received percutaneous enteral

gastrostomy (Atassi, Cudkowicz, & Schoenfeld, 2011).

According to the data of two independent population-based studies, interdisciplinary care (ALS dedicated center) is related to a prolongation of survival (Traynor, Alexander, Corr, Frost, & Hardiman, 2003; Chiò, Bottacchi, Buffa, Mutani, & Mora, 2006), as well as an improvement of patients' perceived quality of life (van den Berg et al., 2005) and a reduction of costs, in particular a reduction of hospital admissions (Chiò et al., 2006). A paper from Southern Italy did not find a benefit for patients followed in an ALS center (Zoccolella et al., 2007), but in this study the rate of patients who underwent enteral nutrition and noninvasive ventilation was relatively reduced both in the cohort of patients attending the ALS center and in that followed by general neurology clinics.

Several studies have been performed on the outcome correlates of biologic markers. A possible protective effect on patients with ALS of an increase of cholesterol levels and a high low-density lipoprotein/high-density lipoprotein ratio has been reported in a series of French patients (Dupuis et al., 2008). However, subsequent papers have given contrasting results (Chiò, Calvo et al., 2009; Dorst et al., 2011; Paganoni et al., 2011; Sutedja et al., 2011; Ikeda, Hirayama, Takazawa, Kawabe, & Iwasaki, 2012; Dedic et al., 2012). Interestingly, reduced cholesterol levels have been related to respiratory failure, a well-known prognostic factor in ALS, maybe through respiratory fatigue and an increase of calories demands (Chiò, Calvo et al., 2009), opening the chance to assess high-calorie/high-fat diets for the treatment of ALS.

A possible protective effect of elevated levels of uric acid has been supported by some studies, but its effect seems to be limited to males (Keizman et al., 2009; Ikeda et al., 2009; Zoccolella et al., 2010; Paganoni et al., 2012; Ikeda et al., 2012). In 2005, a large study from a tertiary referral center in France identified serum creatinine levels as having a prognostic effect in patients with ALS (Paillisse et al., 2005). In summary, all these biologic markers seem to be promising factors for modeling ALS prognosis and also for unveiling mechanisms related the pathogenesis and the progression of ALS pathology, but they need to be confirmed in larger populations and in longitudinal studies.

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Chapter 13

Genetics of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

Janel O. Johnson and Bryan J. Traynor

BACKGROUND AND NEW POINTS

Familial forms of motor neuron disease (MND) were recognized in the 1800s, but the first gene, superoxide dismutase (SOD1), was not identified until 1993. However, SOD1 mutations accounted for only 12–15% of familial amyotrophic lateral sclerosis (ALS). The mechanism of cell death from *SOD1* mutations remains unknown.

The search for other genes has yielded an additional 20 associated with ALS, but this number does not account for all families with ALS. The concept of hereditary ALS has expanded as other disorders, such as frontotemporal lobe dementia (FTLD) and psychiatric disease, are associated in families with ALS, and new genes have been found unique to these combinations. Some mutated genes seem to have roles in RNA processing, suggesting a common mechanism for them, but no mechanism has been firmly determined for any mutation.

INTRODUCTION

The first clinical description of ALS by Charcot in 1869 has served firmly as the basis

for diagnosis of the disease. Charcot discussed the motor symptoms of ALS and described lesions of the motor neurons and spinal cord, but did not link additional symptoms in the progression of the disease. For more than a century, neurology teachings defined ALS as a disease of purely motor neuron degeneration, even though additional features have since been noticed. In fact, cognitive impairment in ALS was described as early as 1892 (Zago, Poletti, Morelli, Doretti, & Silani, 2011). The El Escorial classification system—the most used diagnostic system in place for ALS to date, requires (1) evidence of upper motor neuron involvement, (2) evidence of lower motor neuron involvement, and (3) notably progressive symptoms. It also requires additional testing to rule out mimic syndromes. This is typically done using electrophysiology, neuroimaging, and neuropathologic examination (Brooks, 1994; Brooks, Miller, Swash, & Munsat, 2000).

The level of cognitive impairment in ALS was not truly appreciated until close to the early 1990s. Reports have since indicated that up to 50% of patients with ALS exhibit some degree of cognitive and behavioral impairment. This percentage may be higher, but the symptoms of ALS likely undermine accurate diagnostic testing for these changes. These impairments are shared with those of what had previously been regarded as a completely separate disease, frontotemporal dementia (FTD). FTD is a disease characterized by selective atrophy of the frontal and/or temporal cortices of the brain. Patients with FTD experience progressive changes in behavior, personality, and/or language (Kertesz, McMonagle, Blair, Davidson, & Munoz, 2005; Ringholz et al., 2005).

It has now become widely accepted that ALS and FTD form a clinical continuum with a range from ALS alone, to ALS with FTD (ALS-FTD), to FTD alone. The link between the two diseases was bolstered with the identification of Tar DNA Binding Protein (TDP43) as the major component of ubiquitylated inclusions in both diseases (Neumann et al., 2006). ALS and FTD are genetically heterogeneous, because more than 20 ALS genes and 5 FTD genes have been identified. Some of these genes contribute to both ends of the disease continuum suggesting that they share common pathways to neurodegeneration.

ALS

ALS has an incidence of 2 in 100,000 per year in Western countries (Logroscino et al., 2010). Below the age of 65, ALS seems to be more common in males than in females (incidence of approximately 2:1); however, the gender ratio seems to approach equality thereafter (Wijesekera & Leigh, 2009). A family history is reported in 5–10% of patients with ALS, and this is referred to as familial ALS (FALS). The remaining patients who do not report a family history are classified as sporadic (SALS).

The reason for such a broad range of heritability is that factors conspire to confound the ascertainment of the familial component of ALS. First, FALS and SALS appear essentially clinically indistinguishable, except that the reported mean age of onset in FALS is 56 years while SALS is 63 years, so patient examination is not sufficient to draw any clear boundary. Second, ALS is typically a late-onset, rapidly progressive disease; small family size and/or early death of obligate carriers likely prohibits transmission and detection of mutations in multiple family members. It has repeatedly been demonstrated that the large effect variants inherited in mendelian fashion are also mutated in many sporadic cases, further supporting this possibility. Nonpaternity and incomplete penetrance of variants also likely masks the presence of additional mutation carriers.

Finally, recent studies have shown clinical manifestations of ALS are variable even within families. Because a formal diagnosis of ALS does not include variable symptoms, misdiagnosis may also play a role. A more expansive and accurate knowledge of family history and diagnostic features might reclassify many apparently sporadic cases as familial. The El Escorial diagnostic criteria for ALS (Brooks, 1994) and its revision (Brooks et al., 2000) do not address this issue. Novel criteria have been proposed for this purpose, with the hope of standardization of epidemiologic and genetic studies and also to strengthen predictive models for genetic counseling (Byrne et al., 2011).

Although the distinction between FALS and SALS is not clear, it is routinely quoted that FALS comprises roughly 10% of all ALS cases (Valdmanis, Daoud, Dion, & Rouleau, 2009). Most cases are inherited in an autosomal-dominant fashion, whereas those that are recessive seem to be rare (Albagha

et al., 2011; Andersen et al., 1995; Sugihara, Maruyama, Kamada, Morino, & Kawakami, 2011). However, given the blurred distinction between FALS and SALS, and that homozygous regions identical by descent commonly occur in outbred populations, it is possible that homozygous recessive mutations in genes occur in what is typically viewed as sporadic disease. This is supported by a recent study in which the number and segment lengths of regions of homozygosity are greater in ALS cases than control subjects in European populations (Mok et al., 2013).

Family studies have been critical to identifying the genetic causes underlying most neurodegenerative diseases. Despite the uncertainty of the magnitude of the familial component of ALS, and the difficulty of genome-wide association studies to provide independent replication of some associated loci, the utility of these studies in the disease is clear. Linkage analysis and homozygosity mapping in the rare large families with large effect variants have led to the discovery of several genes that contain mutations causing ALS, many of which cause both familial and apparently sporadic disease. The first genetics milestone was reached in 1993 when linkage analysis was used to identify mutations in *SOD1* (chromosome 21q22.11; Rosen, 1993) and since then, using this technique and more novel ones, more than 20 genes containing mutations have been reported as causative of ALS.

As in several other neurodegenerative diseases, protein aggregates are a pathologic hallmark of ALS. In nearly all ALS cases there are ubiquitylated inclusions in lower motor neurons of the spinal cord and brainstem. In 80–100% of SALS cases, small eosinophilic inclusions called Bunina bodies are found in the cell bodies of spinal motor neurons. Knowledge of the composition of the proteins in inclusions can provided clues as to the underlying genetic causes, because the components conjugated to ubiquitin are often proteins encoded by the mutant genes themselves. One such example is TDP-43, which was determined to be an ALS gene in this fashion (Robberecht, 2000; Talbot & Ansorge, 2006; Sreedharan et al., 2008).

FTD

FTD is a leading cause of dementia (after Alzheimer disease) in patients younger than 65. Neuropsychiatrist Arnold Pick released the first report of this disease in 1892. The clinical syndromes of FTD include a behavioral variant (bvFTD) and two language variants, which include semantic dementia (SD) and progressive nonfluent aphasia. In bvFTD patients exhibit profound changes in personality and social behavior caused by involvement of the orbital and mesial frontal lobes. In SD, patients exhibit loss of conceptual knowledge including lack of memory for words, objects, and/or faces caused by involvement of anterior and inferior temporal lobes. In progressive nonfluent aphasia, patients exhibit loss of expressive language, such as the phonologic and grammatical aspects of language production because of atrophy of the insular and inferior frontal regions of the brain (Lillo & Hodges, 2009; McKhann et al., 2001; Neary, Snowden, & Mann et al., 2005; Neary et al., 1998).

FTD typically has an age at onset in the 60s and it is a common cause of dementia with a prevalence that ranges across populations from 2.7 to 15.1 per 100,000, and incidence of approximately 3.5 in 100,000 per year. Gender ratios vary by clinical syndrome because males tend to be more commonly affected with bvFTD and SD, whereas females are more commonly affected with progressive nonfluent aphasia. Average survival for FTD ranges from 6 to 11 years from symptom onset (Rabinovici & Miller, 2010), although survival varies by clinical syndrome with bvFTD having the shortest and SD the longest survival.

As in ALS, family studies in FTD are useful to aid in the identification of novel genetic causes of disease. About one-third to one-half of FTD is familial. Six FTD genes with mendelian inheritance have been identified using these studies. Transmission in these families occurs in an autosomal-dominant fashion, and the mutations are highly penetrant (≥95% lifetime risk). More than 80% of the autosomal-dominant cases can be explained by mutations in three of the known FTD genes. Mendelian forms of FTD have largely been explained genetically, but individuals from families with mendelian inheritance only account for approximately 10–30% of all of FTD.

Remaining FTD cases are thought to be multifactorial in etiology, meaning that multiple small effect genetic risk variants and/or environmental risk factors combine to cause

disease in most cases. Dementia is a common disease, complicating the ability of the practicing neurologist to distinguish mendelian disease from complex disease. This is important to do from a genetic counseling perspective because lifetime risk of dementia in the general population, those with a positive family history, and mutation carriers with mendelian disease variants have estimated lifetime risks of 10%, 20%, and 95%, respectively. Individuals with at least three affected family members in two generations and early onset disease are more likely to be carriers of high-effect variants (Loy, Schofield, Turner, & Kwok, 2013).

The genetic mutations in FTLD genes lead to differentially distributed neuropathologic protein aggregates. These have been important to classify so that new treatments target and ultimately modulate the proteins involved. Early neuropathologic classification characterized FTLD into groups based on markers detected by way of immunohistochemistry (Dickson, Kouri, Murray, & Josephs, 2011; Wider & Wszolek, 2008). In 1998, the first genetic cause identified as underlying FTLD were mutations in microtubule-associated protein tau (*MAPT*), and these were identified using positional cloning (Hutton et al., 1998). These mutations lead to aggregate formation caused by impaired function of tau in microtubule assembly. Tau aggregates were seen in many cases of FTLD; however, there were several cases lacking mutations in tau. These instead showed ubiquitin immunoreactivity. Cases were separated into two groups: individuals with deposition of microtubule-associated protein tau (FTLD-TAU), and individuals with ubiquitin-positive cytoplasmic inclusions lacking tau (FTLD-U; Dickson et al., 2011; Wider & Wszolek, 2008).

Most in the second group, FTLD-U, were later found to be immunoreactive for TDP-43 (Neumann et al., 2006). A small number of FTLD-U cases lacked TDP-43 immunoreactivity, and the major protein conjugated to ubiquitin in these instances was later determined as fused in sarcoma (FUS; Neumann, Rademakers, Roeber, Baker, & Kretzschmar, 2009). The most recent system now classifies according to the predominant deposition of (1) microtubule-associated protein tau, (2) TDP-43, or (3) FUS. (4) In rare occasions there are other proteins deposited. These are referred to in the literature as FTLD-TAU, FTLD-TDP, FTLD-FUS, or FTLD-Other, respectively (Mackenzie, Foti, Woulfe, & Hurwitz, 2008).

FTLD AND ALS OVERLAP

At the extremes of the continuum, patients with pure ALS only present with symptoms of motor neuron degeneration, whereas those with pure FTD experience dementia characterized by changes in behavior, language, and personality caused by cortical degeneration absent of motor neuron involvement. However, it is more common to have both ALS and FTD symptoms; this can occur within one family or even in one individual. The elucidation of the genetic changes underlying the development of ALS and FTD has begun to explain mechanisms that are both common and unique to the two disorders. These are important to understand because it is has become clear that one treatment is not feasible for all manifestations of disease. A model with complex multisystem involvement is favored and provides clues as to what therapeutic targets are desired over others.

Ends of the Spectrum

SOD1 and *MAPT* in ALS and FTD, respectively, represent extremes of the continuum that do not seem to overlap clinically or neuropathologically. SOD1 clinically features purely upper and lower motor neuron signs, whereas *MAPT* clinically features predominant behavioral variant dementia and parkinsonism in the absence of MND. Neuropathologically, patients with *SOD1* have ubiquitylated inclusions in anterior horn neurons positive for p62 and SOD1, and negative for TDP-43. Patients with *MAPT* have aggregation of hyperphosphorylated tau proteins in neurons and glia.

SOD1 in ALS

SOD1 is one of the few ALS gene that, when mutated, causes pure ALS. *SOD1* mutations

have been estimated to account for variable proportions of cases in different research studies. The only population-based study of *SOD1* mutation frequency suggests that they account for 12–15% of familial cases and 0.7% of sporadic cases (Chio et al., 2008). From 1993 to 2011, *SOD1* was known as the most common cause of ALS. Therefore functional and neuropathologic studies related to *SOD1* have predominated. As of the present, 174 pathogenic *SOD1* mutations have been identified, and these are spread out across the gene. Most of these mutations are missense, but frameshift mutations have also been described. The pathogenic nature of many of the *SOD1* variants described has not been confirmed (http://alsod.iop.kcl.ac.uk), so it is possible that the frequencies of mutations that have been reported are overestimates(Andersen, 2006).

SOD1 contains five exons and spans 11.6 kb across chromosome 21q22.11. It produces a 981-nucleotide transcript in which the longest open reading frame is 464 nucleotides, encoding Cu/Zn SOD, a protein of 153 amino acids. Cu/Zn SOD is a predominantly cytosolic enzyme that functions as a 32 kDa homodimer as it specifically catalyzes the reduction of the superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2). The stability and activity of the enzyme is dependent on the metals Zn^{2+} and Cu^{2+}. Zn^{2+} regulates proper folding and keeps the protein stable. Cu^{2+} binds to its native site on the enzyme and regulates the activity of the enzyme by way of redox reactions at the active site (Fukai & Ushio-Fukai, 2011).

Mutations in *SOD1* lead to dominantly inherited disease with one exception. The D90A mutation results in recessively inherited disease specifically in the Scandinavian population. In Finland and Northern Sweden, D90A is the most common *SOD1* mutation. Up to 3% of people in these areas carry the D90A mutation in the heterozygous state, and carriers seem to be neurologically normal. Scandinavian patients with homozygous D90A have clinically distinguishable symptoms from those carrying other gene mutations including painful muscle spasms, paresthesia in the legs, and longer disease course (Andersen et al., 1996). The most frequent *SOD1* mutation in the United States is the A4V mutation. This is carried by approximately 50% of *SOD1*-positive patients. A4V patients present with predominantly lower motor neuron signs and suffer rapid disease progression (Aggarwal & Nicholson, 2005; Juneja, Pericak-Vance, Laing, Dave, & Siddique, 1997).

Native SOD1 protein has intrinsic structural factors that contribute to its stability. The pathogenic variants promote oxidation and misfolding of the protein (Valentine & Hart, 2003) decrease its stability (Lindberg, Tibell, & Oliveberg, 2002), alter the net charge (Shaw & Valentine, 2007), and increase the aggregation propensity of the molecule (Stathopulos et al., 2003). Insoluble aggregates containing SOD1 protein are found in the postmortem tissues of patients with ALS (Shibata et al., 1996) and animal models (Gurney et al., 1994) positive for *SOD1* mutations.

MAPT in FTLD

The first FTLD linkages were of 13 families with autosomal-dominant FTD and parkinsonism to a 2-cM region on chromosome 17q21.11 (FTDP-17). Many of the linked cases showed immunoreactivity against insoluble hyperphosphorylated microtubule associated protein tau filaments in neuronal and/or glial inclusions. Because the gene for this protein, *MAPT*, is within the chromosome 17 interval, Hutton and coworkers sequenced *MAPT* in FTDP-17 and subsequently identified missense mutations in *MAPT* mutations that underlie this disease (Hutton et al., 1998).

Since 1998, 44 *MAPT* mutations resulting in FTD have been described (http://www.molgen.vib-ua.be). It has been estimated that mutations in *MAPT* cause between 10% and 43% of dominant FTD and 5% and 20% of all FTD cases (Poorkaj et al., 2001; Rizzu et al., 1999; Rosso et al., 2003).

MAPT contains 15 exons and spans 167 kb across chromosome 17q21.1. It produces a 6,816-nucleotide transcript in which the longest open reading frame is 2,330 nucleotides, encoding microtubule-associated protein tau (tau), a protein of 352–441 amino acids (www.ncbi.nlm.nih.gov/gene). The reason for this variability is that tau contains a variable 31 amino acid repeat region near the C-terminus and it is alternatively spliced into six isoforms. The repeat region is the microtubule binding domain and depending on splicing, isoforms either has three or four tandem repeats. These

repeat isoforms exist within defined ratios in specific tissues and are developmentally regulated to execute the functions of balances of these isoforms. The main function of tau is to regulate microtubule dynamics through its association with microtubules. It regulates the assembly and disassembly, and spatial organization of microtubules in axons. Tau function is modulated by phosphorylation and dephosphorylation. Mutations in MAPT are missense, deletions, or splice mutations that differentially alter functional properties of tau. Missense and deletion mutations are mostly located in or close to exon 10, which is in its microtubule-binding domain. Almost all of these mutations reduce the ability of tau to bind microtubules and cannot promote assembly. This promotes tau filament formation. Splicing mutations alter the ratio of four-repeat to three-repeat tau leading to more tau produced than can bind to microtubules and subsequent aggregation (Goode, Chau, Denis, & Feinstein, 2000; Hong, et al., 1998; Spillantini, Van Swieten, & Goedert, 2000).

GENES WITH POSSIBLE ALS AND FTLD OVERLAP

PGRN Mutations

Many of the FTLD families linked to the chromosome 17 region both lacked tau pathology and mutations in *MAPT*. In fact, most cases of FTLD lack tau pathology. About 60% of dominant FTD was not explained by tau mutations. The remaining FTDP-17 families, instead, had ubiquitin pathology (FTLD-U) and intranuclear and intracytoplasmic inclusions in the frontal and temporal cortex and in the dentate fascia of the hippocampus, or they had "dementia lacking distinctive histopathology" (FTLD-Other). In 2006, this was clarified by the finding that the remaining FTLP-17 families carried mutations in another gene, *PGRN*, which is coincidentally near the *MAPT* linked locus. In these early studies, individuals carrying the *PGRN* mutations met the clinical criteria for FTD without MND (FTLD-U and more specifically FTLD-MND-type) and shared the neuropathology of neuronal intranuclear inclusions. These inclusions do not contain the protein encoded by *PGRN* (Baker et al., 2006; Cruts et al., 2006; van der Zee et al., 2006). Only rarely do patients with *PGRN* mutations present with MND. A recent study estimates concomitant MND in 5% of patients with PGRN mutations (Chen-Plotkin et al., 2011).

PGRN contains 13 exons and spans 10 kb across chromosome 17q21.32. It produces a 2,323-nucleotide transcript in which the longest open reading frame is 1,781 nucleotides, encoding progranulin, a protein of 593 amino acids (www.ncbi.nlm.nih.gov/gene). Wild-type progranulin is a 68 kDa glycoprotein that is expressed in peripheral tissues, neurons, and microglia. The functions of progranulin have not been fully elucidated but it has roles in mediating regulation of cell growth, progression of the cell cycle, and wound healing and inflammation (He & Bateman, 2003; He, Ong, Halper, & Bateman, 2003). It has been estimated that mutations in *PGRN* cause between 3.4% and 25.6% of dominant FTD and 1% and 11.7% of all FTD cases (Gass, Prudencio, Stetler, & Petrucelli, 2012). As of the present 69 pathogenic mutations in *PGRN* have been described and they are spread across the entire gene (www.molgen.ua.ac.be). Known pathogenic mutations in *PGRN* include nonsense, frameshift, and splice mutations that lead to truncated protein and a loss of function mechanism. Missense, silent, and intronic mutations occur in PGRN but the pathogenicity of these is less clear.

Clinical phenotype, neuropsychological profiles, and histopathologic profiles are variable with *PGRN* mutations, even though they all cause null alleles. Age of onset of disease and disease duration are also variable within and between families suggesting there is incomplete or age-related penetrance of mutations and other influences on disease course (Gass et al., 2006; Le Ber et al., 2008). In fact, genome-wide association studies of PGRN mutation carriers have shown that single-nucleotide polymorphisms specifically in the *TMEM106B* region affects age of onset and severity of disease (Finch et al., 2011; Nicholson et al., 2013; Van Deerlin et al., 2010).

There are, at present, no effective targeted therapies for FTD caused by *PGRN* mutations. The phenotype with *PGRN* mutations is variable, so genetic testing for mutations in *PGRN* is mainly useful for ruling out other neurodegenerative diseases that might otherwise have viable treatments. It may not be very useful to

perform predictive genetic testing for PGRN mutations for several reasons: (1) most *PGRN* tests do not fully detect all possible genetic rearrangements, (2) there are many variants of unknown significance in which pathogenicity is unclear, and (3) mutations are not fully penetrant (Gijselinck, Broeckhoven, & Cruts, 2008).

TDP-43 Mutations

Shortly after the discovery of mutations in *PGRN* as causative of tau-negative, ubiquitin-positive FTLD, researchers identified TDP-43 as the major component of these types of inclusions. This was in the neuronal cytoplasm in both ALS and FTLD as round Lewy-body like and skein-like inclusions. TDP-43 in inclusions is hyperphosphorylated and there is loss of the protein from the nucleus. Much of the accumulated TDP-43 exists as ~25 kDa C-terminal fragments. This was an important breakthrough because it provided the first pathologic link between FTD-MND-type, FTD-MND, and ALS (Arai et al., 2006; Neumann et al., 2006), motivating the screening of *TDP-43* for genetic mutations in ALS. In 2008, missense mutations in *TDP-43* in both familial and sporadic ALS were identified (Sreedharan et al., 2008).

TDP43 contains six exons and spans 16.1 kb across chromosome 1p36.22. It produces a 4,236-nucleotide transcript in which the longest open reading frame is 1,244 nucleotides, encoding transactive response (TAR) DNA binding protein 43, a protein of 414 amino acids (http://www.ncbi.nlm.nih.gov/gene). TDP-43 is a nuclear RNA and DNA binding protein; it has two RNA binding domains that allow it to form complexes with RNAs and interacting proteins. By way of these complexes, it has roles in processing mRNA and producing microRNAs. It contains both a nuclear localization signal (NLS) and nuclear export signal, allowing it to shuttle between the nucleus and the cytoplasm.

The discovery of TDP-43 in inclusions provided the first evidence for the involvement of RNA metabolism to the pathogenesis of ALS and FTLD and this has become a recurring and important theme in understanding the disease continuum. TDP-43 maintains normal expression levels and splicing patterns of other mRNAs, and it also regulates its own expression level through negative feedback. To date, 32 known pathogenic mutations have been identified in *TDP-43*. All mutations except one (D169G) cluster in exon 6, which encodes the C-terminal glycine-rich domain. This is the region involved in protein-protein interactions. *TDP-43* mutations are especially frequent in patients of southern European origin. The most common mutation overall is A382T. Microsatellite and single-nucleotide polymorphism analysis in Italian patients show that this is usually a founder mutation. It is also probably sometimes a spontaneous mutation hotspot because a different missense mutation (A382P) has been identified at the same position (Fiesel & Kahle, 2011; www.molgen.ua.ac.be; Chio, Borghero, et al., 2011; Corrado et al., 2009; Daoud et al., 2009). With the exception of southern Europe, *TDP-43* mutations are not common. Mutation frequency dramatically varies throughout the world, but in the United States it ranges from 0.72% to 3.3% of familial and sporadic ALS cases combined (Brown et al., 2012).

Most patients with TDP-43 mutations have a pure ALS phenotype, but some patients have been described with FTLD-MND, and even pure FTD, further strengthening the view of ALS and FTD as a continuum (Benajiba et al., 2009; Chio et al., 2010).

It is not yet certain how *TDP-43* mutations cause disease, but a likely mechanism is that mutations cause mislocalization of protein to the cytoplasm, leading to depletion of critical protein in the nucleus, misregulation of RNA processing, and subsequent neuronal degeneration. Protein mislocalized to the cytoplasm might also cause cell death by way of gain of toxic function (Neumann, Kwong, Sampathu, Trojanowski, & Lee, 2007). When TDP-43 was first identified as the major protein component of tau-negative, ubiquitin-positive cases of FTLD and ALS, it was thought that TDP-43 was present in all of these cases. It was later shown, however, that 10–20% of FTLD-U cases is actually TDP-43 inclusion-negative. This form of disease was then referred to as "atypical FTLD-U" (aFTLD-U; Mackenzie et al., 2008; Roeber, Mackenzie, Kretzschmar, & Neumann, 2008).

FUS Mutations

In early 2009, two groups concurrently identified mutations in a new gene causing FALS.

Kwiatkowski et al. (2009) described an ALS family of Cape Verdean descent where the proband's unaffected maternal grandparents were first cousins. Because this suggests autosomal-recessive inheritance, loss-of-het erozygosity mapping was performed and a major loss-of-heterozygosity cluster was identified that overlapped a previously described FALS locus on chromosome 16. Sequencing genes at that locus led to identification of a homozygous missense mutation in *FUS* that segregated with disease. Follow-up sequencing of a total of 17 families (including families linked to the chromosome 16 locus and a cohort of probands) revealed 13 different *FUS* mutations. These families showed autosomal-dominant inheritance. Most of these mutations occurred in exon 15, most commonly affecting residue 521 (Gitcho et al., 2008; Yokoseki et al., 2008). Vance and coworkers discovered *FUS* mutations as a cause of ALS by prioritizing sequencing in a large British ALS family at the linked chromosome 16 region according to structural similarities to TDP-43. R521C was the cause of disease in this family, and this group also found mutation at residue 521 to be most common pathogenic change in *FUS* (Kwiatkowski et al., 2009; Vance et al., 2009). There are several different *FUS* mutations at position 521, suggesting that it is a mutations hotspot (Blair et al., 2010). Recent reports show *de novo* mutations at this position (Chio, Calvo, et al., 2011; DeJesus-Hernandez et al., 2010; Zou et al., 2013).

To date, 23 distinct pathogenic mutations in *FUS* have been described. Like *TDP-43*, the frequencies of mutations in *FUS* vary dramatically around the world. In the United States, *FUS* mutations account for approximately 1.9–5% of familial and sporadic ALS cases combined. Mutations in *FUS* are extremely rare in sporadic ALS (Brown et al., 2012; Lai et al., 2011). Also like TDP-43, *FUS* mutation carriers usually have a pure ALS phenotype, although there are rare instances in which disease can present as ALS-FTD. *FUS* mutations often result in earlier onset disease than what is typical of ALS and the disease course can be particularly severe and rapid, and *FUS* patients have a shortened lifespan. Cognitive impairment is not common with *FUS* mutation (Millecamps et al., 2010).

FUS contains 15 exons and spans 18.4 kb across chromosome 16p11.2. It produces a 5,119-nucleotide transcript in which the longest open reading frame is 1,580 nucleotides, encoding fused in sarcoma, a protein of 526 amino acids (http://www.ncbi.nlm.nih.gov/gene). FUS has an N-terminal transactivating domain, and a glycine rich region followed by an RNA recognition motif. It then has a zinc finger domain and the C-terminus contains a NLS. The common exon 15 mutations are within the NLS. Like TDP-43, FUS is an RNA and DNA binding protein that plays roles in regulation gene expression, splicing, and transcription (Lanson & Pandey, 2012).

Patients with *FUS* mutations have different neuropathologic features than those with classical ALS. Inclusions in the cytoplasm of anterior horn cells rarely and diffusely contain ubiquitin, and neurons and glial cells do not contain TDP-43. They do, however, contain cytoplasmic inclusions of *FUS* protein in spinal cord motor neurons and dystrophic neurites. It is not clear how mutations in FUS lead to disease. However, like TD43, FUS has been shown to be mislocalized to the cytoplasm of cells where it forms aggregates. Nuclear depletion possibly causes motor neuron death, whereas there is a toxic gain of function in the cytoplasm (Blair et al., 2010; Kwiatkowski et al., 2009; Vance et al., 2009).

As described previously, TDP-43 and FUS share several similarities:

1. High degree of functional homology (the presence of RNA binding domains, nuclear export signal, and NLS)
2. Involvement in similar biologic processes
3. Indistinguishable clinical phenotypes that present as classical ALS
4. Formation of pathologic inclusions containing the protein upon mutation

This significant level of overlap between the two proteins led to speculation that FUS, like TDP-43, might be the major pathologic protein in cases of tau-negative, TDP-43-negative disease. Neumann and coworkers confirmed FUS pathology was indeed in aFTLD-U, with a higher ratio of insoluble versus soluble FUS than in control subjects. They also excluded FUS pathology in cases where TDP-43 inclusions were present (Neumann et al., 2009). Understanding the key mechanisms of TDP-43 and FUS (how they overlap and how they differ) is necessary. The roles of these proteins

and how they converge on disease-relevant target RNAs and in RNA metabolism in general should elucidate the spectrum of ALS and FTLD (Fiesel & Kahle, 2011).

CHMP2B Mutations

Mutations in *CHMP2B* were first identified in an autosomal-dominant Danish FTLD family in 2005 (Skibinski et al., 2005). *CHMP2B* mutations are a rare cause of disease because only four confirmed pathogenic variants have been described to date (http://www.molgen.ua.ac.be). *CHMP2B* encodes a 213 amino acid charged multivesicular body protein 2B, a component of the endosomal sorting complex required for transport. CHMP2B protein is a structural protein of the endosome that fuses with the lysosome to degrade endocytosed proteins. Mutations disrupt fusion of endosomes leading to dysfunction in autophagy. It is possible that proper function of multivesicular bodies is required for degradation of proteins and TDP-43. Cytoplasmic inclusions in the frontal and temporal cortices of patients with *CHMP2B* mutations, however, are negative for TDP-43. They are also negative for FUS, and positive for ubiquitin. Thus, they are classified as FTLD-Other. *CHMP2B* mutations have been found in patients with ALS and FTLD but because functional work and neuropathology does not match that found for the Danish family, the pathogenicity of these variants is unclear (Fiesel & Kahle, 2011; Isaacs, Johannsen, Holm, & Nielsen, 2011; Skibinski et al., 2005).

OPTN

Mutations in *OPTN* were first described in Japanese ALS families in 2010 in autosomal-recessive cases and in a sporadic case (Maruyama & Kawakami, 2013). OPTN mutations more commonly cause primary open-angle glaucoma (Fuse, 2010). Heterozygous OPTN mutations causing ALS have since been identified in additional Japanese and European FALS and SALS cases, but these mutations are rare. OPTN-positive patients show onset with lower-limb involvement with slow progression (>10 years) to predominantly upper motor neuron involvement. Age of onset is highly variable (from the second to seventh decade), and familial and sporadic forms are clinically indistinguishable. No clinical overlap with primary open-angle glaucoma is evident (Del Bo et al., 2011; Maruyama & Kawakami, 2013; Solski, Williams, Yang, Nicholson, & Blair, 2012).

OPTN encodes optineuron, a 67-kDa protein that functions as an autophagy receptor that binds ubiquitin. Mutations in OPTN lead to dysfunctions in autophagy (Wild et al., 2011). Neuropathologic data show that optineuron is sometimes, albeit rarely, present in ubiquitylated inclusions, and in SOD1-, TDP-43-, and FUS-positive inclusions in patients with ALS without OPTN mutations (Hortobagyi et al., 2011). It is also distributed in inclusions in several other neurodegenerative diseases and even inclusion body myositis (Yamashita et al., 2013). Although there is neuropathologic overlap, OPTN mutations are not found in patients with ALS-FTD or frank FTD (Del Bo et al., 2011; Maruyama & Kawakami, 2013; Solski et al., 2012; Ying & Yue, 2012). OPTN was also identified in a genome-wide association study as a susceptibility locus for Paget disease of bone (Albagha et al., 2011), which brings us to mutations in VCP.

VCP

VCP mutations were first identified as causative of autosomal-dominant inclusion body myopathy with Paget disease of bone and FTD (IBMFTD). IBMFTD is an autosomal-dominant, progressive disorder characterized clinically by adult-onset muscle weakness and early onset FTD, and neuropathologically by TDP-43 inclusions in muscle and frontal cortex (Watts et al., 2004). In 2010, exome sequencing revealed VCP mutations as a cause of FALS (Johnson et al., 2010). This is yet another finding that supports ALS and FTLD as a continuum. Not only do ALS and IBMFTD share TDP-43 as a hallmark, but ALS, FTD, and Paget disease manifested distinctly or together even within the same family.

VCP encodes valosin-containing protein, a ubiquitously expressed AAA+ protein that functions in multiple cellular processes of which the most well-characterized is to regulate degradation of misfolded proteins, and has

more recently been shown to play a role in regulating protein degradation at the outer mitochondrial membrane (Bartolome et al., 2013; Johnson et al., 2010; Meyer, Bug, & Bremer, 2012).

Mutations in VCP account for 1–2% of FALS and less than 1% of SALS in the absence of FTLD (Abramzon et al., 2012), and less than 1% of familial FTLD. To date, 18 pathogenic mutations have been described in *VCP* (http://www.molgen.ua.ac.be). Patients with VCP mutations typically experience disease onset in the third to fifth decade, and several of the mutations are not fully penetrant. It is possible that mutations in VCP disrupt its normal function and lead to the accumulation of ubiquitinated inclusions within cells. Most pathogenic variants lie between the D1 and N domains of the protein, and possibly interfere with the relative movement of the domains as it coordinates its interactions. Patients with *VCP* mutations have prominent neuronal intranuclear, neuronal cytoplasmic inclusions, and dystrophic neuritis that are TDP-43-positive. Some of these inclusions also contain *VCP* protein (Ju et al., 2009).

SQSTM1

SQSTM1 is a gene on chromosome 5q35 that encodes p62. This protein has been found in inclusions in several neurodegenerative diseases including ALS and, for this reason, was selected as a candidate gene for ALS. Several mutations were indeed identified for both FALS and SALS (Fecto et al., 2011). SQSTM1 mutations are now estimated to account for approximately 1–5% of FALS and 2–4% of SALS (Chen et al., 2014), although this is likely to be an overestimate. P62 has multiple functions but its most well-characterized function is as a scaffolding protein that binds ubiquitin to shuttle proteins to the proteasome for degradation. Mutations probably cause disease by impairing the ability of p62 to bind ubiquitin (Fecto et al., 2011; Seibenhener et al., 2004). Like *VCP, SQSTM1* mutations were previously identified as causative of both familial and sporadic Paget disease of bone, and some of the same mutations cause both diseases.

To date, 24 mutations have been described in patients with ALS and these are distributed across the entire protein. Age of onset is extremely variable in patients with ALS with *SQSTM1* mutations, but in general patients have late ages of onset (>60 years). Clinical features are also highly variable (Chen et al., 2014; Fecto et al., 2011; Teyssou et al., 2013). SQSTM1 mutations have also been described in families with FTLD and ALS-FTLD. Intrafamilial clinical variability of these conditions and Paget disease occurs as in VCP. Like ALS, age of onset for FTD is late (≥70 years) in several cases of *SQSTM1* mutation. Disease manifests as bvFTD. SQSTM1 mutation carriers have aggregates of TDP-43 and p62 in and atrophy of neurons of the spinal cord and frontal cortex (Le Ber et al., 2008).

UBQLN2

In late 2011, UBQLN2 mutations were identified in an X-linked dominant ALS and ALS-FTLD family. As might be expected in X-linked disease, there was reduced penetrance in female mutation carriers with no male-to-male transmission. Linkage analysis and sequencing revealed a missense mutation of a proline residue at position 497. Sequencing of UBQLN2 in additional families with ALS and ALS/dementia revealed additional mutations. Notably these mutations occurred only at proline residues with a unique repeat region (Deng et al., 2011). Since this finding, 20 different mutations in UBQLN2 have been described, with variable pathogenicity. Most of mutations involve proline changes, although other pathogenic missense mutations are described (Daoud et al., 2012; Deng et al., 2011; Gellera et al., 2013; Millecamps et al., 2012; Synofzik et al., 2012; van Doormaal et al., 2012; Williams et al., 2012).

UBQLN2 encodes Ubiquilin 2, a 624 amino acid protein that is a member of the ubiquitin-like protein family. Like p62, it functions to deliver ubiquinated proteins to the proteasome to be degraded. Ubiquilin 2 binds at its N-terminus to polyubiquitin chains conjugated to protein to be degraded. It also has a PXX tandem repeat region; this is the region most commonly mutated. The frequencies of *UBQLN2* mutations vary according to population studied, but in general mutations are very rare. Ubiquilin 2 is a component of ubiquinated inclusions in UBQNL2-positive patients, in SALS and

FALS with SOD1 or TDP-43, and also in FALS with unknown cause (Deng et al., 2011; Williams et al., 2012).

C9orf72

The search for the gene harbored by the chromosome 9p locus began in 2006, when there were reports of linkage in families with pure ALS, ALS-FTD, and pure FTD (Morita et al., 2006; Vance et al., 2006). The genetic lesion managed to elude discovery for years because of limitations on conventional genetic methods, but in 2011 this search came to an end when two groups simultaneously published a breakthrough finding in the journal *Neuron* that a large intronic hexanucleotide repeat expansion within intron 1 of *C9orf72* is a common cause of both ALS and FTLD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Patients with chromosome 9p disease carry between 700 and 1,600 copies of the repetitive GGGGCC sequence, whereas control individuals carry a maximum of 23 copies. Intrafamilial manifestations of the ALS and FTD spectrum often occur (DeJesus-Hernandez et al., 2011).

Patients with ALS with C9orf72 expansions have classical symptoms with earlier onset, more rapid progression, and greater frequency of bulbar onset than patients with mutations in other genes (Chio et al., 2012; Cooper-Knock et al., 2012). Assessment of frequencies of mutations in ALS or FTD genes can be difficult, mainly because frequencies are population-specific. Data are largely unavailable for individuals not descended from Europe. The most comprehensive study of global frequency of *C9orf72* repeat expansions is by Majounie et al. (2012). Here it is estimated that for sporadic ALS, *C9orf72* repeat expansions account for 7% of white individuals from the United States, Europe, and Australia, ~4% of black individuals from the United States, and ~8% of Hispanic individuals from the United States. For FALS, expansions account for ~40% of white individuals from Europe and the United States. For sporadic FTD, *C9orf72* expansions account for 6% of white individuals from Europe. For familial FTD, expansions account for ~25% of white individuals from Europe. No expansions were found in smaller cohorts of other backgrounds, possibly highlighting the need for larger sample sizes from groups other than what is more commonly studied.

The disease haplotype, which seems to have originated from a common founder in Finland about 1,500 years ago, as it is the same for all expansion carriers (Majounie et al., 2012; Mok et al., 2012). Expansion mutations show incomplete penetrance that varies with age. Disease does not occur before age 35 and is fully penetrant by age 80. The issue of whether or not to perform genetic testing for *C9Orf72* repeat expansions for patients with sporadic ALS is a matter of debate in the literature at the present and revised guidelines have been proposed (Chio, Hammond, Mora, Bonito, & Filippini, 2013).

Even though repeat expansion mechanisms have been described in neurodegenerative disease before (e.g., myotonic dystrophy, Huntington disease, spinocerebellar ataxia), the *C9Orf72* expansion discovery has been a game changer in ALS/FTD study. Inclusions containing RNA binding proteins TDP-43 and FUS provided the first pathologic link that bolstered the continuum hypothesis, but the high frequency of C9ORF72 mutation, its involvement of both familial and sporadic disease, and its broad influence on other forms of neurodegeneration have provided an undeniably strong molecular link between ALS and FTD (van Swieten & Grossman, 2012).

The function of the 481 amino acid C9Orf72 protein is largely unknown, because the domains are largely unannotated, although a recent report based on bioinformatics analysis suggested the presence of DENN domains (Levine, Daniels, Gatta, Wong, & Hayes, 2013). Loss of function of C9Orf72, or gain of function by toxic RNA or protein may both play large roles (La Spada & Taylor, 2010), and these continue to be investigated. Both are possible as evidenced by loss of one alternatively spliced transcript, and the production of RNA foci in the nucleus (DeJesus-Hernandez et al., 2011).

hnRNPAB1/2

The most recent genetic discovery in the ALS-FTLD disease spectrum highlights the idea that each genetic discovery unravels

pathways and provides strong clues guiding the discoveries of novel ones. The finding that *VCP* mutations can cause multisystem proteinopathy demonstrates that these diseases provide opportunities to isolate molecular defects causing spectrum diseases like ALS, FTLD, and Paget disease. In late 2013, mutations in hnRNPA2B1 and hnRNPA1 were determined as causative of a multisystem proteinopathy, providing a clue that these might, like *VCP*, be defective in cases of ALS. This led to the discovery of a pathogenic mutation in hnRNPA1 (c.784/940G>A; p.D262/314N) and a potentially pathogenic mutation in hnRNPA1 (c.800/956A>G, p.N267/319S). Further study of the proteins encoded by these two genes is needed; however, they are excellent candidates for disease because they interact with TDP-43 directly to function in RNA metabolism. They have also been implicated as suppressors of VCP-mediated degeneration, and are sequestered in another neurodegenerative disorder (Kim et al., 2013).

GENERAL CONSIDERATIONS FOR GENETIC TESTING IN ALS AND FTLD

The purposes of genetic testing are to inform patients of their risk for developing disease, provide accurate diagnosis, and provide targeted therapies. Given the utility of this information, the accuracy of information disseminated is of extreme importance. There are difficulties that arise with regard to genetic testing in ALS and FTLD.

First, the original classification in ALS simplified the disease to two groups: those with a family history, and those without a family history. The fact that mutations in genes leading to mendelian patterns of inheritance also occur in apparently sporadic disease has led to difficulty in reporting an accurate assessment of disease risk. The discovery of C9Orf72 expansions as a common cause of ALS, FTD, and various other neurodegenerative syndromes in both sporadic and familial disease underscores that this separation is problematic.

Second, because about 90% of patients with ALS and 30–50% of patients with FTLD have no affected first-degree relatives, it may be tempting for specialists to ensure these patients that there is low risk that their children will inherit these disorders. The problem is that it is likely that apparently sporadic disease has a significant genetic contribution from common variants that contribute small effects that collectively lead to disease. It has also been demonstrated multiple times that mutations in genes causing familial disease have been found in patients with no reported family history, suggesting that rare variants can act as low-penetrance dominant alleles. A concrete distinction between familial and sporadic disease is difficult, but recent genetic discoveries are now providing clues about the earliest pathologic steps in these diseases and also provide opportunities for presymptomatic study. With this information, it is hoped that longitudinal biomarker studies will ultimately result in therapies (Talbot, 2011).

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Chapter 14

Genome-Wide Approaches to Identify Amyotrophic Lateral Sclerosis-Associated Genes

John E. Landers and Nicola Ticozzi

BACKGROUND AND NEW POINTS

Genetic factors have long been recognized in amyotrophic lateral sclerosis (ALS) and their identification is essential toward understanding the mechanisms of pathogenesis. Over time, new technologies have emerged for genome-wide screening for of factors contributing to ALS. As a result, the discovery of new genes now account for ~65% of familial ALS (FALS). Unfortunately, these advancements can only account for ~5% of sporadic ALS (SALS) despite its high heritability. Future efforts will be devoted toward to the identification of these missing heritability factors. This will likely rely on the development of novel models for the disease, advancements in high-throughput technologies, and innovative approaches to data analysis.

INTRODUCTION

The origin of human disease is often the result of a combination of genetic and environmental factors. Diseases with a strong genetic factor can usually be identified through familial clustering or segregation, as is the case for FALS, and may be the result of a single gene mutation. In contrast, diseases with a stronger environmental influence may present as sporadic in origin, such as in SALS. Here, the contributing genetic factors may be represented by several genes each resulting in an increased or decreased risk. The identification of genetic factors in FALS and SALS can contribute tremendously to the understanding of disease pathogenesis. Over the years, a wide variety of genetic techniques have been used to identify ALS-associated genes, both in sporadic and familial cases. Furthermore, technologic advances in genome-wide single-nucleotide polymorphism (SNP) genotyping and next-generation sequencing has expanded the capabilities towards this goal. This chapter describes several of these genetic techniques and how they were successfully applied to the identification of ALS-associated genes (Fig. 14–1, Table 14–1). Also discussed is the future of genetics

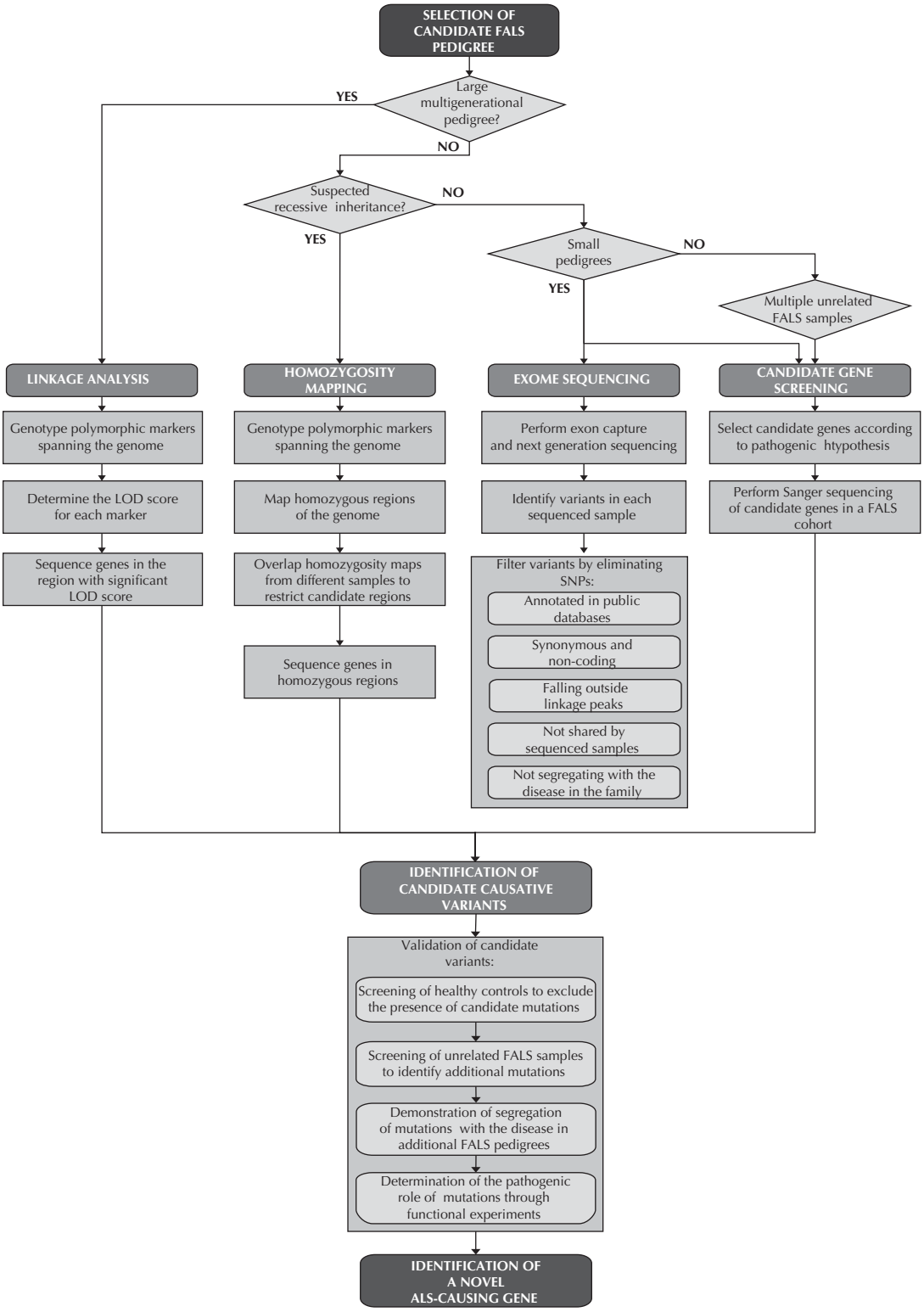

Figure 14–1. Flowchart describing approaches toward the identification of novel ALS associated genes.

Table 14–1 **Identified Genes and Loci Linked to ALS**

ALS Type	Onset	Inheritance	Locus	Gene	Protein	Year of Discovery	Identification Approaches
ALS1	Adult	AD (AR)	21q22.1	*SOD1*	Cu/Zn superoxide dismutase	1993	Linkage analysis
ALS2	Juvenile	AR	2q33-35	*ALS2*	Alsin	2001	Linkage analysis
ALS3	Adult	AD	18q21	Unknown	—		
ALS4	Juvenile	AD	9q34	*SETX*	Senataxin	2004	Linkage analysis
ALS5	Juvenile	AR	15q15-21	*SPG11*	Spatacsin	2010	Candidate gene screening
ALS(FTD)6	Adult	AD (AR)	16p11.2	*FUS*	Fused in sarcoma	2009	Linkage analysis, homozygosity mapping
ALS7	Adult	AD	20p13	Unknown	—		
ALS8	Adult	AD	20q13.33	*VAPB*	VAMP-associated protein B	2004	Linkage analysis
ALS9	Adult	AD	14q11	*ANG*	Angiogenin	2006	Candidate gene screening
ALS(FTD)10	Adult	AD	1q36	*TARDBP*	TAR DNA-binding protein	2008	Candidate gene screening, linkage analysis
ALS11	Adult	AD	6q21	*FIG4*	PI(3,5)P(2)5-phosphatase	2009	Candidate gene screening
ALS12	Adult	AR (AD)	10p15-p14	*OPTN*	Optineurin	2010	Homozygosity mapping
ALS13	Adult	Susceptibility	12q24.12	*ATXN2*	Ataxin-2	2010	Candidate gene screening
ALS(FTD)14	Adult	AD	9p13.3	*VCP*	Valosin-Containing Protein	2010	Exome sequencing, candidate gene screening
ALS(FTD)15	Adult	AD	Xp11.23-p11.1	*UBQLN2*	Ubiquilin-2	2011	Linkage analysis
ALS(FTD)17	Adult	AD	3p11.2	*CHMP2B*	Charged Multivescicular Body Protein 2B	2005	Linkage analysis
ALS-FTD1	Adult	AD	9p21.2	*C9ORF72*	C9ORF72	2011	Linkage analysis, GWAS
ALS18	Adult	AD	17p13.2	*PFN1*	Profilin 1	2012	Exome sequencing, linkage analysis

AD = autosomal-dominant; AR = autosomal-recessive; GWAS = genome-wide association studies.

in ALS research and the goals researchers hope to obtain.

LINKAGE ANALYSIS

Linkage analysis represents one of the earliest approaches to identify diseases resulting from mutations in a single gene, otherwise known as Mendelian diseases. The overall concept is based on the fact that all affected members in a given family are the result of the same genetic mutation that is passed on from generation to generation. As such, if we establish how each chromosomal segment in the genome is passed on to the progeny, we can determine the region that segregates in an identical manner as the disease phenotype. This region should define the portion of the genome that harbors the causative mutation. We can then focus on this region to identify the causative change.

To determine the segregation of each region of the genome, polymorphic DNA markers are used. In the past, DNA markers would typically be microsatellite containing dinucleotide, trinucleotide, or tetranucleotide repeat elements. The number of repeats is variable throughout the population due to the instability of the repeat elements. The markers are amplified by polymerase chain reaction using primers on either side of the repeat element and the products are separated on a polyacrylamide gel. By doing so, the size of the polymerase chain reaction product reflects the number of the repeat elements and thus the genotype at each location. Because the markers are multiallelic, individuals are often heterozygous, which simplifies the tracking of DNA segments through a multigenerational family. Typically, 400 microsatellite markers would yield sufficient coverage of the entire genome. The segregation of each DNA fragment is then established for each marker and compared with the segregation of the disease phenotype through the family. Due to the number and complexity of the calculations, these comparisons are often performed by software packages, such as Allegro (Gudbjartsson et al., 2005) or LINKAGE (Lathrop et al., 1984). The level of linkage of each genetic marker to the causative mutation is scored based on the odds of observing the segregation versus observing the pattern by chance. The $\log_{10}$ of this value represents what is known as the log of odds ratio (LOD) score for that genetic marker. The higher the LOD score, the greater is the probability that the DNA marker is located near the causative mutation. As expected, the larger the affected family, the greater is the LOD score that can be achieved. It is established in the scientific field that a LOD score of 3 (1000:1 odds) is highly suggestive of linkage. Linkage analysis typically narrows the region of interest to about a 2- to 20-Mb region.

Although a fairly straightforward methodology, linkage analysis has proved to be a difficult task when applied to ALS genetics because it requires large multigenerational pedigrees with multiple affected individuals, which are hard to find in a disease of adult life and with a rapid lethal course. For this reason, many initial attempts at identifying ALS-associated genes through linkage analysis were carried on in multiple unrelated families, with the hope of increasing the resulting LOD score. In 1991, by studying 23 pedigrees with autosomal-dominant ALS, a positive LOD score of 2.89, which was suggestive, but not conclusive for linkage, was obtained for a single marker on chromosome 21. Multipoint analysis to examine linkage of FALS to a set of four adjacent chromosome markers boosted the LOD score to 5.03 for a region 10 cM telomeric to the marker D21S58, with the highest LOD score for a single family being 2.58 (Siddique et al., 1991). Interestingly, not every pedigree examined by the researchers displayed a linkage with chromosome 21, suggesting the existence of genetic heterogeneity in FALS. In the following years, positional cloning was used to narrow the candidate region in order to identify the putative FALS-causing gene. In 1993, linkage with a marker located near the *SOD1* gene was eventually found, and the subsequent screening of *SOD1* revealed the presence of several missense mutations in FALS pedigrees linked to markers on chromosome 21 (Rosen et al., 1993). In the following years, many studies on ALS cohorts of different geographic origins confirmed that mutations in *SOD1* were a major genetic cause of ALS, being detected in ~20% of all familial cases (Robberecht & Philips, 2013).

As mentioned, linkage analysis can restrict the region of interest where a gene is located to approximately 2–20 Mb depending on the size of the analyzed family. Analyzing multiple

families with the same linkage peak could help to minimize this region further, as with the discovery of *SOD1*, but often this was not possible. Unfortunately, prior to the sequencing of the human genome, relatively few genes had been discovered and their genomic location characterized. This often left researchers at an impasse because they did not know what genes or how many genes were in a given region. Within 2–20 Mb, it was not unusual to have more than 100 genes with only a small number identified. As a result, researchers were forced to screen their region of interest for previously unidentified genes. The methodologies to identify novel genes in a region were tedious, time consuming, and technically challenging. As a result, several linkage projects were unsuccessful in identifying the causative gene for long periods of time. In some respects, the identification of *SOD1* was facilitated by the fact that this gene was one of the few that had already been discovered and confidently mapped to chr21q22.1 (Wulfsberg et al., 1983; Huret et al., 1987), whereas most neighboring genes were still unknown. As such, it is not surprising that more than 10 years and the completion of the Human Genome Project had to happen before the identification of a second causative gene in FALS. The human genome sequence helped researchers to overcome this obstacle and led to a boom to the identification of novel Mendelian genes. Researchers can now, through the Internet, quickly visualize their region of interest, identify all candidate genes in the region, and even develop primers to screen these genes for mutations. These processes could literally replace years of work in just a matter of minutes.

The field of ALS genetics benefited greatly from the Human Genome Project, as shown by the number of causative genes identified in the last decade. To find novel ALS genes, researchers mostly adopted the same multistep approach, which can be summarily broken into six subsequent procedures: (1) identification of candidate loci through linkage analysis on large ALS families, (2) screening by Sanger sequencing of the genes mapping to the region, (3) screening of healthy control subjects to exclude the presence of candidate mutations, (4) validation of mutations on unrelated ALS families, (5) demonstration of segregation with the disease in additional families, and (6) determination of the pathogenic role of mutations through functional experiments. To show how successful this approach has been in identifying ALS-associated genes, two examples are provided.

In 2003, by performing linkage analysis using polymorphic microsatellite markers on a multigenerational North American pedigree with 13 members affected by autosomal-dominant typical ALS, Sapp and coworkers identified a novel putative locus on chr16p12.1-q21 (ALS6) spanning 37.8 Mb from markers D16S764 to D16S3053 with a multipoint LOD score of 3.29. The association was confirmed in a second North American pedigree (Sapp et al., 2003). Unfortunately, more than 200 genes mapped to the ALS6 locus, making the screening for the causative mutation an almost impossible task. In 2009, however, by performing a loss-of-heterozygosity (LOH) analysis (discussed in detail later), Kwiatkowski and coworkers identified a major LOH cluster within the ALS6 locus in a Cape Verdean inbred family, thus narrowing the candidate region to 4.4 Mb on chr16p11.2 consisting of 56 genes. By screening these genes, missense mutations in the nuclear localization signal of the *FUS* gene were found in all the previously identified ALS6 pedigrees (Kwiatkowski et al., 2009; Vance et al., 2009). Interestingly, although most *FUS* mutations display a dominant transmission pattern, the H517Q variant found in the Cape Verdean pedigree is inherited in a recessive fashion. The identified mutations were shown to be absent in healthy control subjects and to segregate with the disease in unrelated ALS families. Following the original reports, several other groups identified additional variants in ALS cohorts of different ethnicities, proposing an overall mutational frequency of ~4% in FALS and ~1% in SALS (Robberecht & Philips, 2013). To date, more than 30 pathogenic variants have been described, most of which are missense substitutions and the rest are frameshift or nonsense mutations. Functional data strongly supported the pathogenic role of mutations in the *FUS* gene, because postmortem analysis of patients showed FUS-immunoreactive inclusions within the cytoplasm of motor neurons, and *in vitro* experiments suggested that mutations induce a nucleocytoplasmic redistribution of the protein with formation of insoluble aggregates (Kwiatkowski et al., 2009).

A similar approach has led in 2011 to the discovery of pathogenic mutations in the *UBQLN2* gene in a large multigenerational ALS family with 19 affected individuals displaying an X-linked dominant mode of inheritance (Deng et al., 2011). Linkage analysis using chromosome X microsatellite markers identified a candidate region spanning 21.3 Mb between markers rs6417786 and DXS1275 on chrXp11.23-q13.1. The genes mapping to this region were then filtered based on their expression profile, function, structure, and potential relevance to ALS pathogenesis, thus leaving 41 candidates. The screening of these genes by Sanger sequencing revealed the presence of a missense substitution in the PXX domain of the *UBQLN2* gene. Similarly to what has been described for *FUS*, *UBQLN2* mutations have been shown to segregate with ALS in unrelated families, and to be absent in healthy control subjects. Additionally, ubiquilin2 pathology has been described in motor neurons of mutated patients, and *UBQLN2* mutations have been shown to lead to an ubiquitin-mediated impairment of protein degradation.

CANDIDATE GENE APPROACH

An alternative method that has been taken to identify causative genes for ALS is the candidate gene approach. Here, scientists test for mutations based on some specific characteristic of the gene, such as decreased expression in patients with ALS or functional relationships to pathways altered in ALS. This approach can be dangerous and result in false-positives. As shown by recent studies, most variants in the genome are actually rare, and each individual carries several hundreds of novel variants. In fact, over 80% of coding variants have allele frequencies less than 0.5% (Tennessen et al., 2012). In addition, many genes demonstrate an increased rate of variation, possibly due to a lack of selective pressure. Based on this knowledge, identifying variants in single cases that are not seen in control cases is possible, if not probable. This possibility increases with the number of cases sequenced.

To date, only a single major ALS gene has been convincingly identified through this approach, namely *TARDBP*. The gene was a natural candidate to hunt for disease-associated mutations, because it encodes for the protein TDP-43, which has been conclusively shown to be the major proteinaceous component of cytoplasmic ubiquitinated inclusions in motor neurons of patients with ALS (Neumann et al., 2006). In fact, learning the lesson from other neurodegenerative diseases, pathogenic mutations have been consistently found in genes encoding for proteins forming neuronal inclusions, such as *PRNP* in prion diseases (Owen et al., 1989), *APP* in Alzheimer disease (Goate et al., 1991), *HTT* in Huntington disease (Group 1993), *SNCA* in Parkinson disease (Polymeropoulos et al., 1997), and *MAPT* in frontotemporal dementia (FTD; Hutton et al., 1998). The screening of the *TARDBP* gene in several ALS cohorts of different geographic origin revealed the presence of missense mutations mostly clustered in exon 6, which encodes for the C-terminal domain of TDP-43. Similarly to *FUS*, *TARDBP* mutations lead to a nucleocytoplasmic redistribution of the protein with formation of TDP-43 immunoreactive inclusions within the cytoplasm of motor neurons of mutated patients. However, to underscore how tenuous is the candidate gene approach, the authors of the original report felt confident that *TARDBP* was indeed a causative ALS gene only after demonstrating linkage to chr1p36, which contains the *TARDBP* locus (Sreedharan et al., 2008). In summary, few ALS genes have been discovered through the candidate approach, which remains the least reliable and most prone to false-positive hits among all methods used for identification of novel genes so far.

GENOME-WIDE ASSOCIATION STUDIES

Linkage analysis is ideal for the identification of a single gene trait with a high penetrance and low environmental component. However, in the case of complex disorders, this approach is not always the best choice. Complex diseases are often characterized by the contribution of multiple genes contributing to an increased risk and a higher environmental influence. Initial attempts to determine whether variants in a gene predisposed to susceptibility to complex diseases were modeled on case-control association studies. In brief, association studies

would measure the allele frequencies of variants present within a candidate gene in a case (affected) and control (unaffected) population. A significantly increased allele frequency is indicative that a variant in a gene is associated to disease susceptibility. It should be clarified that the variant displaying the increased frequency may not necessarily be the variant functionally contributing to the increased risk. Rather, the genotyped variant may solely represent a surrogate marker for the functional variant on the same genetic background (haplotype) that is the actual contributor to the increased risk.

Association testing was very prevalent due to its simplicity and the relatively low cost of genotyping. In fact, prior to 2005, there were more than 9,000 published gene association studies. These studies were focused on a single or small number of candidate genes. The reason for this is that the cost of genotyping enough SNPs to cover all genes in the genome (several hundred thousands) was cost-prohibitive. Unfortunately, despite the large number of publications, nearly all association studies (>95%) cannot be consistently replicated and likely represent false-positives (Hirschhorn et al., 2002). Several reasons contribute to this lack of replication. First, association studies are subject to publication bias. That is, positive results for association studies can be more easily published compared with negative association. As such, if numerous researchers are testing for association to the same candidate gene, it is quite likely that one study will show a significant association by chance alone. This study will likely be published, whereas the others will not. Second, due to the ease and relatively low cost of association studies, researchers can test numerous genes for association. However, often a multiple testing correction is not applied. As a result, association results that are again based on type I errors are published. Finally, association studies are highly dependent on homogeneity of the genetic background of cases and control subjects, because differences in allele frequencies of variants are routinely observed in differing ethnic populations. Controlling this factor is extremely difficult because tests for population stratification require genotyping a large numbers of variants.

Many of these issues were alleviated in the late 1990s with the introduction of products that genotyped hundreds of thousands of SNPs at a reasonable cost allowing genome-wide association studies (GWAS) to be performed. First, because all genes are tested simultaneously, there is no bias of gene selection. Furthermore, all tests are performed simultaneously permitting a comparison of every *P* value with all others and applying multiple test corrections can also minimize false-positives. Additionally, the large number of genotyped SNPs allows a comparison of the ethnic background of the case and control group such that outliers can be thrown out and positive hits can be further tested to ensure they are not due to population stratification.

In the past 5 years, several large case-control GWAS have been performed with the hope of identifying common genetic variants associated to SALS susceptibility and/or phenotypic traits. Unfortunately, these massive, costly efforts led by multinational teams have mostly met with disappointing results. Several candidate SNPs have been identified in nongenic regions of the genome, thus making their association with SALS susceptibility difficult to demonstrate. Also, odds ratios associated to candidate SNPs were often negligible. Even more disturbing, with few notable exceptions, most variants that reached statistical significance after correction for multiple testing could not be replicated throughout different GWAS. The major conceptual flaw responsible for this outcome is in all likelihood the fact that GWAS are designed to identify common risk variants, on the assumption that complex disorders are influenced by common, weakly associated alleles (common disease-common variant hypothesis), whereas recent evidence points out that rare genetic variants may be responsible for a large part of ALS heritability.

Two notable exceptions that stand out from the otherwise unsatisfactory GWAS in ALS are represented by the identification of an association with two loci on chr19p13.3 and chr9p21. In 2009, a GWAS conducted on 2,323 patients with SALS and 9,013 control subjects, followed by replication in a second independent cohort of 2,532 cases and 5,940 control subjects, revealed genome-wide significance for the SNP rs12608932 on chr19p13.3, with $p = 1.30 \times 10^{-9}$ (van Es et al., 2009). Interestingly, rs12608932 lies within the *UNC13A* gene that encodes for a protein regulating the release of neurotransmitters at neuromuscular synapses. After the

original report, the association with *UNC13A* has been confirmed by another GWAS (Shatunov et al., 2010), and rs12608932 has also been proposed as a modifier of survival in two independent ALS cohorts (Diekstra et al., 2012; Chio et al., 2013).

However, the major contribution of GWAS was surprisingly not toward the identification of risk factors associated to SALS susceptibility, rather than to the discovery of a novel FALS gene. Starting from 2006, several research groups identified through linkage analysis a major disease locus spanning 3.7 Mb on chr9p21 in multiple ALS, FTD, and ALS-FTD families of different geographic origin (Boxer et al., 2011; Gijselinck et al., 2010; Le Ber et al., 2009; Luty et al., 2008; Morita et al., 2006; Pearson et al., 2011; Valdmanis et al., 2007; Vance et al., 2006). At the same time, several GWAS on both ALS and FTD pointed to the chr9p21 locus, further restricting the candidate region to 232 kb (van Es et al., 2009; Shatunov et al., 2010; Van Deerlin et al., 2010; Laaksovirta et al., 2010; Mok et al., 2012). Although the region was small enough to be studied by Sanger sequencing, the causative mutation eluded identification for quite some time.

Eventually, two groups independently reported that a large hexanucleotide GGGGCC repeat within the first intron of the *C9orf72* gene was responsible for ALS and/or FTD cases previously linked to the chr9p21 locus. Observing that patients within chr9p21-linked families seemed to be homozygous for the C9orf72 locus, and that the affected progeny seemed not to inherit an allele from the affected parent, DeJesus-Hernandez and coworkers hypothesized that the apparent lack of segregation could be due to the presence of an unamplifiable repeat expansion on one allele (DeJesus-Hernandez et al., 2011). Renton and coworkers reached the same conclusion by performing a targeted resequencing of the chr9p21 region, and noticing a misalignment of sequencing reads within the first intron of *C9orf72* (Renton et al., 2011). Since the original reports, the hexanucleotide repeat expansion in the *C9orf72* gene has been found to be the major genetic cause of ALS in many populations, with a mutational frequency of 20-40% in familial cases and 5% in sporadic ones (Robberecht & Philips, 2013).

HOMOZYGOZITY MAPPING

To date, most causative mutations identified for FALS display a dominant mode of inheritance. In other words, a single mutant allele is sufficient to produce the disease. Often, these mutations are due to a toxic gain of function or a dominant negative effect on the protein produced from the normal allele. In some cases, dominant diseases are due to a haploinsufficiency of the wild-type protein. Alternatively, there are many diseases that display a recessive mode of inheritance. These diseases require mutations in both maternal and paternal alleles. Cystic fibrosis is a classic example of a recessive disorder. Linkage analysis can be used for the identification of recessive causative genes. However, because both parents are required to harbor a mutant allele, and assuming the mutant allele is rare in the population, the disease is typically not passed down through multiple generations. As a result, linkage analysis for recessive disorders can be more difficult. Furthermore, due the late-onset of ALS, and that often individuals that harbor a mutation do not always develop the disease (incomplete penetrance) the analysis is even more complicated.

Due to these difficulties, many researchers have taken an alternative approach to identify recessive disease-causing genes. This approach, homozygozity mapping, is focused on using consanguineous families afflicted with a recessive disorder, which occur more commonly in a context of consanguinity. Typically, a person with a recessive disorder would require both parents to harbor a rare mutant allele. Because these alleles are rare, the likelihood of two such individuals mating is low. However, if an ancestor of a consanguineous marriage harbors a mutant allele, the chance of the progeny receiving a homozygous mutant allele is greatly increased. This is due to the fact that in consanguineous matings, the parents share a significant amount of DNA from the same ancestral background. The amount of shared material is dependent on how distantly related are the parents. For instance, siblings share 50% of their genetic material and first cousins share 12.5%. Geneticists are able to take advantage of consanguineous matings to identify the location of a recessive disease gene. Homozygozity mapping is based

on the premise that the homozygous mutation in progeny is present within a large segment of the genome that is ancestrally shared and thus homozygous. Similar to traditional linkage analysis, polymorphic DNA markers are genotyped throughout the genome to map the homozygous regions within the progeny's genome likely harboring the recessive genetic mutation. If multiple affected siblings are observed, the overlapping homozygous regions from each individual can help further narrowing the region of interest. Additionally, if multiple consanguineous families that are thought to originate from a common ancestral mutation (such as a confined geographic area) are available, the overlapping region from the different families may be used to narrow the region harboring the gene mutation even further. Once the region of interest is established, the genes within can then be screened for homozygous mutations.

The application of homozygosity mapping to the field of ALS genetics has been already mentioned while discussing the discovery of the *FUS* gene, where this approach was crucial in restricting the candidate region obtained from linkage analysis, thus allowing the researchers to sequence the genes mapping to it. More recently, by performing a genome-wide scan followed by selecting runs of homozygous SNPs in Japanese patients from consanguineous pedigrees, Maruyama and coworkers were able to identify a 2.5-Mb LOH region on chr10p13 shared by four individuals belonging to three apparently unrelated families (Maruyama et al., 2010). In two consanguineous siblings, the sequencing of the 17 genes mapping to the region revealed the presence of a homozygous 1.8-kb deletion encompassing exon 5 of the *OPTN* gene. Two additional unrelated patients were found harboring a homozygous Q398X nonsense mutation in exon 12, leading to premature truncation of the mature protein. These individuals shared a 0.9-Mb haplotype containing the *OPTN* gene, strongly suggesting that Q398X derives from a common ancestor. Lastly, a missense heterozygous mutation was also identified in two other families. *OPTN* mutations are believed to disrupt the nuclear factor-kB signaling pathway and, similarly to other ALS-causing genes, OPTN-immunoreactive cytoplasmic inclusions have been found in motor neurons of mutated patients.

EXOME SEQUENCING

It is estimated that the Human Genome Project cost nearly $3 billion and more than 10 years to complete. Although this accomplishment had a strong impact on scientific research, it was economically unfeasible for individual researchers to perform whole-genome sequencing on any population or sample of interest. However, major technological developments over the past several years have dramatically reduced the cost, time, and labor required to sequence the human genome. Although methodologies vary, these approaches are focused on obtaining small amounts of sequence information on millions of fragments simultaneously (massively parallel short-read sequencing). As a result, the cost of whole-genome sequencing in 2013 has been reduced to less than $10,000 per sample. The dramatic price reduction caused by next-generation sequencing methodologies has had a profound effect on scientific research.

Although the cost of whole-genome sequencing has dropped dramatically, it is still prohibitive for many scientific projects. Furthermore, the understanding of alterations outside of the coding/untranslated region of genes is still in its infancy. Due to these limitations, several products have been developed that are able to restrict the process of next-generation sequencing to the protein coding regions of the genome. Given that these regions represent only ~2% of the entire genome, this approach cuts sequencing costs dramatically, thus permitting projects that were otherwise prohibitive. These exome capture products function in a very similar manner. In essence, sequences representing the coding or exonic region of the genome (exome) are attached to a solid surface (e.g., beads). The genomic DNA of interest is then sheared and hybridized to the beads. The beads are then washed and the remaining DNA is eluted. The resultant DNA is enriched for the exome region and can then be subject to next-generation sequencing. Exome sequencing results in tens of millions of short reads of raw DNA sequencing. The sequences must then be aligned to the genome and further analyzed to establish the location of DNA variants and their annotations (gene location, effect of variants, and so forth). As a result, the identification of novel causative genes using exome sequencing has a much stronger bioinformatics component compared with previous methodologies.

One advantage of exome sequencing is that it can be combined with linkage analysis to further increase the speed of identifying mendelian genes. As described previously, linkage analysis often restricts the region of interest to about 2–20 Mb. Previously this required researchers to then sequence each individual gene in the region to identify the causative mutation. Furthermore, if a candidate causative mutation was identified, it required researchers to genotype or sequence that gene in a large control population. Through exome sequencing, this approach is simplified because it can sequence ~80–90% of all genes in a single experiment. Additionally, several large-scale sequencing projects have led to the development of public databases containing variants observed in general population. These databases can easily be surveyed to establish whether a candidate mutation is present in the general population and thus likely to be benign. Two such examples are the National Heart, Lung, and Blood Institute's Exome Sequencing Project and the 1000 Genomes Project (1000 Genomes Project Consortium et al., 2010; Tennessen et al., 2012). The Exome Sequencing Project currently consists of exome sequencing data from more than 6,300 individuals that can easily be queried through an easy-to-use World Wide Web interface. Similarly, the 1000 Genome project currently has data on 1,092 individuals; however, this information is a combination of whole-genome data, exome data, and low-coverage sequencing data. It should be noted that screening of these databases should not be the sole source of variant testing of control subjects. The lack of a variant in a database may be the result of several factors including poor capture, low quality control, or difficulties in the alignment of the sequencing reads due to homologous gene family members. It is essential to confirm any result through the direct analysis of control samples.

A well-established analytical pipeline has been adopted by most exome sequencing studies to identify causative mutations in Mendelian diseases. In general, the two most distantly related affected individuals of a family are sequenced to reduce the background of noncausal variants. Variants are then filtered through several steps, by eliminating those already annotated in public databases, synonymous and noncoding SNPs, and those not shared by both individuals sequenced. If the pedigree under study contains multiple affected individuals, the variants not segregating with the disease and those falling outside linkage peaks are also eliminated. Through this filtering process, hundreds of thousands of SNPs identified through exome sequencing can be reduced to a handful of candidate variants that can be further validated.

Applying this approach to a multigenerational Italian ALS-FTD pedigree, Johnson and coworkers identified six heterozygous variants of interest, one of which was contained in the *VCP* gene (Johnson et al., 2010). *VCP* was an obvious candidate, because missense mutations in the gene had already been proved to be causative for inclusion body myopathy, Paget disease of bone, and FTD, a disease that shares significant phenotypic overlap with ALS-FTD. Thus, several methods can be applied to the identification of novel ALS genes, in this case exome sequencing and the candidate gene approach, to maximize the chances of success. Since the original report, *VCP* mutations have been described in less than 1% of patients with FALS with or without associated FTD (Robberecht & Philips, 2013).

The first ALS gene identified through a "pure" exome sequencing approach was *PFN1*. By performing exome sequencing on affected individuals from two large Caucasian and Sephardic Jewish autosomal-dominant FALS pedigrees, and applying the filtering procedures aforementioned, Wu and coworkers discovered that the two families harbored two different missense mutations within a single common gene, *PFN1*, which encodes for the actin-binding protein profilin1 (Wu et al., 2012). The screening of a large cohort of FALS index cases further revealed additional families harboring mutations in the *PFN1* gene, and cosegregation with the disease was proven in two of them. With the exception of a single E117G variant, which was found in healthy control subjects, albeit with a reduced frequency compared with FALS, *PFN1* mutations were not present in the normal population. Functional experiments confirmed the pathogenic role of mutations, by demonstrating that, similarly to other ALS-causing genes, *PFN1* mutants form insoluble aggregates in the cytoplasm of transfected cells, which in many cases often contain TDP-43. Additionally, in primary motor neurons, mutant *PFN1* displays decreased bound actin levels, inhibits axon outgrowth, and

leads to smaller growth cones with a reduced F/G-actin ratio. These observations helped to document that cytoskeletal pathway alterations contribute to ALS pathogenesis.

FUTURE DIRECTIONS

The future identification of additional ALS-associated genes will undoubtedly be based on large-scale genome-wide sequencing efforts. We have already seen the beginnings of this contribution through the identification of two ALS genes by exome sequencing. Over time, the price of whole-genome sequencing efforts will continue to drop and permit gene-identification efforts that were previously cost-prohibitive. One major area where these advances in sequencing will contribute is in the understanding of SALS. Currently, few genetic components of SALS are known. Repeat expansions within *C9orf72* are observed in ~5–10% and mutations in *SOD1* account for ~2% of SALS cases. Given that SALS reflects 90% of all ALS cases, it is imperative to identify additional genetic defects contributing to it. Twin studies have found a high heritability for SALS (h = 0.38–0.85), thus suggesting that genetic factors have a significant role in determining the susceptibility to the disease (Graham, Macdonald, & Hawkes, 1997). It is likely that numerous genetic factors may impart an increased (or decreased) risk for ALS. However, detecting these risk factors will not be simple. This has been illustrated by the GWAS in SALS that ultimately required many thousands of samples to be analyzed before an association with *UNC13A* and the *C9orf72* region could be observed.

As indicated previously, GWAS studies are indirect association tests that are dependent on linkage disequilibrium between the actual risk factor and the genotyped allele. They also rely, in part, on the presence of a single risk allele of significant higher abundance than the general population. However, if an ALS-associated gene contains numerous different variants of lower allele frequencies, derived from different ancestral events (and thus on differing haplotype backgrounds), it will be more difficult to discover through GWAS study. For example, the repeat expansion within chromosome 9 seems to have occurred in a single ancestral event because all cases were observed on the same haplotypic background (Smith et al., 2013). As a result, identifying indirect association with a common allele on the expanded repeat haplotype background was indeed possible.

In the future, whole-genome sequencing efforts will alleviate some of the issues of indirect association studies. Scientists will be able to conduct direct association testing for risk alleles thus eliminating the need for linkage disequilibrium with a common genotyped allele. Furthermore, it is anticipated that testing of multiple alleles within a given gene region will increase the power of these studies. However, several new technical and statistical challenges will also need to be overcome. The analysis of genome-wide variants, which will be several million per individual, will require very powerful computer resources. Additionally, analyzing individual SNPs will likely increase the number of false-positives per study. Current GWAS studies attempt to address this problem by incorporating a Bonferroni multiple-test correction. This is typically based on ~500,000 tests (the number of genotyped SNPs for the study) thus requiring a p value less than 10^{-7} for significance. With genome-wide sequencing, the number of variants, and thus the number of tests, will expand exponentially. As a result, it is possible that many more samples will be needed to identify risk factors using genome-wide sequencing approaches.

In addition to SALS, it is anticipated that increased effort will be devoted toward identifying modifying genes for ALS. In fact, patients with ALS can be grouped into several subphenotypes according to age of onset, site of onset, survival, distribution of signs of upper and lower motor neuron dysfunction, and presence of cognitive impairment. Identifying the genetic factors contributing to these subphenotypes can help us understand the progression and pathogenesis of the disease itself.

In conclusion, the different techniques described in this chapter allowed for the identification of the pathogenic mutations responsible for ~65% of FALS and ~5% of SALS cases. Undoubtedly, a clever combination of these approaches on a case-by-case basis will still be instrumental in the future to the discovery of novel ALS-related genes, as demonstrated by the identification of *FUS*,

C9orf72, and *PFN1*. However, the key to the resolution of ALS missing heritability will likely rely on the development of novel epidemiologic and genetic models for the disease, high-throughput and low-cost sequencing platforms, and innovative statistical tools for data handling and analysis.

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Chapter 15

Pathology of Amyotrophic Lateral Sclerosis and Frontotemporal Lobar Degeneration

Andrew King and Safa Al-Sarraj

BACKGROUND AND NEW POINTS

Basic microscopic findings in amyotrophic lateral sclerosis (ALS) were noted long ago, but were supplemented with the discovery of ubiquitinated inclusions in neurons using modern immunohistochemical staining techniques.

The new staining techniques have led to discoveries of specific abnormal protein aggregations associated not only with ALS but also with frontotemporal lobar degeneration (FTLD), and combinations of the two in specific patients have defined ALS as a proteinopathy. Some of the protein inclusions are associated with mutations in associated genes. At this time, the role aggregated proteins play in the pathogenesis of ALS remains unclear.

INTRODUCTION

Marked advances in describing the pathologic features of ALS have occurred over the past 5–10 years. Although the first pathologic descriptions of the disease date back to Charcot and colleagues in the 19th century, advances lagged behind other neurodegenerative diseases because of difficulty finding specific protein abnormalities associated with the disease. Leigh et al. (1988) and Lowe et al. (1988) described ubiquitinated inclusions in anterior horn neurons in 1988, but whereas tau protein was described in Alzheimer disease and α-synuclein in Lewy body disease, the protein abnormality associated with ALS remained elusive: a "proteinopathy without a protein." This all changed in 2006 when two groups independently described the ubiquitinated form of a protein called TAR DNA-binding protein 43 (TDP-43) in the central nervous system of patients with ALS and in some cases of FTLD (Neumann et al., 2006; Arai et al., 2006). This not only allowed ALS research to advance at a more rapid pace, it also provided further evidence for the existence of a clinicopathologic spectrum incorporating both ALS and FTLD.

DEFINITIONS

Before one can attempt to understand some of the pathologic features of ALS and FTLD one must first understand the various definitions. These are presented in this chapter and include the concept that there is very probably a clinicopathologic spectrum that exists between the conditions.

ALS/Motor Neuron Disease

ALS, or motor neuron disease (MND), is a progressive neurodegenerative disorder characterized by degeneration of motor neurons of the motor cortex, brainstem, and spinal cord. Clinically and pathologically there is usually evidence of both upper and lower motor neuron degeneration, and sometimes with degeneration of brainstem motor nuclei. There is associated atrophy of skeletal muscles, including respiratory muscles, leading to respiratory failure, usually in a relatively short time (median survival about 3 years). By convention, excluded are forms of MND that have clear genetic causes, such as bulbospinal muscular atrophy (Kennedy disease) and spinal muscular atrophy (Werdnig-Hoffmann disease and Kugelberg-Welander syndrome).

FTLD

FTLD is a pathologic description of a neurodegenerative process where there is preferential atrophy of the frontal and/or temporal elements of the cerebral hemispheres. The pathologic term "frontotemporal lobar degeneration" is not synonymous with the clinical term frontotemporal dementia (FTD).

Cortical atrophy in FTLD can be symmetrical or asymmetrical, and the pathology is usually visualized microscopically in the form of neuronal loss in affected lobes. The cases are usually associated with accumulation of an abnormal protein (e.g., TDP-43 or tau), and are referred to as FTLD-TDP or FTLD-tau. When considering the relationship with ALS it is FTLD-TDP (or FTLD-MND/ALS), or occasionally FTLD-FUS that one is concerned with, and not FTLD-tau.

Clinically FTLD usually presents as one of three syndromes (although there may sometimes be overlap):

1. FTD (sometimes called behavioral type [bv-FTD]). A key symptom is loss of social inhibition.
2. Progressive nonfluent aphasia. There is a severe language disturbance, but sometimes with relative preservation of memory.
3. Semantic dementia. Semantic memory is particularly affected but with relative preservation of episodic memory.

FTLD-MND/ALS

It was reported many years ago by Jackson, Lennox, and Lowe (1996) and others that some patients with ALS develop a frontotemporal type dementia, and some patients with FTD (usually bv-FTD) later develop symptoms of ALS. This led to the suggestion of a clinicopathologic spectrum: when these processes occur together it is described as FTLD-MND/ALS, or sometimes as FTLD-MND or FTLD-ALS.

There is some disagreement as to what actually constitutes this combined process because many patients with ALS have some degree of cognitive decline. It is probably best to confine the entity of FTLD-MND/ALS to those with clinical evidence of FTD (or language disturbance or semantic dementia) with clinical evidence of ALS and pathologic evidence of FTLD (usually TDP-43 positive inclusions in frontal/temporal lobes) with pathologic evidence of ALS (usually TDP-43–positive inclusions in motor neurons of cord, brainstem, and motor cortex; Table 15–1).

PROTEIN INCLUSIONS

Four major protein inclusions (excluding tau, which is not associated with ALS) are found in neurons in cases of ALS and FTLD. They are detected by an immunohistochemical staining technique where an antibody previously raised against a particular protein is used to expose that protein in tissue sections.

Ubiquitin

Ubiquitin is an important protein that the cell uses to rid itself of toxic or unwanted proteins. In the process known as "ubiquitination" the small protein ubiquitin becomes attached to the target protein, which therefore becomes "ubiquitinated." Subsequently, this target protein is shuttled to a protease complex called the 26S proteasome where it is degraded (Lehman, 2009). As such, ubiquitin is therefore not a specific marker in ALS or FTLD, because it is seen also in neurofibrillary tangles in Alzheimer disease and in Lewy bodies.

p62

p62 (also known as sequestosome 1) is an ubiquitin-binding protein that may aid in protein destruction by the proteasome system. It has a similar immunohistochemical staining pattern to ubiquitin, and is also not a specific marker. It is often assessed because it gives a "cleaner" picture on stains than does ubiquitin.

TDP-43

TDP-43 is a nuclear protein that can bind with DNA and RNA and is believed to have a role in transcription and translation. In ALS and FTLD, however, abnormal forms of the protein can be detected in the cytoplasm. Its presence is a (relatively) specific marker for most cases of ALS, FTLD-MND/ALS, and FTLD-TDP.

FUS

Fused in sarcoma (FUS) protein is another nuclear protein that can bind DNA and RNA, and may have roles in translation/transcription and nuclear transport. In a small proportion of ALS and FTLD cases, abnormal forms of the

Table 15–1 **Proposed Clinicopathologic Spectrum of ALS, FTLD-MND/ALS, and FTLD-TDP**

ALS	FTLD-MND/ALS	FTLD-TDP
← **Clinical Features** →		
Muscle weakness/wasting Bulbar symptoms Upper motor symptoms	Loss of inhibition Cognitive decline Muscle weakness/wasting Bulbar symptoms Upper motor symptoms	Loss of inhibition Cognitive decline
← **Pathologic Features** →		
Loss of motor neurons in cord, brainstem, motor cortex Inclusions (usually TDP-43) in motor neurons. Sometimes limbic regions	Loss of neurons: frontal, temporal lobes Inclusions (usually TDP-43) in frontal/temporal cortex and limbic regions Loss of motor neurons in spinal cord, brainstem, motor cortex Inclusions (usually TDP-43) in motor neurons	Loss of neurons frontal, temporal lobes Inclusions (usually TDP-43) in frontal/temporal cortex and limbic regions

protein are found in the cytoplasm, and therefore it is a marker for occasional cases of familial ALS (FALS) and occasional cases of FTLD (FTLD-FUS).

PATHOLOGY OF SPORADIC ALS

Although most cases of ALS are sporadic, approximately 10% of cases have some family history. This distinction has been further complicated by the recent discovery of mutations in the *C9ORF72* gene, which is also found in occasional cases of sporadic ALS.

The pathologic features in ALS include neuronal loss in the anterior horn neurons of the spinal cord, the motor cortex, and the brainstem. The anatomic sites of the pathology are illustrated in Figure 15–1.

Spinal Cord

Macroscopically there may be thinning and a greyish appearance of anterior nerve roots. There may also be discoloration in the region of the lateral corticospinal tracts on the cut surface of the spinal cord.

Microscopically the regions most affected are the cervical and lumbar regions because they innervate muscles in the upper and lower limbs, respectively. There is usually evidence of loss of anterior horn neurons, and is often associated with reactive changes, such as reactive astrocytosis and microglial activation. Surviving neurons are often shrunken (Fig. 15–2) and show chromatolysis (pink appearance on conventional hematoxylin and eosin stains). There may be evidence of neuronophagia (neurons being attacked by cells, such as macrophages) and the presence of small intraneuronal pink bodies called Bunina bodies.

A myelin stain (Luxol fast blue/Nissl) usually reveals focal loss of myelin, especially in the lateral corticospinal tracts, which in turn indicates loss of neurons in the motor cortex (upper motor neuron damage) (Fig. 15–3).

In the surviving neurons of the anterior horns there are inclusions that are not visible on hematoxylin and eosin stains but are detected on antibodies to ubiquitin, p62, and TDP-43 (Fig. 15–4). Classically they are in the form of skeins

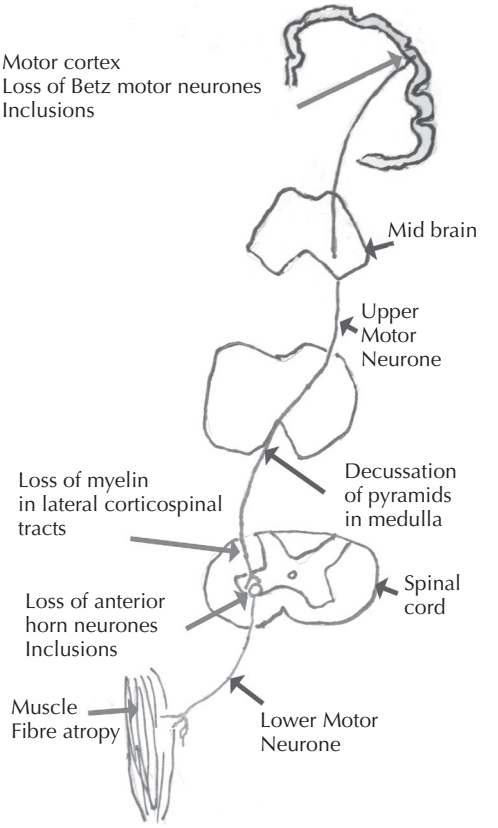

Figure 15–1. Diagram showing the areas where pathologic features are usually seen in cases of ALS.

(threadlike) (Fig. 15–4a), but can be more globular in form (Fig. 15–4b). The different shapes are considered to reflect different stages in the formation and processing of the abnormal protein. This protein can in many cases also be seen in the glial cells of the cord (Fig. 15–4 inset).

Occasionally, in advanced cases of ALS, the situation develops where the neuronal loss is so advanced that no neuronal inclusions can be detected. In such cases TDP-43–positive glial inclusions can often still be seen.

Brainstem

Macroscopically usually no abnormalities are evident.

Microscopically the most affected region is the XIIth nerve nucleus (pure motor nerve innervating the tongue) in the medulla (the floor of IVth ventricle). There may be evidence of neuronal loss, and gliosis. In surviving neurons

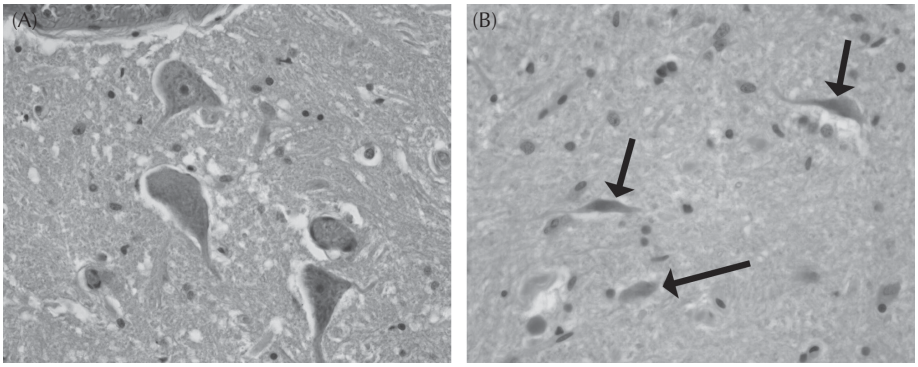

Figure 15–2. Spinal cord (anterior horn) motor neurons from (A) control case, and (B) sporadic ALS. Note atrophic neurons in the ALS case (arrows).

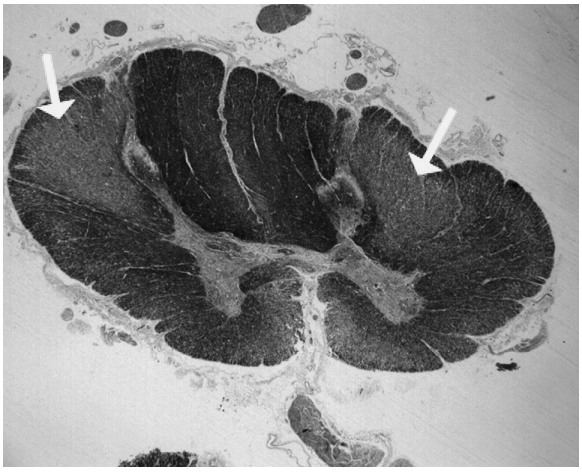

Figure 15–3. A section through spinal cord and stained for myelin (Luxol fast blue/Nissl). The arrows indicate loss of myelin over the regions of the lateral (crossed) corticospinal tracts, indicating upper motor neuron damage and anterograde loss of axons and myelin.

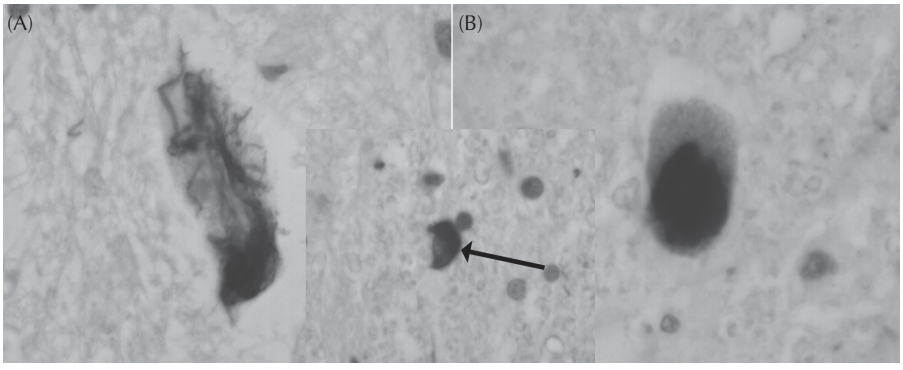

Figure 15–4. (A) TDP-43–positive skein and (B) TDP-43–positive globular inclusion within anterior horn neurons of the spinal cord in cases of sporadic ALS. The inset shows a TDP-43–positive glial cytoplasmic inclusion (arrow) in the cord in a case of sporadic ALS (antibody to TDP-43).

there are often ubiquitin-, p62-, and TDP-43–positive neuronal cytoplasmic inclusions. There also may be TDP-43–positive glial cytoplasmic inclusions similar to those seen in the cord.

Motor Cortex

Macroscopically usually no abnormalities are evident. Although textbooks sometimes suggest that there is thinning of the precentral gyrus (the motor strip), in our experience this is rare.

Microscopically there is usually evidence of loss of the large motor neurons (Betz cells) in the motor cortex. Some experience is needed seeing them in normal motor strips before one can confidently assess whether they are reduced in numbers in pathologic cases. Often the neuronal loss is accompanied by activation of microglial cells, which can be demonstrated immunohistochemically. Ubiquitin-, p62-, and TDP-43–positive neuronal cytoplasmic inclusions and glial inclusions are sometimes seen, but are often more difficult to detect than in the spinal cord and brainstem.

There are occasional cases of so-called primary lateral sclerosis where only upper motor neuron pathology is observed, and also cases of severe progressive muscular atrophy with only lower motor neuron pathology evident.

Extramotor Structures

Occasional cases show extramotor inclusions, TDP-43–positive neuronal cytoplasmic inclusions usually in the hippocampus (dentate fascia and pyramidal layer) and amygdala with no significant associated neuronal loss and also not affecting the cerebral cortex. The significance of these features is uncertain, but may provide a pathologic link with FTLD-MND/ALS (see Table 15–1) or it may just be incidental. Interestingly, occasional cases of Alzheimer disease also show similar TDP-43–positive inclusions in the amygdala and/or hippocampus. The significance of this is also uncertain.

Muscle

Affected skeletal muscle in ALS usually shows evidence of denervation with small angulated fibers, small fiber group atrophy (Fig. 15–5a), and "target" fibers (on special NADH-TR stains; Fig. 15–5b). If the process is relatively longer term there may be evidence of fiber-type grouping (grouping together of similar fiber types) indicating extensive reinnervation of the muscle.

PATHOLOGY OF FAMILIAL ALS (FALS)

The nature of pathologic findings depends upon the mutation, and some changes are identical to sporadic cases of ALS, whereas others have additional or totally different pathologic features (Ince et al., 2011; Al-Chalabi et al., 2012).

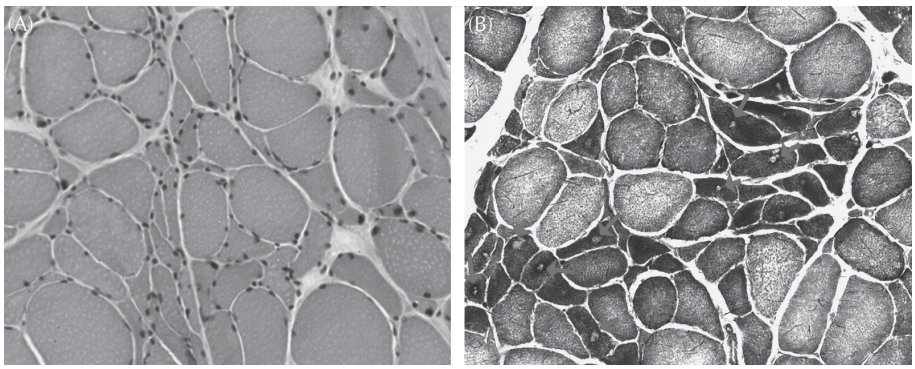

Figure 15–5. (A) Skeletal muscle showing neurogenic atrophy of fibers with group atrophy present, and (B) NADH-TR stain revealing target fibers (in the darker staining fibers) an indication of neurogenic change.

SOD1

Superoxide dismutase 1 (SOD1; chromosome 21) was the first gene abnormality described for ALS, and mutations are widely used as animal models. Clinically, it presents as pure ALS with no dementia. Pathologically, there are skeins in the spinal cord and inclusions in the cerebral motor cortex. These are ubiquitin- and p62-positive but they are TDP-43–negative and, therefore it is fundamentally different from sporadic ALS.

C9ORF72

Relatively recently expansions in a hexanucleotide repeat (GGGGCC) in the *C9ORF72* gene (chromosome 9 open reading frame 72) were discovered to be associated with familial FTLD and/or ALS (Vance et al., 2006; Dejesus-Hernandez et al., 2011; Renton et al., 2011). It is now believed to be the most common cause of FALS, and also involved in some sporadic cases. It often presents with an FTLD-MND/ALS picture, but can present as either ALS or FTD.

Pathologically, TDP-43–positive neuronal cytoplasmic inclusions are seen in the spinal cord in the form of skeins. In the brain, there is very often extensive TDP-43 neuronal positivity not only in the brainstem and limbic areas but also the neocortex. Most significantly, in addition to TDP-43 pathology there is also excess ubiquitin and particularly p62 immunopositivity, which seems to be out of proportion to the TDP-43 inclusions. Furthermore, there are numerous p62- and ubiquitin-positive, TDP-43–negative, inclusions in the cerebellum (especially the granule cell layer) and the CA4 region of the hippocampus (Murray et al., 2011; Al-Sarraj et al., 2011; Figs. 15–6 and 15–7). The cerebellar inclusions have also been seen to be labelled with RNA-binding proteins (Mori et al., 2013), and antibodies to dipeptide repeat proteins.

FUS

FUS (chromosome 16) mutations are a relatively rare cause of FALS (Vance et al., 2009). The inclusions seen in the cord tend to be less skein-like and more globular (Fig. 15–8) and although the inclusions are immunopositive to ubiquitin, p62, and FUS they are TDP-43–negative.

CHMP2B, TARDP, p62, UBQLN2

CHMP2B (chromosome 3), Optineurin (chromosome 10), TARDP (chromosome 1 [the gene coding for TDP-43]), and p62/sequestosome 1 (chromosome 5) are genes that show mutations in rare cases of FALS. The cases have TDP-43–positive inclusions similar to the sporadic disease. Cases with Ubiquilin 2 (*UBQLN2*–X chromosome) mutations also have TDP-43–positive inclusions but occasionally the inclusions are also positive for FUS.

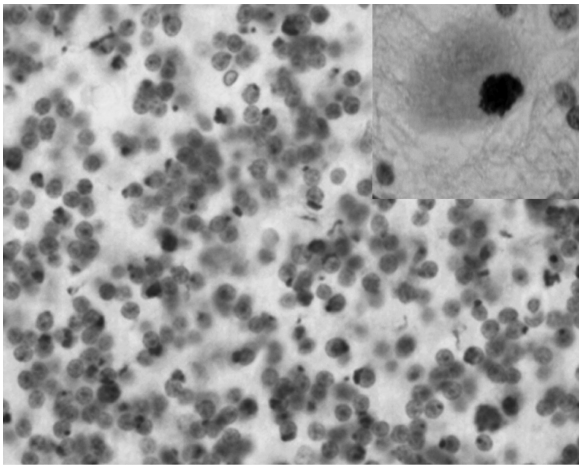

Figure 15–6. FTLD-MND/ALS patient with *C9ORF72* repeat expansion. The main picture reveals p62-positive neuronal inclusions within the cerebellar granule cell layer. These inclusions are negative for TDP-43. The inset reveals a p62-positive neuronal cytoplasmic inclusion within a Purkinje cell within the cerebellum (antibody to p62).

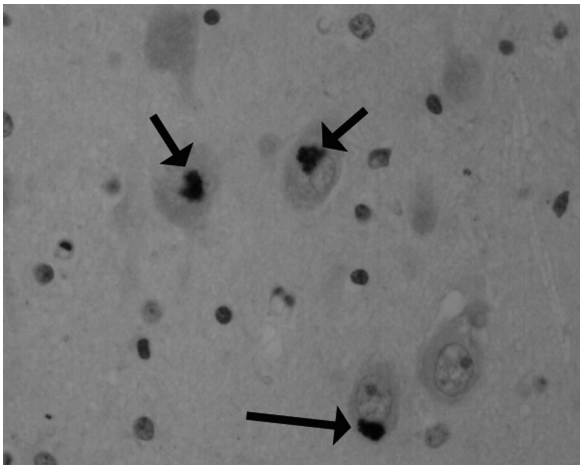

Figure 15–7. FTLD-MND/ALS patient with *C9ORF72* repeat expansion. The pyramidal cells within the CA4 region of the hippocampus have unusual p62-positive cytoplasmic inclusions described as "starburst" (arrows). These are TDP-43–negative (antibody to p62).

ALS-Parkinson Dementia Complex

This condition is probably not genetic. The pathology in ALS-Parkinson dementia complex of Guam reveals TDP-43–positive inclusions similar to sporadic ALS. Elsewhere in the brain there is abnormality of tau, which is thought to be responsible for the parkinsonian and dementia features.

Spinal Bulbar Muscular Atrophy

Spinal bulbar muscular atrophy (Kennedy disease, X linked) is associated with mutations in the androgen receptor gene and does not show TDP-43 positivity.

PATHOLOGY OF FTLD

Macroscopically the picture of the brain can be variable ranging from a relatively normal appearance, to cases with severe atrophy of the frontal and/or temporal lobe (Fig. 15–9). The atrophy can be symmetrical or markedly asymmetrical.

Microscopically there is neuronal loss in the temporal and/or frontal cortices. FTLD is subdivided into different types according to the associated protein accumulation: FTLD-TDP

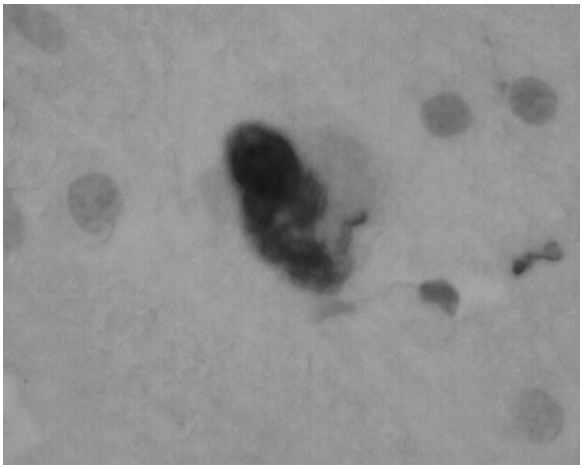

Figure 15–8. FUS-positive neuronal cytoplasmic inclusions within an anterior horn neuron of the spinal cord in a case of familial ALS with a FUS mutation (antibody to FUS).

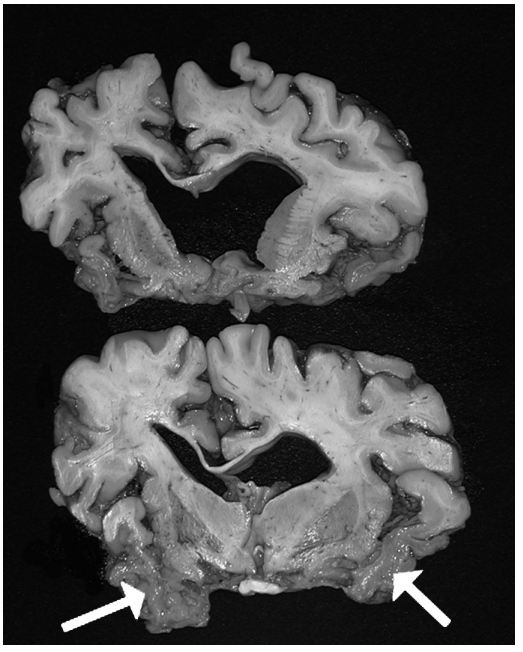

Figure 15–9. Coronal slices from a brain in a case of FTLD-TDP. There is marked cerebral atrophy. In this case the middle and inferior temporal gyri are severely atrophic (arrows) but with some preservation of the superior temporal gyri.

(and FTLD-MND/ALS), where there is abnormal TDP-43 accumulation; FTLD-tau when there is abnormal tau accumulation (FTLD-tau was sometimes called Pick disease when sporadic); and FTLD-FUS.

It is to be noted that not all FTLD cases are associated with ALS (e.g., FTLD-tau is not usually associated). Those that are associated tend to have TDP-43 (or FUS) neuronal cytoplasmic inclusions not only in the hippocampus and/or amygdala but also the frontal and temporal neocortex.

FTLD-TDP and FTLD-MND/ALS can either be sporadic or familial.

SUBTYPING OF CEREBRAL PATHOLOGY OF FTLD-TDP AND FTLD-MND/ALS

To make matters more complex, once a case has been pathologically established as FTLD-TDP (or FTLD-MND/ALS) then the TDP-43 pathology in the cerebrum can be further subcategorized. This is not just an academic exercise because there seems to be some correlation with clinical features and genetic abnormalities. The subtypes are now designated as A, B, C, and D (Mackenzie et al., 2011; Fig. 15–10).

Type A

This subtype is often associated with progranulin (GRN) mutations on chromosome 17. Usually, it presents as pure FTLD (bvFTD or

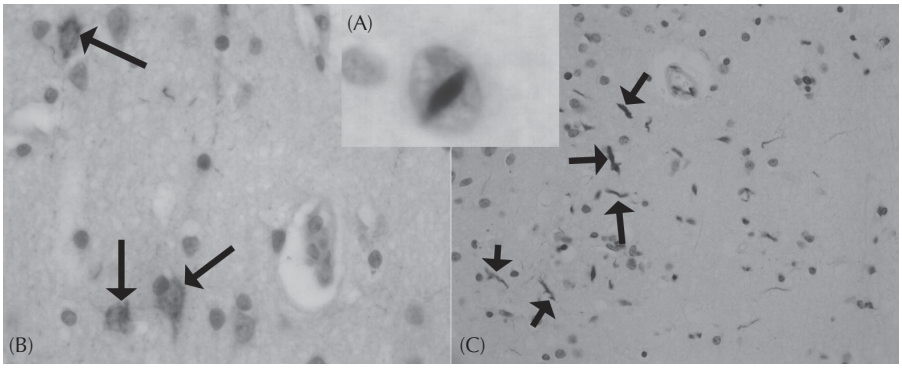

Figure 15–10. Different pathologic patterns seen in cases of FTLD-TDP and FTLD-MND/ALS. (A) TDP-43–positive neuronal intranuclear inclusion characteristic of subtype A, which is often associated with progranulin mutations. (B) TDP-43–positive neuronal cytoplasmic inclusions (arrows) within the neocortex. This is characteristic of the subtype B. These inclusions may be transcortical, and are not usually associated with prominent neurites or neuronal intranuclear inclusions. This pattern is most often associated with FTLD-MND/ALS, especially cases with a *C9ORF72* repeat expansion. (C) Prominent thick TDP-43–positive neurites (arrows) in the superficial temporal cortex of a case of FTLD-TDP. Few neuronal cytoplasmic inclusions are seen. This is subtype C, often correlating with semantic dementia clinically and not usually associated with pathologic or clinical features of ALS (antibody to TDP-43 in A–C).

occasionally progressive nonfluent aphasia clinically) with no ALS features. Intracytoplasmic and intranuclear TDP-43–positive neuronal inclusions are relatively numerous in the cerebral cortex, sometimes associated with small neurites in the superficial cortex.

Type B

This subtype is often associated with FTLD-MND/ALS, especially those with the *C9ORF72* repeat expansion. The neuronal cytoplasmic TDP-43–positive inclusions are transcortical and not usually associated with intranuclear inclusions or numerous neurites.

Type C

This subtype is often associated with semantic dementia. It is very rarely seen in association with ALS. Pathologically, there are numerous superficial thick neurites positive for TDP-43.

Type D

This subtype is rare and reserved for mutations in valosin-containing protein (VCP, chromosome 9), which itself is associated with FTLD (occasionally ALS), inclusion body myopathy in muscle, and Paget disease of bone. Pathologically there are numerous neuronal intranuclear inclusions, which are TDP-43– and VCP-positive.

FTLD-FUS

Unlike ALS cases associated with FUS the entity FTLD-FUS is only very rarely associated with mutations. Clinically, it occurs in the age range of younger to middle-age (Lashley et al., 2011). Usually there is a bvFTD clinical picture. Pathologically there are ubiquitin- positive, p62-positive, FUS-positive, TDP-43–negative inclusions and there is overlap pathologically with basophilic inclusion body disease and neurofilament inclusion disease.

PATHOLOGY OF FAMILIAL FTLD

Familial cases include progranulin mutations, cases with the *C9ORF72* repeat expansion, and VCP mutations. There is another group that is associated with microtubular-associated protein tau mutations on chromosome 17 (but at a different locus to the progranulin gene). Clinically they have a dementia and/or atypical parkinsonian features, but very rarely have ALS features. They generally have extensive tau pathology (e.g., FTLD-tau).

HEAD TRAUMA AND TDP-43

It has been appreciated that head trauma may be a risk factor for the development of ALS in later life. Similarities have been found between the neuropathologic changes seen in Alzheimer disease and the features seen in boxers who had later developed the neurodegenerative process called dementia pugilistica (now called chronic traumatic encephalopathy). Recent work has shown that exposure to long-term repetitive head injury, which includes not just boxers but also American football players and wrestlers, is associated with not only accumulations of abnormal tau, common to Alzheimer disease, but also TDP-43 (McKee et al., 2009, 2010; King et al., 2010). The brains and spinal cords of some of these sportsmen have pathologic similarities to cases of FTLD-TDP and/or ALS. This does not prove that trauma causes FTLD-TDP or ALS any more than trauma causes Alzheimer disease. It does suggest that the abnormal proteins generated in these diseases may have some common pathologic pathways in their development and processing. This is now well set to become a particularly fruitful area of research in the next few years.

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Chapter 16

Convergence and Divergence in Amyotrophic Lateral Sclerosis Syndrome

Genetics, Molecular Pathways, and Molecular Mechanisms

Lucie I. Bruijn and John Ravits

BACKGROUND AND NEW POINTS

The pathophysiology of amyotrophic lateral sclerosis (ALS) has been challenging. Initial clues were investigated with transgenic superoxide dismutase mouse models.

The pathology of motor neuron disease has expanded with the appreciation that frontotemporal degeneration occurs in patients with ALS and families, the identification of protein aggregates in neurons, and the discovery new genes linked to ALS. There is new information on how degeneration might spread across susceptible neurons.

INTRODUCTION

The chief characteristic that defines ALS is progressive weakness from neurodegeneration

of the upper motor neuron (UMN) and lower motor neuron (LMN). Clinically, this is defined by a history establishing weakness over time and space, and by an examination showing signs of both UMN and LMN dysfunction in one or more body regions. Neuropathologically, ALS is defined by loss of motor neurons in brain, brainstem, and spinal cord and now increasingly by a sophisticated repertoire of neuropathologic markers. Clinical phenotypes are determined by the anatomic location of neuropathology, and during life, this anatomic pathology can be imputed clinically.

Heterogeneity of clinical phenotypes is characteristic—there are vastly different degrees of involvement of UMN and LMN, body regions that are affected, degrees of involvement of other systems especially cognition and behavior, and progression rates. Although there are highly distinctive molecular neuropathologic subtypes of ALS (patterns), in fact most of the known neuropathology seems more homogeneous than heterogeneous and does not clearly correlate with the various clinical phenotypes. This is the main mystery: is it one disease with shared fundamental biologic mechanisms or is it many diseases with different fundamental mechanisms and if so, how do they relate?

Genetics is giving us clues—clinical phenotype both masks and unmasks essential elements and there must be both single mechanisms and multiple mechanisms! Sporadic ALS (SALS), which is 90% of cases, and familial ALS (FALS), which is 10% (up to 18–20% when seemingly sporadic cases are tested for the known genetic mutations), are indistinguishable from each other by phenotype. The fact that many different gene mutations have identical or at least highly similar clinical phenotypes tells us there must be multiple mechanisms that cause ALS. But the fact that one single genotype (the same mutation in the same gene in the same family) causes many different phenotypes tell us that there must be single common mechanisms that lead to multiple phenotypes. Thus, there must be both convergence and divergence of mechanisms.

MOLECULAR NEUROPATHOLOGY

Five to 10% of ALS is genetically transmitted by way of dominant gene mutations and these numbers increase to as high as 15–20% when known genes are tested in patients who were thought to have sporadic disease. Approximately 60–70% of the genes have now been identified, the main ones being *SOD1, TARDBP, FUS*, and *C9ORF72* (reviewed in Anderson & Al-Chalabi, 2011). Clinical phenotype heterogeneity is characteristic of FALS, the range of clinical phenotypes is as vast in FALS as SALS, and no features definitely distinguish FALS from SALS. Remarkably, marked clinical phenotype heterogeneity is even seen in the same mutation in the same gene in the same kinship, implying phenotype is determined by other factors. But some trends exist. Mutations in *SOD1* and *FUS* tend to cause predominantly LMN syndromes. Mutations in *TARDBP* tend to begin in the upper extremity and to progress slower than average (Corcia et al., 2012). Mutations in *SOD1, TARDBP*, and *FUS* cause mostly motor syndromes and are only rarely associated with frontotemporal dementia (FTD). Mutations in *FUS* cause a juvenile and adult motor neuron disease syndrome. Mutations in *C9ORF72* are as likely to cause FTD as ALS, often with psychosis. The "A4V" mutation in *SOD1* ALS is rapid, whereas the (unusually) recessive "D90A" is slow and indolent.

Over the last decade there has been an exponential growth of the identification of gene mutations linked to FALS and some cases of SALS. The known functions of these gene products have provided insight into potential disease mechanisms and can be grouped into distinct areas: genes that are involved in protein regulation and stability, those involved in RNA biology, and those that are involved in cytoskeletal functions (Table 16–1). A clearer understanding of disease mechanisms, disease onset and variability, and differential cell vulnerability will provide insight into therapies. In 1988, Leigh et al. and Lowe et al. independently identified depositions of ubiquitin in the cytoplasm of ALS motor neurons using immunohistochemistry. The morphologies of the deposits were either skein-like or dense and round. Ubiquitin is a housekeeping protein involved in protein homeostasis and the finding suggested an unknown pathologic protein was being tagged for removal by the cell. Similar changes of ubiquitinated aggregates were soon identified in about 50% of brains from patients with FTD known as frontotemporal lobar dementia (FTLD) with ubiquitinated

Table 16–1 **ALS and FTLD Proteinopathies: Main Molecular Neuropathologic Features**

			Main Molecular Features				
Proteinopathy	**Phenotypes**	**Genes**	**Motor Cortex (UMN)**	**Spinal Anterior Horn or Brainstem Motor Nuclei (LMN)**	**Frontotemporal Regions**	**Miscellaneous (cerebellum, hippocampus)**	**Descending Axonal Pathways (e.g., lateral columns)**
FUS	• Juvenile ALS • Rare adult ALS (usually with atypical sxs, such as oculomotility, autonomic, cerebellar, or cognitive dysfunction) • FTD	• FUS-TLS	• Basophilic inclusions especially juvenile cases • FUS+, TDP-43- NCIs especially juvenile cases • FUS+, TDP-43- GCIs especially adult cases	• Basophilic inclusions especially juvenile cases • FUS+, TDP-43- NCIs all cases • FUS+, TDP-43- GCIs especially adult cases	• Rare or none basophilic inclusions • Rare or none FUS+, TDP-43- NCIs • FUS+, TDP-43- GCIs in adult cases	• Rare basophilic inclusions • FUS+ TDP-43- NCIs and GCIs in other regions including substantia nigra, nuclei raphe, inferior olives, and dentate nucleus in adult cases	• Degeneration and sclerosis
SOD1*	• ALS, usually LMN predominant features • Very rare FTD	• SOD1	• Infrequent abnormalities as seen in spinal anterior horns	• Weakly, ubiquitin+, TDP-43-, SOD1-, neurofilament+ intracytoplasmic hyaline conglomerates	• Few reports, presumptively same as motor cortex	• Changes also in Clarke nucleus, dorsal horn, nucleus ambiguous, and Onuf nucleus	• Distal axonal degeneration • Also, degeneration in dorsal columns
TDP-43 (non-C9ORF72 related)	• ALS • ALS-FTD • FTD	• Most non–SOD1-associated FALS, including TARDBP • All SALS	• Ubiquitin+, TDP-43+ NCIs, and GCIs	• Ubiquitin+, TDP-43+ NCIs, and GCIs	• Ubiquitin+, TDP-43+ NCIs, and GCIs	• No significant p62+ or UBQLN+ NCIs or GCIs in cerebellum and hippocampus	• Degeneration and sclerosis

TDP-43 (C9ORF72 variant)	• ALS • ALS-FTD • FTD	• C9ORF72	• Ubiquitin+, TDP-43+ NCI, and GCI • Dipeptide repeat proteins • RNA nuclear inclusions mainly in neurons but rarely in glia	• Ubiquitin+, TDP-43+ NCI, and GCI • Dipeptide repeat proteins (probably) • RNA nuclear inclusions mainly in neurons but rarely in glia	• Ubiquitin+, TDP-43+ NCI, and GCI • Dipeptide repeat proteins • RNA nuclear inclusions mainly in neurons but rarely in glia	• p62+, UBQLN+, TDP-43- NCIs, and GCIs in cerebellum and hippocampus; TDP+ pathology is present but separable from p62 and UBQLN • Dipeptide repeat proteins • RNA nuclear inclusions mainly in neurons but rarely in glia	• Degeneration and sclerosis
Tau (included for comparison)[†]	• FTD • Progressive supranuclear palsy • Corticobasal syndrome • Multiple system atrophy	• MAPT	• Signature tau+, ubiquitin- TDP-43- NCIs, and GCIs	• Few reports, presumptively negative (see 3)	• Signature tau+, ubiquitin-, TDP-43-, NCIs and GCIs	• Pick bodies = 3R tau+ globular or spherical NCIs in the granule cells of dentate gyrus	• Presumptively negative

GCI = glial cytoplasmic inclusions; NCI = nuclear cytoplasmic inclusions.
* No primary FTD phenotypes have been defined by SOD1 pathology.
[†]No primary ALS phenotypes have been defined by tau+ neuropathology.

pathology (FTLD-U). In 2006, the identity of the ubiquitinated protein in ALS and in FTLD-U was found to be TDP-43, a nuclear protein that is involved in DNA and RNA processing and that in ALS translocates to the cytoplasm where it is also found to be cleaved, hyperphosphorylated, and insoluble (Neumann et al., 2006; Arai et al., 2006). The current molecular neuropathologic classification is likely to be continually modified: it now seems that the other proteins beside TDP-43 may be ubiquitinated in ALS and FTD; newer markers are being identified; and abnormal TDP-43 may be seen in other neurodegenerations. But overall, all SALS and nearly all FALS, except SOD1-associated ALS, regardless of clinical phenotype, seems to have as its hallmark neuropathologic pattern deposition of ubiquitinated TDP-43 in the cytoplasm of central nervous system (CNS) cells. This feature is increasingly regarded as TDP-43 proteinopathy. Heat maps of the distribution of TDP-43 pathology show that abnormalities are widely present in the brain, and not just in motor regions (Geser et al., 2008; Brettschneider et al., 2013).

Whereas gene mutations do not directly correlate with clinical phenotype, they better correlate with molecular neuropathology, which seems to be distinctive among various genes (Table 16–2). The first and main molecular neuropathologic subtype is TDP-43 proteinopathy as defined above. This applies to all SALS and most non-*SOD1* FALS. It is defined by ubiquitin+/TDP-43+ skeins and dense round inclusions deposited in the cytoplasm in the spinal motor neurons and the cortex, where they are primarily localized in motor areas (Mackenzie et al., 2007). The inclusions are also seen glial cells.

The second and newest defined neuropathologic subtype of ALS is a variation on TDP-43 proteinopathy related to an intronic hexanucleotide repeat expansion in C9*orf72*, which represents 33–40% of FALS and up to 7% of SALS. *C9ORF72*-associated cases have all the hallmark features of TDP-43 proteinopathy, and, in addition, there are also abundant deposits of p62 and dipeptide repeat proteins in the cytoplasm in the cerebellum, basal ganglia, and hippocampus, features that are not seen or minimally present in non–*C9ORF72*-associated cases (Al-Sarraj et al., 2011; Ash et al., 2013; Troakes et al., 2011; Gendron et al., 2013; Mori et al., 2013). p62 is a protein involved in both the proteosomal pathway and in autophagy, and this has relevance to the growing interest in these pathways in neurodegeneration. A signature feature of the subset of repeat expansion diseases characterized by RNA-mediated toxicity is RNA foci in the nucleus of neurons and rarely of glia and this has also been identified (DeJesus et al., 2011) and recently characterized neuropathologically (Lagier-Tourenne et al., 2013; Zu et al., 2013).

The third main molecular neuropathologic signature of ALS applies to mutations in *SOD1*, which represents up to 20% of FALS and 1–2% of SALS. Most of the *SOD1*-associated neuropathology was reported prior to 2006 before TDP-43 and the *C9ORF72* expanded hexanucleotide repeat were identified and sorely needs to be updated (discussed by Ince et al., 2011). In general, it is characterized by deposition in the cytoplasm of sometimes large amorphous conglomerates of ubiquitinated SOD1 protein that are negative for TDP-43 (Mackenzie et al., 2007). There seems to be a greater burden on the LMN than UMN (Cudkowicz, McKenna-Yasek, Chen, Hedley-Whyte, & Brown, 1998) and the degree of axon loss seems to be greater than neuronal loss, leading to the concept of it being a distal axonopathy (Ince et al., 1998). Misfolded SOD1 is present in *SOD1*-associated FALS but whether or not this is present in the SALS remains disputed, although emerging evidence suggests that, if it is present at all, it is not prominent.

The fourth main molecular neuropathologic signature of ALS is FUS proteinopathy, which represents up to 3% of FALS and less than 1% of SALS. This neuropathologic subtype is characterized by basophilic inclusions in the cytoplasm of neurons of the motor cortex and of spinal anterior horns, and by FUS-positive, TDP-43-negative immunoreactive inclusions in the cytoplasm of neurons and glia in the motor cortex, spinal anterior horns, and various nonmotor regions (Blair et al., 2010; Mackenzie et al., 2011). There seem to be different signatures in juvenile and adult forms of disease (Baumer et al., 2010; Mackenzie et al., 2011). One recent report indicated FUS proteinopathy may be more prominent in TDP-43-proteinopathies if optimal technical protocols are used for detection, but so far this has not been verified by other groups (Deng et al., 2010).

Table 16–2 **Genetic Causes of FALS**

Mutant Molecule	Gene Locus	Inheritance	Estimate % of FALS
Enzyme			
Superoxide dismutase 1 (SOD1)	21q22.1	Dominant except for D90A	20%
RNA processing and RNA toxicity			
TAR DNA-binding protein-43 (TDP-43)	1p36.2	Dominant	1–5%
FUS	16p11.2	Dominant	1–5%
Senataxin (SETX)	9q34	Dominant	Unknown
TATA-binding protein associated factor 15 (TAF15)	17q11.1-q11.2	Unknown	Unknown
Repeat expansions			
Chromsome 9 open reading frame 72 (C9orf72)	9p21.3-p13.3	Dominant	40–50%
Ataxin 2 (ATXN2)	12q24	Dominant	<1%
Protein homeostasis			
Ubiquilin 2 (UBQLN2)	Xp11	Dominant	<1%
Optineurin (OPTN)	10p15-p14	Dominant	<1%
Valosin-containing protein (VCP)	9p13	Dominant	<1%
Disruption of axonal dynamics			
Vesicle-associated membrane protein-associated protein B and C (VAPB and VAPC)	20q13.3	Dominant	<1%
Dynactin 1 (DCTN1)	2p13	Dominant	Unknown
Profilin 1 (PFN1)	17p13.2	Dominant	Unknown

For comparison to FTLD, it is worth noting that there are three main neuropathologic molecular subtypes of FTLD: FTLD-U or TDP-43 (about 50% of FTLD), FTLD-FUS (about 3–5% of FTLD), and FTLD-tau (about 40–50% of FTLD). The former two overlap significantly with ALS, suggesting they belong in a clinical pathologic spectrum. But, the overlap does not seem to include FTLD-tau, except in one recent report, suggesting a complex correlation (Yang & Strong, 2012).

CLINICAL AND PATHOLOGIC HOMOGENEITY AND UNDERLYING MECHANISMS

Hypotheses of Propagation

The correlations between clinical phenotype, molecular neuropathology, and genetics are not understood and pathobiology is undoubtedly complex. But propagation of pathology is one theme that is emerging as a possible key. One hypothesis posits neuroanatomic propagation (Ravits & La Spada, 2009). ALS usually begins in discrete body regions and for these regions, the degree to which UMNs and LMNs are affected (the distribution of disease burden) is variably distributed (Ravits, Paul, & Jorg, 2007). Once triggered, disease propagates to proximate neuroanatomic regions independently at the two levels and progressive motor neuron loss summates and then saturates neuropathologically (Ravits, Laurie, Fan, & Moore, 2007). The rate of disease progression reflects both the kinetics of propagation and the distribution of the disease burden between UMN and LMN. In this light, primary lateral sclerosis and progressive muscular atrophy differ primarily in distribution of the pathologic burden between UMN and LMN levels, limb variants differ by neuroanatomic location of pathology, and FTD and ALS differ in the cerebral distribution of pathology. A dramatic example is repeat-expanded *C9ORF72*-associated ALS/FTD, where a single disease mechanism leads to either ALS or FTD phenotype, each with its own heterogeneity. Different from neuroanatomic propagation is a hypothesis of propagation within neuronal networks. According to

this hypothesis, the vast functional and structural networks in the CNS create a connectome (Brooks, 1991; van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009). Neuronal networks may have selective vulnerabilities through natural anatomic patterns that underlie different neurodegenerative syndromes (Seeley, Crawford, Zhou, Miller, & Greicius, 2009), possibly through preferential spread (Zhou, Gennatas, Kramer, Miller, & Seeley, 2012). In support of this, advanced magnetic resonance imaging data demonstrating ALS-specific neurodegeneration within motor and extramotor networks is emerging (Douaud, Filippini, Knight, Talbot, & Turner, 2011; Mohammadi, Kollewe Samii, Dengler, & Munte, 2011; Verstraete, Veldink, Mandl, van den Berg, & van den Heuvel, 2011).

Although propagation patterns have now been defined by many groups (Turner et al., 2010; Körner et al., 2011; Chiò, Calvo, Moglia, Mazzini, & Mora, 2011; Fujimura-Kiyono et al., 2011; Gargiulo-Monachelli et al., 2012), other contributions to phenotype heterogeneity are also emerging. One recent study identified that up to 14% of second regions involved in disease progression were not contiguous (Gargiulo-Monachelli et al., 2012). Bifocal or multifocal onset has been proposed (Kanouchi, Ohkubo, & Yokota, 2012). Two recent studies using different approaches, one traditional groupings and one unbiased cluster analysis, identified a variety of demographic factors that were significant determinants of phenotype, (Chiò et al., 2011; Ganesalingam et al., 2009). Genetic syndromes, which are often focal in onset, also have important biologic determinants other than propagation.

Parallels to FTD

As noted, FTD and ALS overlap clinically, genetically, and neuropathologically. FTD has three main clinical phenotypes (primary progressive aphasia, semantic dementia, and behavioral variant) and FTLD has three main neuropathologic subtypes (FTLD tau, FTLD ubiquitin or TDP-43, and FTLD FUS). Extensive studies over the last several years have sought correlations between FTD clinical phenotypes with FTLD neuropathologic subtypes, and although trends have been identified, there are no clear predictors and the correlations are complex. Selective involvement within neuronal networks seems clear. In addition, because the clinical phenotypes can be as defined by the focal neuroanatomic site of onset (left or right, frontal or temporal) as by the molecular nature of the pathologic process (tau, TDP-43, or FUS) FTD, like ALS, seems to be a focally beginning and neuroanatomically propagating disease and thus neuroanatomic propagation also seems to be a principle component of its biology.

PATHOGENESIS

Prime Observations

Because, on the one hand, multiple gene defects lead to any of the ALS clinical phenotypes and, on the other hand, each gene defect can lead to multiple clinical phenotypes, there must be both convergence and divergence of pathogenic mechanisms. It is reasonable to divide these mechanisms into three separable components: (1) triggers, (2) progression (or propagation), and (3) neuronal death. The separable importance of disease trigger is highlighted by the observation that onset is highly variable in site and in distribution between UMN and LMN, in penetrance, and in age of onset (even within the same gene mutation in the same kinship). The separable importance of disease progression is highlighted by the highly variable patterns and rates of progression, which suggest variable kinetics of propagation both in space and time. The separable importance of cell death is highlighted by the observation that select motor neuron degeneration is the ultimate result neuropathologically and that this summates over time. Many themes about molecular mechanisms of neurodegeneration have emerged over the past two decades and these are highlighted next and schematically represented in Fig. 16–1. Whether these molecular mechanisms are a gain of a novel function or a loss of normal protein function, or both, remains unclear in some cases of ALS. For SOD1 mutations linked to ALS this is clearer: disease is due to a gain of a novel function rather than a loss of function. For the more recent mutations, however, of TDP-43, FUS, and C9orf72 this is less defined and disease is likely due to both a gain and a loss of function.

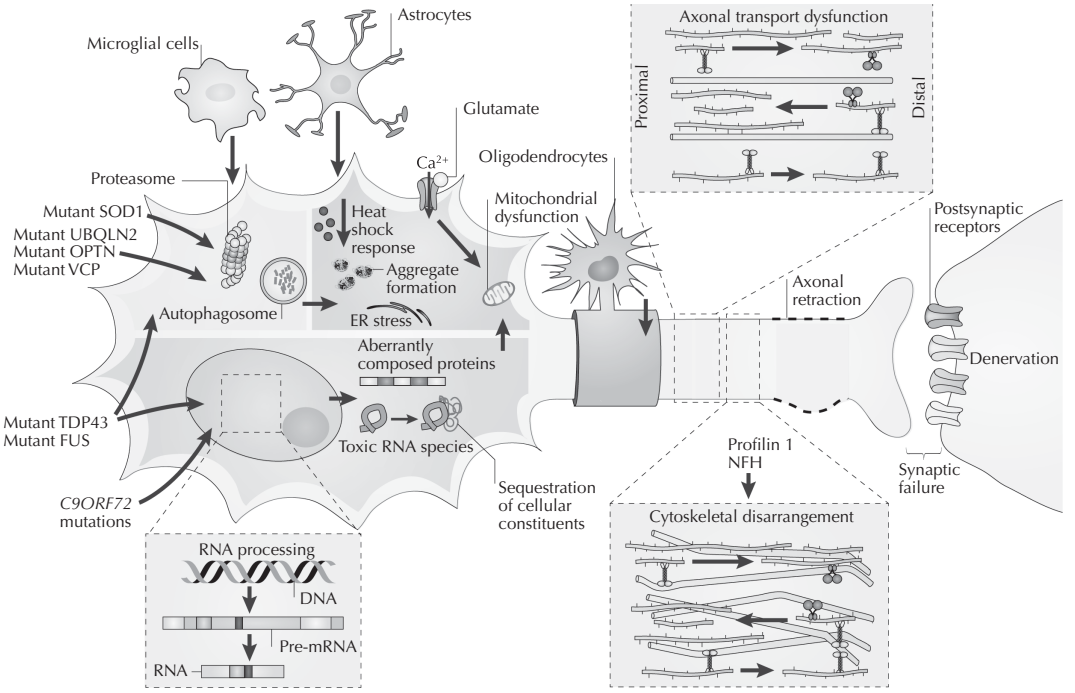

Figure 16–1. Overview of events in the pathogenesis of ALS. Interference with normal proteasomal or autophagic protein degradation is caused by mutations in superoxide dismutase 1 (SOD1), valosin-containing protein (VCP), ubiquilin 2 (UBQLN2), charged multivesicular body protein 2b (CHMP2B), optineurin (OPTN), and, potentially, TAR DNA-binding protein 43 (TDP-43) and FUS (ALS as a proteinopathy; shaded yellow). Disturbance of normal RNA processing, which yields erroneously assembled proteins and toxic RNA species, is caused by mutations in chromosome 9 open reading frame 72 (*C9ORF72*) and potentially *TARDBP*, which encodes TDP-43, and *FUS* (ALS as an RNopathy; shaded purple). Through both gain- and loss-of-function mechanisms, these primary pathogenic changes result in progressive cellular failure (shaded light red) that is characterized by protein clumping, aggregate formation, endoplasmic reticulum (ER) stress, and Golgi and mitochondrial failure. Axonal architecture (cytoskeleton) and function (transport) fail, and axonal retraction results in denervation of neurons (such as the lower motor neuron) or muscle. Nonneuronal cells modify this process through the loss of their normal effect on the neuron and/or the gain of a toxic effect. Vulnerability factors, such as stress response capacity (e.g., by the capability to activate heat shock proteins) and susceptibility to excitotoxicity (e.g., through the permeability characteristics of the glutamate receptor) determine (or codetermine) which neurons are (mostly) susceptible to these processes. Some ALS-causing mutant proteins may act more downstream in this model (e.g., profilin 1 and neurofilament heavy chain [NFH] through a direct effect on the cytoskeleton and D-amino-acid oxidase on excitotoxicity). Axonal attraction systems (e.g., semaphorin and vascular endothelial growth factor) and repellent systems (e.g., NOGO and ephrins) seem to modify the processes of axonal retraction and denervation. (Courtesy of W. Robberecht and T. Philips, 2013.)

Motor Neuron Resistance and Vulnerability in ALS

In ALS, the most vulnerable UMNs are layer V projection neurons in the primary motor cortex, and spinal motor neurons of the ventral horn. The most vulnerable regions and neurons in FTD are less clearly defined, but anterior cingulate and frontoinsular regions show early involvement, and these regions contain unique layer V projection neurons (von Economo neurons and fork cells). The coincidence of ALS and FTD in some patients raises the possibility that a shared cellular or molecular feature is present in cortical motor neurons and subspecialized layer V neurons of other cortical regions, which defines the sensitivity to degeneration in ALS-FTD. The prominent layer V degeneration in TDP-43 transgenic models suggests the determinants of this shared vulnerability of layer V neurons may be present in rodents and be accessible to study. Improving the understanding of whether subspecialized layer V cells really are selectively vulnerable

in ALS and FTD, and why, could provide a unique angle for understanding pathogenesis of these diseases.

Among different LMN populations, the motor neurons subserving eye movements and pelvic sphincters are highly resistant compared with typical spinal motor neurons. Although often considered as a group, spinal motor neurons are highly diverse in terms of their morphology, connectivity, and functional properties and differ significantly in their response to disease. Recent studies of motor neuron diversity have clarified developmental mechanisms and provided novel insights into their neurodegeneration. Motor neurons of different classes and subtypes (fast/slow, alpha/gamma) are grouped together into motor pools, each of which innervates a single skeletal muscle. Distinct mechanisms regulate their development. In multiple contexts including ALS, spinal muscular atrophy, and aging, fast-fatigable motor units degenerate early compared with motor neurons innervating slow muscles. Mechanisms for this could also relate to those conferring resistance to of those subserving eye movement and pelvic sphincter control. If we could understand why populations and subpopulations of motor neurons are resistant or vulnerable, we would have a strong rationale approach for intervention. One approach is through functional genomics using laser capturing and new genomic technologies. Extrinsic and intrinsic mechanisms that confer resistance represent promising therapeutic targets in these currently incurable diseases.

ALS as a Systemic Disease

As previously stated, there is a growing body of knowledge about the systemic changes that are occurring in ALS. These include ultrastructural abnormalities in hepatic cells, skin cells, muscle mitochondria, systemic glutamate metabolism, inflammatory cytokine production, immunologic changes, glucose metabolism, and lipid metabolism. Skeletal muscle is the single largest organ by mass, constituting 40–45% of the entire body mass and is the end-organ of the motor neurons. Skeletal muscles generate target-derived neurotrophic factors that can substantially affect motor neuron survival. Part of the hypermetabolism that is becoming defined in patients with ALS may be caused by abnormal mitochondrial energy production in skeletal muscle (Desport, Torny, Lacoste, Preux, & Couratier, 2005), generating a large amount of radical oxygen species (Muller et al., 2007) that could interact with those from inside the CNS (Bogdanov et al., 2000). Lipid peroxidase products are highly biologically active, causing cellular damage via apoptosis or nucleophilic action and these could be connected to ALS by way of APOE isoforms and/or paraoxonase I (PON1) or other pathways.

The Cellular Neighborhood Matters: Nonautonomous Cell Death

It is now clear that ALS associated with *SOD1* mutations are non–cell autonomous, that is damage of the population of affected neurons depends upon complex interactions between them and their surrounding cells (Clement et al., 2003; Yamanaka et al., 2008). From analysis of mice that are mixtures of mutant-expressing SOD1 and normal cells, gene inactivation in selected cell types, and cell grafting to replace mutant-expressing cells with normal ones, it seems mutant damage within motor neurons determines the timing of disease onset, and mutant damage within astrocytes and microglia drives disease progression. Thus, the cellular neighborhood matters. The exact roles of the different cell types are complex. Astrocytes expressing ALS-related *SOD1* mutations can kill neighboring spinal motor neurons and are crucial to drive disease progression. This mechanism is unknown but the preponderance of evidence from SALS and FALS and rodent models suggests a common loss of function of glutamate handling, through decreased expression and function of glutamate transporters (Rothstein, Martin, & Kuncl, 1992; Rothstein et al., 1994; Howland et al., 2002), which is neurotoxic. Another could be mediated by soluble toxic factors that are protein in nature, thermo-labile, and negatively charged, but no in vivo evidence has emerged for this gain-of-toxicity mechanism. The identity of these toxic factors, the molecular pathways engaged, and protective small molecules have not yet identified exactly how this occurs. Microglia, the resident innate immune sentinels of the CNS, become

activated, and evidence from both in vivo and in vitro models suggests that they can be either neuroprotective or cytotoxic, probably through the release of neurotrophic factors and cytokines (Beers et al., 2006, 2011). Activated microglia may switch from anti-inflammatory and neuroprotective to proinflammatory and neurotoxic, and a greater understanding of the numerous pathways involved could provide opportunities for novel therapeutic intervention. Oligodendroglia in the grey matter has recently been found to have a significant role in ALS. They are derived from NG2 cells, which are adult stem cells located throughout the neural axis. Oligodendrocytes provide trophic and possibly metabolic support to neurons and axons. They are massively proliferating in ALS, both mouse models and human disease, either because of some unknown signal or oligodendrocyte injury. Their exact role in ALS neurodegeneration and how this discovery may impact therapy remains to be determined.

RNA Processing and RNA Toxicity

Views on pathogenesis are undergoing a sea-change from the recognition of the importance of the two RNA/DNA-binding proteins TDP-43 and FUS. Both are widely expressed, predominantly nuclear, have similar domains and prion-like properties, and have ALS mutations localized in the C-terminal region. They are both structurally close to the family of heterogeneous ribonucleoproteins (hnRNPs) and have been involved in multiple levels of RNA metabolism including transcription, RNA splicing, RNA transport, translation, and microRNA processing (reviewed in Buratti & Baralle, 2008; Lagier-Tourenne & Cleveland, 2009; Lagier-Tourenne, Polymenidou, & Cleveland, 2010). Importantly, splicing alterations (Lin et al., 1998; Rabin et al., 2010) and mRNA-editing errors (Aizawa et al., 2010; Kawahara et al., 2004) have been reported in sporadic patients with ALS, albeit a role of TDP-43 or FUS in these modifications has not been defined. The emerging TDP-43 and FUS stories have led to the proposal that defects in RNA processing play a central role in neurodegeneration and this was further underscored by the recent recognition of intermediate length polyglutamine expansions in ataxin-2, another RNA binding protein, as a risk factor for ALS (Elden et al., 2010). At present, it is unresolved as to whether neurodegeneration is caused by a loss of TDP-43 or FUS function, a gain of toxicity, or a combination of the two. The nuclear clearance of TDP-43 and to less extent FUS in neurons containing cytoplasmic aggregates is consistent with pathogenesis driven, at least in part, by a loss of TDP-43 or FUS nuclear function. An alternative (not mutually exclusive) hypothesis, however, is that the accumulated proteins acquire a toxic function in the cytoplasm of affected neurons. This acquired toxic function may also rely on the RNA-binding properties of these proteins, as suggested by recent works in yeast, fruit fly, and chick showing that the toxicity of TDP-43 aggregates is abolished when the RNA-binding property of the protein was removed (Elden et al., 2010).

In October 2011, an expanded hexanucleotide repeat in *C9ORF72* was identified in chromosome 9-linked ALS, FTD, and their overlap, thus identifying the single most common genetic mechanism in ALS-FTD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). This identification has three immediate implications. First, the same genetic defect can cause either ALS or FTD phenotype, thus re-enforcing that clinical phenotype does not directly reflect underlying molecular mechanism. Second, ALS-FTD now joins the group of expansion repeat disorders, a group of more than 22 inherited neurodegenerative diseases characterized by expanded nucleotide repeat sequences (microsatellites) in the genome. Third, three mechanisms seem most reasonable: gain-of-function caused by production of toxic RNA (Donnelly et al., 2013; Lagier-Tourenne et al., 2013; Sareen et al., 2013); or loss of gene function, although no known functions of the *C9ORF72* protein are currently known. Several groups have shown that mRNA levels of certain C9orf772 variants are decreased in c9FTD-ALS (Belzil et al., 2013). A third possibility is that the protein acquires a toxic function. Investigators have identified the presence of aggregation-prone proteins by repeat-associated non-ATG (RAN) translation. (GGGGCC) exp RNA foci are observed in c9FTD-ALS (Ash et al., 2013; Mori et al., 2013; Gendron et al., 2013).

Disruption of Protein Homeostasis

The identification of mutations in SOD1 paved the way for the hypothesis that protein homeostasis is disrupted. Mutant SOD1 misfolds; does not get appropriately degraded through the ubiquitin pathway; and disrupts two important components of the cells degradation machinery, namely the proteosomal pathway and autophagy (Chen et al., 2012). This is further supported by an increase in the number of autophagosomes in motor neurons in the spinal cord of both animal models and patients with ALS (Sasaki 2011; Morimoto et al., 2007). Further evidence for impaired protein degradation in ALS comes from more recent mutations in ubiquilin 2 (UBQLN2) linked to ALS and FTD (Deng et al., 2011). UBQLN2 delivers ubiquitinylated proteins to the proteasome for degradation. Mutations in the gene encoding valosin-containing protein are also linked to ALS (Johnson et al., 2010). Valosin-containing protein is a multifunctional ubiquitin-sensitive chaperone that unfolds proteins and disassembles complexes and also plays a role in autophagy. In addition, p62 is a protein involved in both the proteosomal pathway and in autophagy. Abundant deposits of p62 are found in the cytoplasm and nucleus of neurons in the cerebellum, basal ganglia, and hippocampus of *C9ORF72*-associated cases of ALS (Al-Sarraj et al., 2011; Troakes et al., 2011). Disruption of protein homeostasis is likely to be multifactorial in origin involving both gain of function and loss of function mechanisms.

Prion-like Propagation

ALS and the linkage of ALS to FTLD could be explained on the basis of disease proteins, such as SOD1 and TDP-43, propagating pathologically from cell to cell. This theme is emerging in a variety of different neurodegenerative diseases, such as Alzheimer disease, Parkinson disease, and FTD and ALS, as it emerges that a number of proteins including alpha-synuclein, tau, abeta, and mutant SOD1 may propagate within the CNS. Transmission or propagation is not the same as infectivity and the terms being used for these properties are "prion-like" or "prionoids" (Aguzzi, 2009), to distinguish them from bona fide prions, which are infectious. To date, there is no evidence that any other neurodegenerative disease besides prion diseases can be acquired by infection in humans (reviewed in Polymenidou & Cleveland, 2011; see Fig. 16–2). Nevertheless, disease progression within the same individual from a focal site of initiating damage throughout the CNS has been described for many neurodegenerative diseases, including ALS. Although the molecular basis of these observations is not well understood, the propagation of pathologic conformation of disease-related proteins (pathologic templating) could underlie this phenomenon. Indeed, misfolded SOD1 and TDP-43 were recently shown to induce a pathologic conformation on their natively folded counterparts when introduced on cells in culture (reviewed in Polymenidou & Cleveland, 2011). This behavior is reminiscent of the pathologic prion protein and has now been demonstrated for several proteins that misfold and accumulate in neurodegeneration, including SOD1 and TDP-43 and A-beta, tau, and alpha synuclein (Furukawa, Kaneko, Watanabe, Yamanaka, & Nukina, 2011; Grad et al., 2011; Münch, O'Brien, & Bertolotti, 2011). Preformed fibrils generated from recombinant alpha-synuclein, for example, when dripped onto primary cultures of wild-type neurons induce alpha-synuclein Lewy neurite pathology in processes, and this gets transported retrogradely back to the cell body where Lewy bodies are formed (Volpicelli-Daley et al., 2011). Physical application to the cell bodies results in its transportation in the opposite direction, and there seems to be transmission throughout other parts of the brain. Not every neuron is affected in the neuroanatomic pathways that connect one part of the brain to the other, but many are. Glial cells also can be induced to form alpha-synuclein pathology, at least in transgenic mice.

Corticospinal Motor Neuron Development, Degeneration, and Subcerebral Projections

Interesting suggestions have been put forth that common molecular origins during the development of corticospinal motor neurons (CSMN), related subsets of cortical nonmotor neurons, and cognitive association projection neurons might share a fatal vulnerability

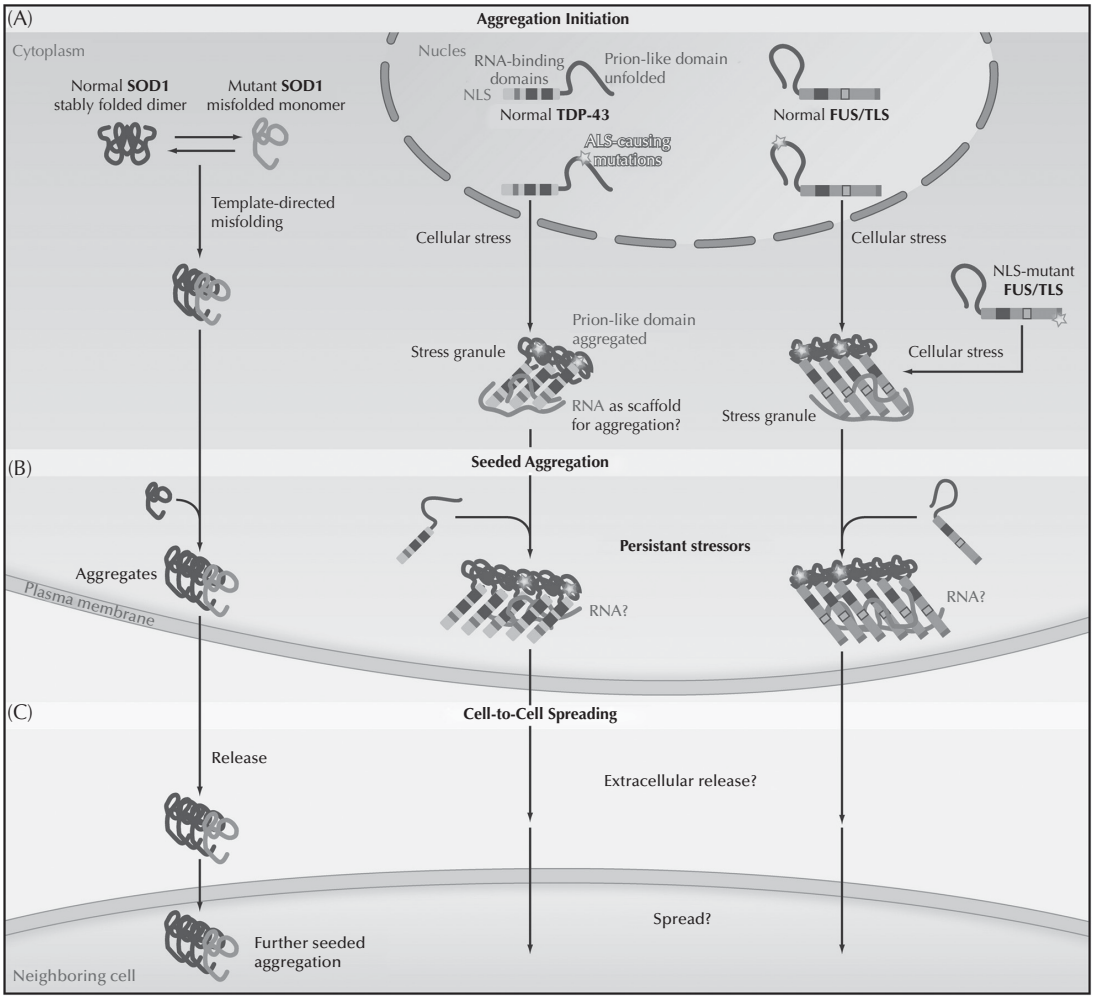

Figure 16–2. Schematic representation of prion-like phenomena in ALS. (A) Mutant, misfolded SOD1 was shown to induce the misfolding of its native counterpart, in a template-directed reaction, thereby forming a seed of aggregated protein. TDP-43 and FUS/TLS are both incorporated in stress granules, which form through the ordered aggregation of several RNA-binding proteins complexed with RNA molecules. This physiologic reaction to cellular stress may be the initial trigger for pathogenic inclusion formation because the increased local protein concentration and RNA scaffolding molecules may facilitate ordered aggregation of TDP-43 and/or FUS/TLS. Mutations in the prion-like domain of TDP-43 (and maybe also FUS/TLS) enhance its aggregation propensity, whereas mutations in the nuclear localization domain (NLS) of FUS/TLS increase its cytoplasmic localization. (B) Misfolded SOD1 follows a self-perpetuating seeding reaction in cell culture. Upon chronic cellular stress and defects in stress granule disassembly occurring with aging, the functional prion-like conformational changes of TDP-43 and FUS/TLS associated with their physiologic roles in stress granule formation may transform into pathogenic self-perpetuating, irreversible aggregation. It is unknown whether cellular RNA is occasionally trapped within the cytoplasmic FUS/TLS and/or TDP-43 inclusions, thereby depleting the cell of essential RNA components. (C) SOD1 aggregates transfer from cell-to-cell to initiate misfolding and aggregation of native SOD1 in neighboring cells (shown in cell culture). It is currently not known whether TDP-43 and/or FUS/TLS can spread from cell to cell by a similar mechanism. Filled blue boxes on TDP-43 and FUS/TLS molecules indicate RNA-recognition motifs and the striped blue box on FUS/TLS refers to the zinc finger domain that can also bind RNA. (Courtesy of M. Polymenidou and D.W. Cleveland, 2011.)

caused by errors introduced during neurodevelopment that could explain at least some of the aspects of ALS (Ozdinler et al., 2011). Complex molecular controls regulate specification, differentiation, connectivity, and survival to create enormous complexity of CNS neuronal subtypes and their connections. Results over the past several years identify that the development and maintenance of CSMNs and other neocortical projection

neuron populations are controlled by a set of complexly interacting developmental molecular regulators, largely transcription factors, and coregulators (MacDonald et al., 2011). A specific subtype of progenitors generates the entire set of CSMN, related subcerebrals, and corticothalamic projection neurons, all "corticofugal projection neurons." This supports the ideas that CSMN and nonmotor, cognitive, and sensory subcerebrals are built on a "common chassis," and common molecular abnormalities can predispose this broader population, or many narrower and more specific populations, to selective disease vulnerability (e.g., UMN disease with more or less nonmotor involvement). Many developmental genes have now been identified as being associated with classical ALS. Thus, during initial development, errors might be introduced that lead to selective vulnerability and later degeneration.

Disruption of Axonal Dynamics

Several studies in rodent models and in humans have demonstrated that axonal retraction from the muscle target occurs early in the disease process (Fischer et al., 2004). Disruption of axonal transport is thought to contribute to axon failure in ALS. Several genes linked with axonal dynamics have been found associated with ALS. Mutations in the neurofilament heavy chain and in peripherin have been found in a small number of patients with ALS (Figlewicz et al., 1994). Most recently mutations in profilin 1 (PFN1) have been linked to ALS (Wu et al., 2012). PFN1 is essential for the polymerization of actin. Mutations in this gene lead to inhibition of axonal outgrowth through decreasing actin polymerization in embryonic motor neurons in vitro. This inhibitory affect may enhance retraction and denervation in the adult neuromuscular system. EPHA4 has also recently been identified as having a potential role in axonal dynamics. EPHA4 is a receptor of the ephrin axonal repellent system and induces cytoskeletal rearrangements. Studies in fish and rodents demonstrated that genetic or pharmacologic inhibition of EPHA4 improved axonal outgrowth (Van Hoecke et al., 2012) and mutations in EPHA4 were found in two patients with ALS with an unusually longer survival. NOGO-A, an axonal outgrowth inhibitor, is up-regulated in muscle of patients with ALS (Dupuis et al., 2002). Finally, a genome-wide association study identified kinesin-associated protein 3 (KIFAP3), a protein that forms a complex with motor proteins KIF3A and KIF3B, associated with increased survival (Landers et al., 2009). Together, these data suggest that factors involved in cytoskeletal structure and cellular transport are involved in the pathogenesis of ALS.

CONCLUDING REMARKS

Clinical phenotypes of ALS are vastly heterogeneous. Yet their correlations with molecular pathology have not been defined. Genetics, by contrast, is not distinguishable by phenotype but is by molecular pathology. The fact that many different gene mutations cause identical clinical phenotypes means that multiple mechanisms exist and ALS is a syndrome. But the fact that one single gene mutation (even the same mutation in the same gene in the same family) causes many different ALS phenotypes means that there must be single common mechanisms that lead to multiple phenotypes. Propagation of pathology is emerging as a principle component in pathobiology. Thus, it is reasonable to divide pathobiology: triggers, progression (or propagation), and neuronal death. The holy grail of ALS is rationally designed therapy that effectively stops ALS neurodegeneration in its advance. With the transforming understanding of clinical, neuropathologic, genetic, and molecular ALS over the last 5 years, this quest has become a realistic hope.

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Chapter 17

Immunologic Aspects of Amyotrophic Lateral Sclerosis

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BACKGROUND AND NEW POINTS

The immune system can have a causative or consequential role in amyotrophic lateral sclerosis (ALS). Data support active participation for both protection and toxicity. Investigations, aided by the transgenic superoxide dismutase (mSOD1) mouse model of ALS, show early neuroprotective microglia and T regulatory lymphocytes and late neurotoxic microglia, astrocytes, and T effector lymphocytes. In mice, transplantation of T regulatory (Treg) cells prolongs survival; in human ALS increased Tregs is also associated with slower progression. Therapies that could up-regulate neuroprotective microglia and Tregs and down-regulate neurotoxic microglia and T effector cells may be effective.

INTRODUCTION

This chapter focuses on the role of neuroinflammation and immunomodulation in the pathogenesis of ALS, the most common of the motor neuron diseases. In ALS, upper and lower motor neuron injury is associated with inflammatory changes characterized by microglia, the innate immune cells of the central nervous system (CNS), and infiltrating T lymphocytes, the adaptive immune cells. A key question is whether this inflammatory process of activated microglia and T lymphocytes is the cause or the consequence of motor neuron injury in ALS. Can it initiate neurodegeneration or is it a secondary response to neuronal injury?

Much of the work directed at answering these questions is derived from a transgenic mouse model that overexpresses human mutant Cu^{2+}/Zn^{2+} superoxide dismutase (mSOD1). Mutations in SOD1 were one of the earliest discoveries proven to cause familial ALS and led to the development of several different mSOD1 transgenic mouse models and an exponential increase in the understanding of disease pathogenesis. The mouse model is characterized by progressive weakness and death secondary to motor neuron degeneration. Information gleaned from both animal

models and human ALS tissues has suggested that multiple pathways contribute to the pathogenesis of neurodegeneration, including mitochondrial dysfunction, increased reactive oxygen species (ROS), misfolded and aggregated proteins, dysfunction of the ubiquitin proteosome/lysosomal pathways, and neuroinflammation. However, in the last decade, deciphering the role of neuroinflammation in ALS as modulator of disease has become a prominent area of research investigation and has expanded the understanding of the specific cells and signals that impact clinical outcomes.

In ALS spinal cord tissue, microglial activation and T-cell and dendritic cell infiltration are significant pathologic hallmarks at sites of neuronal injury. In the mSOD1 transgenic mouse, immune/inflammatory responses are present early in the disease prior to any evidence of motor dysfunction, suggesting that the inflammatory responses could contribute to the pathogenesis of motor neuron injury. Yet such neuroinflammation has been considered the consequence and not the cause of neuronal injury. The present review challenges this dogma and suggests that both innate and adaptive immune systems respond to and contribute to the pathology and tissue destruction.

The involvement of nonneuronal cells in the pathogenesis of ALS was established with experiments that documented that expression of mSOD1 solely in motor neurons or in astrocytes did not cause motor neuron disease, nor did expression of mSOD1 in microglia alone cause motor neuron disease (Clement et al., 2003; Gong et al., 2000; Beers et al., 2006). Instead mSOD1 had to be expressed in motor neurons and glia. These experiments provided compelling evidence that neurons do not die alone, but depend on the active participation of nonneuronal cells, such as microglia, astrocytes, and T cells. Thus, although the disease process is initiated by mutant SOD1 in the mouse model, compromise of the motor neuron is necessary, albeit not sufficient per se, to cause neurodegeneration. Microglia, T lymphocytes, and dendritic cells actively contribute to neurodegeneration, and at the very least amplify and exacerbate an ongoing inflammatory process, triggering extensive neuronal injury and cell death.

ROLE OF MICROGLIA IN ALS

Microglia are the resident innate immune cells of the CNS that continuously monitor the environment and are activated in response to changes in the extracellular milieu. Upon activation, microglia undergo changes in shape, expression of surface receptors, and secretion of anti-inflammatory or proinflammatory molecules. These CNS innate immune cells are similar to macrophages and are capable of exhibiting a spectrum of responses ranging from the classically activated proinflammatory M1 phenotype with the secretion of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and ROS including nitric oxide (NO) and superoxide ($O_2^{\bullet -}$), and an anti-inflammatory alternatively activated M2 phenotype with the secretion of neurotrophic factors, such as insulinlike growth factor-1 and anti-inflammatory cytokines, such as IL-10 (Fig. 17–1).

Under normal physiologic conditions, microglia serve to protect the neuronal milieu by secreting anti-inflammatory factors. However, in pathologic conditions, such as ALS, a growing body of evidence suggests that microglia exert toxic effects through the increased expression of proinflammatory gene products leading to the release of inflammatory mediators. Postmortem studies have demonstrated a significant number of activated microglia in the brains and spinal cords of patients with ALS compared with control subjects (Henkel et al., 2004; McGeer & McGeer, 2002).

In mSOD1 transgenic mice wild-type microglia or microglia expressing less mSOD1 promoted neuroprotection and extended survival (Beers et al., 2006). During the early, slowly progressing stage of disease in mSOD1 mice, microglia are predominantly M2 that mediate neuroprotection, whereas in the later, rapidly progressing stage microglia are predominantly neurotoxic M1 (Liao, Zhao, Beers, Henkel, & Appel, 2012). What triggers the neurotoxic microglial phenotype in mSOD1 transgenic mice has not been definitively established. Misfolded and aggregated mSOD1 has been demonstrated to activate microglia and mediate motor neuron injury in vitro. Furthermore, misfolded mSOD1 accumulates as aggregates in motor neurons in vivo enhanced by mitochondrial dysfunction and

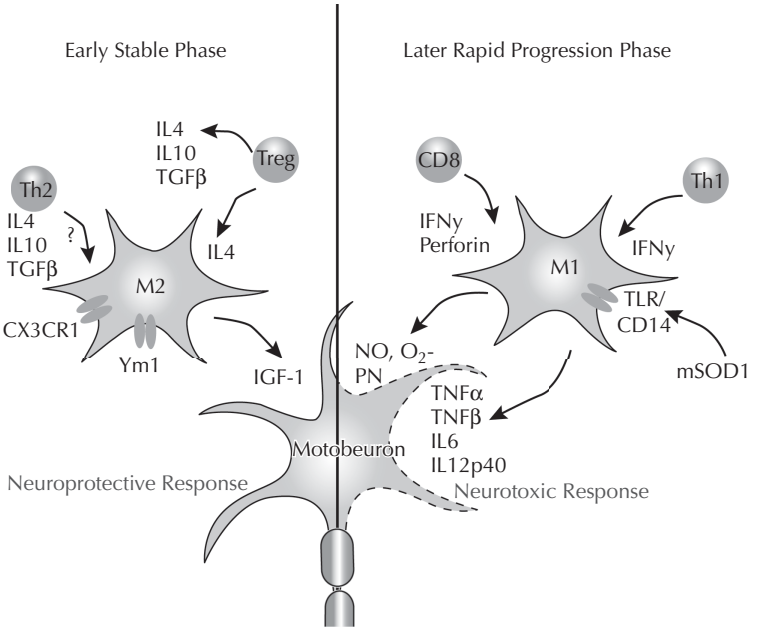

Figure 17–1. Microglia and T cells in the early and late phases of ALS. IFN, interferon; TGF, transforming growth factor. (Adapted from Beers DR, Henkel JS, Zhao W, Wang J, Huang A, Wen S, Liao B, Appel SH. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain. 2011;134(pt 5):1293-314.)

endoplasmic reticulum stress. These misfolded proteins themselves or related signals released from motor neurons could activate microglia shifting them from an anti-inflammatory neuroprotective M2 phenotype associated with the release of cytokines that suppress inflammation toward a proinflammatory neurotoxic M1 phenotype. The neurotoxic M1 microglia in turn release ROS and proinflammatory cytokines, further increasing motor neuron stress and cell injury, and initiate a self-propagating cycle of motor neuron injury and cell death (Appel, Zhao, Beers, & Henkel, 2011).

ASTROCYTES AND ALS

Astrocytes are the most abundant glial cells in the CNS. They perform many functions, including support of endothelial cells that form the blood-brain barrier, provide nutrients to the nervous tissue, and aid in the repair process of the brain and spinal cord. Under normal conditions, astrocytes provide neurons with structural, metabolic, and trophic support. A prominent example is the glutathione dipeptide precursor CysGly, which is secreted by astrocytes, taken up by neurons, and converted into glutathione, which serves an important antioxidant function. Astrocytes also mediate neuroprotection by removing excess glutamate through the EAAT2 transporter (Kim et al., 2011). In the injured brain, however, astrocytes become reactive and respond to proinflammatory cytokines with morphologic changes and increased proliferation. Although astrocytes are not classically professional immune cells, upon activation they can contribute to the local innate immune response. When activated, astrocytes can produce several toxic molecules, including a variety of ROS and NO. ROS can promote excitotoxicity to neighboring neurons, through damage to the glutamate uptake molecule EAAT2, which is present on astrocytes (Trotti, Danbolt, & Volterra, 1998). In vitro, it has been shown that activated mSOD1 astrocytes are capable of killing even previously healthy neighboring motor neurons (Di Giorgio et al., 2007; Nagai et al., 2007). In ALS, areas of neuroinflammation contain an abundance of activated

astrocytes and microglia. Collectively, data from previous studies have suggested that mutant SOD1-expressing astrocytes, at baseline, may be intrinsically not quiescent and more prone to enter an activated neuroinflammatory state compared with their wild-type counterparts (Papadimitriou et al., 2010). Thus, even though astrocytes may not initiate the disease, there is evidence that in the mutant SOD1 mouse model of ALS, the expression of mutant SOD1 in astrocytes may amplify motor neuron injury, adding to the neurotoxicity promoted by activated microglia (Clement et al., 2003).

CYTOKINES, CHEMOKINES, AND OTHER MARKERS OF INFLAMMATION IN ALS

Levels of numerous proinflammatory and anti-inflammatory cytokines and chemokines are increased in patients with ALS and ALS mouse models. The proinflammatory moieties include IL-6, TNF-α, IL-1β, IL-12, IL-17, IL-23, interferon-γ; the anti-inflammatory cytokines include IL-4, IL-10, and transforming growth factor-β, most of which derive from activated glia, monocytes/macrophages, T cells, or dendritic cells. IL-17 and IL-23 were found to be elevated in serum and cerebrospinal fluid of patients with ALS (Fiala et al., 2010; Rentzos et al., 2010), and it has been suggested that these elevations are a reflection of T_h17 cell activation.

None of these cytokines are specific for ALS or ALS models and several, such as TNF-α and transforming growth factor-β, can contribute to either protection or toxicity. Furthermore, the multiplicity of such cytokines and pathways suggests that no single factor per se mediates either protection or toxicity; deletion or inhibition of no single factor can dramatically change the course of disease. Yet collectively their presence supports the involvement of immune/inflammatory processes in the pathogenesis of disease.

Chemokines are critical signals for the recruitment of immune cells to areas of tissue injury and promote differentiation of recruited cells. Chemokines, such as fractalkine (CX3C1) and monocyte chemotatic protein-1 (CCL2), also have a role in modulating neuroinflammation in ALS that promotes neuroprotection or propagation of inflammation. Microglia are the only CNS cells that express the chemokine receptor to fractalkine (CX3CR1). In the absence of the CX3CR1 fractalkine receptor, M1 neurotoxic microglial activation is significantly increased and associated with extensive neuronal loss in the mutant SOD transgenic mouse (Cardona et al., 2006). CCL2 is another potent chemoattractant and activating peptide that is expressed mostly in astrocytes but also in neurons, microglia, and macrophages following diverse injury. CCL2 attracts myeloid dendritic cells, monocyte/macrophage/microglia, and activated T cells through expression of cell surface chemokine (C-C motif) receptor 2 (CCR2), a dominant chemotaxis receptor that mediates chemotactic movement of monocytes in response to CCL2. Thus, the recruitment of immune/inflammatory cells into the CNS, particularly of dendritic cells and monocytes, is dependent on CCL2. Higher levels of the chemokine CCL2 are present in ALS spinal cord tissue and in the cerebrospinal fluid of patients with ALS compared with patients with noninflammatory neurologic disease (Kuhle et al., 2009). Patients with ALS with higher CCL2 values trended toward a shorter diagnostic delay and a shorter survival. The association of higher CCL2 with faster disease progression suggests that enhanced trafficking of activated monocytes/macrophages might contribute to the pathogenesis of disease. It has also been reported that the CCL2 receptor, CCR2, was reduced on circulating monocytes in ALS (Zhang et al., 2006) and associated with a slower rate of disease progression. Thus, the loss of CCR2 expression might be an essential protective reaction of the host immune response to macrophage-mediated CNS damage in ALS.

Other markers of inflammation noted in ALS include those of the classical complement pathway. Levels of mRNA and proteins of C1q and C4, and the downstream complement components C3 and C5b-9, were found to be elevated in ALS samples in comparison with controls (Sta et al., 2011). There is also evidence of low level systemic inflammation with increased levels of C-reactive protein and erythrocyte sedimentation rate in subjects with ALS compared with control subjects, with the levels correlating with levels of disability as measured by the ALS functional rating scale (Keizman et al., 2009).

T CELLS IN ALS

Perivascular and intraparenchymal infiltrates of $CD4^+$ and $CD8^+$ T cells have been demonstrated in autopsy specimens of ALS spinal cords. Expression of the T-cell receptor (TCR-BV2) was also significantly increased in spinal cord specimens (Panzara et al., 1999). However, the presence of such cells does not define their function, and $CD4^+$ T cells have varying roles in the immune system depending on the subtype. $CD4^+$ Th1 and Th17 T cells promote cytotoxicity and contribute to neuroinflammation through molecules, such as IL-1, IL- 6, IL-17, TNF-α, and interferon-γ, that enhance microglia-mediated neurotoxicity following the release of ROS and NO. By contrast, Th2 and Tregs produce the anti-inflammatory cytokines IL-4 and IL-10, which enhance microglia-mediated neuroprotection (Xin et al., 2011; Zhao, Beers, Liao, Henkel, & Appel, 2012).

$CD4^+CD25^+FoxP3^+$ Tregs contribute to suppression of immune cell activation and maintenance of immunologic tolerance. They also contribute to neuroprotection in ALS by promoting microglial secretion of anti-inflammatory cytokines and neurotrophic factors, and/or by promoting a shift from pro-inflammatory Th1 to anti-inflammatory Th2 cells (Kipnis et al., 2004). Most relevant is the ratio of Treg/Th2 cells to Th1/Th17 cells, which can mediate the balance of neuroprotection versus neurotoxicity. The greater the number and function of Treg/Th2 cells the greater the neuroprotection; the greater the number and function of Th1/Th17 cells the greater the neurotoxicity. Experiments with mSOD1 transgenic mice have provided significant insights. $CD4^+$ T cells have been observed in the spinal cord during all phases of mSOD1 transgenic mouse disease, including disease onset and during the early slowly progressing stage of disease, whereas $CD8^+$ T cells were present only in terminal stages. In the early slowly progressing stages, $CD4^+$ Tregs were increased along with M2 microglia. When the disease began to accelerate, Treg and Th2 cells were significantly decreased, and Th1 and M1 microglia were more prominent (Beers et al., 2011).

To determine whether the T cells were protective or toxic, mSOD1 mice were bred with mice lacking functional $CD4^+$ T cells (Beers et al., 2008). Removing CD4 T cells accelerated motor neuron disease in these transgenic mice. Thus, T cells are neuroprotective in mSOD1 mice, and the lack of functional $CD4^+$ T cells results in accelerated disease accompanied by spinal cord markers of increased mRNA levels for proinflammatory cytokines and NOX2, and decreased levels of trophic factors and glial glutamate transporters. Reconstitution of the transgenic mice with T cells, especially $CD4^+$ Treg cells, prolonged survival, suppressed cytotoxicity, and restored the M2 neuroprotective phenotype. These results suggest that $CD4^+$ Treg and Th2 cells are neuroprotective possibly by enhancing the neuroprotective functions of glia (Beers et al., 2008).

In the peripheral blood, patients with ALS with faster progressing disease had decreased numbers of Treg lymphocytes and Th2 cells, and decreased RNA expression of FoxP3 and Gata3, aTh2 transcription factor. In fact, both numbers of Treg and their FoxP3 expression as well as Th2 cells and Gata3 expression inversely correlated with progression rates (Henkel et al., 2013). These data suggest that in patients with ALS as in mSOD1 transgenic mice, $CD4^+$ T lymphocytes actively contribute to disease progression. However, it is the subpopulation of $CD4^+$ T lymphocytes that is most important; decreased levels of Treg/Th2 lymphocytes relative to Th1/Th17 lymphocytes were associated with significantly faster disease progression. Thus, the therapeutic goal would be to increase Treg/Th2 cell populations and decrease the Th1/Th17 cell populations (Beers et al., 2011).

DENDRITIC CELLS

Dendritic cells are potent antigen-presenting cells that regulate the innate and adaptive immune systems. Dendritic cells can present antigen and stimulate naive adaptive immune T cells. There are two main subclasses of dendritic cells: myeloid dendritic cells and plasmacytoid dendritic cells. Myeloid dendritic cells are particularly sensitive to CCL2, through their expression of CCR2 on their cell surface. Both immature and activated dendritic cell transcripts were significantly elevated in sporadic and familial ALS spinal cord tissues, but the specific antigens being present are

not presently defined (Henkel et al., 2004). Furthermore, increased expression of dendritic cell transcripts, but not monocytic/macrophage/microglial, cytokine, or chemokine transcripts, seemed to correlate with more rapidly progressing disease, thus suggesting that dendritic cells may exacerbate motor neuron injury in ALS. Increased dendritic cells were also seen in the spinal cord tissue of mSOD1 mouse model (Henkel, Beers, Siklos, & Appel, 2006).

TREATMENT TARGETING IMMUNE MECHANISMS AND NEUROINFLAMMATION

Several older therapeutic trials used immunosuppressant agents as potential treatments of ALS, including cyclophosphamide, intravenous immunoglobulin, prednisone, cyclosporine, plasmapheresis, thalidomide, and total-body irradiation. In a recent study, glatiramer acetate, a well-known immunomodulating agent used in multiple sclerosis, failed to show any impact on disease progression in a double-blind, randomized, placebo-controlled, multicenter trial (Meininger et al., 2009). Thus, no studies have supported the efficacy of immunosuppression, and as a result the immune system has been rejected as contributing significantly to the pathogenesis of ALS. However, recent data suggest that the immune system does play a role, but why conventional immunosuppression has been ineffective is not clear. Certainly the heterogeneity of ALS could contribute and the immune system might make a significant contribution only in a subpopulation of patients with ALS. Furthermore, it is now clear that immune cells can be neurotoxic and neuroprotective, and simultaneously suppressing both arms might not change the relative balance of neurotoxicity and neuroprotection.

Given that macrophage activation is believed to be a primary contributor to the pathology underlying ALS and other neurodegenerative diseases, medications are being developed that target this mechanism. NP001 is a pharmaceutical that can transform macrophages from a neurotoxic state to a neuroprotective state. A double-blinded, placebo-controlled, phase 1, single ascending dose study demonstrated safety and tolerability of NP001 at four different dose levels in patients with ALS. A phase II trial has been completed and despite the small size of the trial, there is a suggestion of efficacy, warranting a more extensive evaluation.

Hematopoietic stem cell transplantation has been associated with suppression of neuroinflammation in various diseases, and is currently being researched in ALS. Mesenchymal stem cells possess immunomodulatory effects, specifically by secreting neurotrophic and anti-inflammatory factors. Several studies have demonstrated successful transplantation of stem cells in mouse models of ALS, and prevention of the degeneration of motor neurons and improved motor function in mouse models (Lepore et al., 2008; Suzuki et al., 2007; Pastor et al., 2011). However, to date, stem cell transplantation studies in patients with ALS have failed to demonstrate any evidence of improved strength or prolonged survival (Appel et al., 2008; Karussis et al., 2010; Mazzini et al., 2011). In a study to determine whether allogeneic human hematopoietic stem cells could engraft at sites of injury within the spinal cord and improve clinical outcomes in ALS (Appel et al., 2008), six patients with definite ALS received peripheral blood hematopoietic stem cell transplantation infusion from identically matched sibling donors. The study demonstrated that donor hematopoietic stem cells can enter the human CNS and engraft as immunomodulatory cells. However, no clinical benefit was seen. Currently, a phase I study is underway examining the safety of surgical implantation of fetal-derived neural stem cells in patients with ALS. In addition to safety concerns, multiple questions still need to be addressed including how long such implanted cells survive in the ALS spinal cord, whether such cells mediate neuroprotection, and whether the implants promote anti-inflammatory or neurotrophic repair mechanisms.

CONCLUSION

It is clear that the immune system plays an important role in the pathogenesis of ALS. The role of inflammation in the initiation of disease, if any, remains unclear. However, research has supported that activated

microglia and $CD4^+$ T cells contribute significantly to disease progression. In the transgenic mutant SOD1 mouse model of ALS, misfolded and aggregated proteins are secreted from neurons, promoting proinflammatory M1 microglia and cytotoxic T cells, and amplifying neuronal injury. In the absence of known mutations, it is not clear how the disease is initiated. However, oxidized nonmutant SOD1 has been detected in the motor neurons of patients with sporadic ALS, and aggregated SOD1 could transform neuroprotective microglia and T cells into cytotoxic cells, thereby accelerating disease progression. Further studies are certainly mandated in patients with sporadic ALS to determine what initiates the oxidative stress and how such stress is translated into misfolded SOD1 or other misfolded proteins or peptides that promote the microglial/monocyte/macrophage and T-cell involvement in disease pathogenesis. Transgenic models of ALS mice provide clear evidence for neuronal-microglial and T cell-microglial dialogues mediating both neuroprotection and neurotoxicity at different stages of disease. Thus the immune response is not merely a consequence of injury, but actively influences and significantly contributes to the balance of neuroprotection and neurotoxicity.

At present no medications are known to change the fundamental course of ALS. However, therapies that can up-regulate Treg lymphocytes and M2 microglia and down-regulate Th1 lymphocytes and M1 microglia have the potential to convey significant benefit.

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Chapter 18

Cyanobacteria, Amyotrophic Lateral Sclerosis, and the BMAA Hypothesis

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BACKGROUND AND NEW POINTS

The high incidence of amyotrophic lateral sclerosis (ALS) and parkinsonism dementia complex (PDC) in the Marianas Islands, which later declined with cultural changes of the indigenous population, raised the strong possibility of an environmental factor underlying the onset of these neurodegenerative diseases. Initial investigations led to consideration of β-*N*-methylamino-L-alanine (BMAA) from cycad seeds, but investigations failed to document sufficient intake.

The concept of biomagnification of BMAA by indigenous people eating bats, who in turn consume cycad seeds, renewed the issue of an environmental factor (Banack & Cox, 2003a). Further investigations raised the possibility that BMAA could be an environmental factor for ALS worldwide. BMAA is produced by cyanobacteria (Cox, Banack, & Murch, 2003; Cox et al., 2005), which is ubiquitous, and possible epidemiologic clusters of ALS have been noted around bodies of water which can have high concentrations of cyanobacterium (Caller, Chipman, Field, & Stommel, 2013; Torbick, Hesssion, Stommel, & Caller, 2014).

INTRODUCTION

Considering that a genetic cause for ALS has not been identified in most patients, it is highly likely that one or more environmental toxins contribute to the etiology of sporadic ALS, most likely interacting with underlying genetic susceptibility factors. A neurotoxin produced by cyanobacteria has been implicated as a potential environmental risk factor for ALS (Cox, Banack, & Murch, 2003; Bradley & Mash, 2009; Banack, Caller, & Stommel, 2010). Cyanobacteria are ubiquitous throughout all ecosystems, most commonly in marine and freshwater environments (Carmichael, 2008). They pose a health risk when large concentrations of cyanobacteria form "blooms" on the surface of water bodies as a result of both natural and human-related eutrophication (Stewart, Webb, Schluter, & Shaw, 2006; Carmichael, 2008). Cyanobacteria are well-known to produce toxins that have human health implications, including microcystins, cylindrospermopsins, lyngbyatoxins, anatoxins, lipopolysaccharide endotoxins, and a neurotoxin of interest (BMAA) (Fig. 18–1) (Cox et al., 2005; Funari & Testai, 2008).

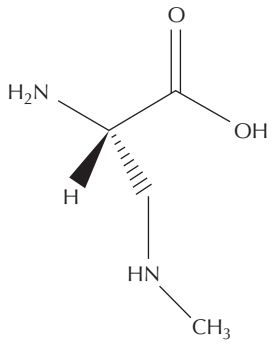

Figure 18–1. BMAA structure.

THE GUAM HYPOTHESIS

Following World War II, an extremely high incidence of ALS and ALS-like conditions (ALS/PDC) was observed in the Marianas Islands, particularly in Guam, where it was estimated to be 50–100 times higher than industrialized nations, and later declined with cultural changes of the indigenous population. (Zimmerman, 1945; Arnold, Edgren, & Palladino, 1953; Kurland & Mulder, 1954; Reed, Plato, Elizan, & Kurland, 1966). The decline in ALS incidence over the subsequent five decades to levels similar to the rest of the world suggested that the cause of the disease was environmental and perhaps related to the rapid westernization of Guam (Garruto, Yanagihara, & Gajdusek, 1985; Plato et al., 2003). Initial research suggested that cycad seeds, a dietary staple used by the indigenous Chamorro people to make flour, might be the environmental exposure of interest (Whiting, 1963, 1964; Dastur, 1964; Spencer, Nunn, Hugon, Ludolph, & Roy, 1986). A neurotoxic nonprotein amino acid, BMAA, was discovered in cycad seeds (Vega & Bell, 1967; Kisby, Ellison, & Spencer, 1992), which is derived from cyanobacteria existing symbiotically in the coralloid roots of *Cycas micronesica* (Cox, Banack, & Murch, 2003; Banack & Cox, 2003b). Initial studies of BMAA concentration in cycad flour determined that concentrations were too low to cause human disease (Duncan, Kopin, Garruto, Lavine, & Markey, 1988). Further investigation demonstrated that BMAA is mainly concentrated in proteins and was consumed by the Chamorros through multiple dietary sources, including cycad flour, flying foxes (a type of fruit bat), and other animals that fed on cycad seeds (Banack, Murch, & Cox, 2006; Banack & Murch, 2009; Cheng & Banack, 2009). BMAA was shown to biomagnify through the food chain, leading to potentially large doses being consumed by the Chamorros (Banack & Cox, 2003a; Murch, Cox, & Banack, 2004). Accumulation of BMAA in the brains of Chamorro patients with ALS/PDC further supported this hypothesis (Murch, Cox, Banack, Steele, & Sacks, 2004). Demonstrating generalizability of this finding to other ALS populations, BMAA was tested for and identified in the brains of patients with ALS in the United States; it was not generally found in brain tissue of nonneurologic control subjects or in patients with Huntington disease, a genetic neurodegenerative disease, suggesting that BMAA does not occur as a by-product of neurodegeneration (Pablo et al., 2009). The decrease of both cycad seeds and flying foxes in the Chamorro diet correlated with the previously mentioned decline in the prevalence of neurodegenerative disease on Guam (Cox & Sacks, 2002; Monson, Banack, & Cox, 2003; Plato et al., 2003; Bradley, Banack, & Cox, 2009).

Of interest is the lag time noted between exposure and disease onset. Emigrants from Guam, who lived on Guam during their childhood and adolescence for at least 18 years, developed ALS 1–34 years after leaving the island, suggesting a possible latency period of more than 30 years (Torres, Iriarte, & Kurland, 1957; Eldridge, Ryan, Rosario, & Brody, 1969; Garruto, Gajdusek, & Chen, 1980). Likewise, Filipino residents in Guam developed ALS 1–29 years after migrating to the island (Garruto, Gajdusek, & Chen, 1981). These latency periods are consistent with the time-dependent latency of deployed Gulf War veterans from the 1990–1991 Persian Gulf war who had higher incidence rates of ALS in the 10-year period following the war (Horner et al., 2008). Those deployed to the Gulf had ALS incidence rates two-fold higher than veterans who were trained at the same time but were not deployed (Haley, 2003; Horner et al., 2003; Coffman, Horner, Grambow, & Lindquist, 2005). These observations have implications for the development of an animal model related to chronic exposure to the neurotoxin.

Mechanisms of BMAA Toxicity

BMAA is considered a nonprotein amino acid (Fig. 18–1) as it is not one of the coded

amino acids that make up normal proteins. The evidence that BMAA could produce neurodegeneration, as seen in ALS, is intriguing. BMAA has the capability of crossing the blood-brain barrier through an active transport mechanism and seems to have a predilection for central nervous system tissue, as shown in rodent models (Duncan et al., 1991; Xie, Basile, & Mash, 2013). *In vitro* work has shown that the acute neurotoxicity of BMAA has multiple modes of action including binding to *N*-methyl-d-aspartate, AMPA/kainite, and metabotropic receptors; affecting the cystine/glutamate antiporter system (system xc^-); induction of oxidative stress and mitochondrial dysfunction; disregulation of cellular protein homeostasis; endoplasmic reticulum stress; and gliotoxicity (Weiss, Koh, & Choi, 1989; Manzoni, Prezeau, & Bockaert, 1991; Rao, Banack, Cox, & Weiss, 2006; Lobner, Piana, Salous, & Peoples, 2007; Liu, Rush, Zapata, & Lobner, 2009; Okle, Stemmer, Deschl, & Dietrich, 2012; Chiu et al., 2013; Muñoz-Saez et al., 2013). Glutamate receptor activity has been implicated previously in the pathophysiology of ALS, and the only Food and Drug Administration approved medication for ALS in the United States, riluzole, acts on glutamate receptors (Zoing, Burke, Pamphlett, & Kiernan, 2006; le Verche, Ikiz, Jacquier, Przedborski, & Re, 2011). *In vivo* research has confirmed that BMAA acts as a glutamate agonist causing *N*-methyl-d-aspartate channels to remain open for prolonged periods; alters protein expression; changes energy metabolism and amino acid metabolism; and induces oxidative stress, mitochondrial damage, and endoplasmic reticulum fragmentation (Goto, Koenig, & Ikeda, 2012; de Munck, et al., 2013; Engskog, et al., 2013; Karlsson et al., 2013). A large portion of BMAA remains protein bound (Murch, Cox, & Banack, 2004), and BMAA may also lead to neurodegeneration through protein misfolding, which might provide a mechanism for neurotoxicity through chronic exposure. Elegant work by Rogers et al. has shown that BMAA can be misincorporated into proteins during protein synthesis by being erroneously substituted for L-serine, which triggers protein misfolding, accumulation of protein aggregates, and apoptosis, all of which are hallmarks of neurodegeneration (Lee et al., 2006; Rogers, 2011; Dunlop, Cox, Banack, & Rodgers, 2013). The incorporation of amino acid analogues into proteins has been previously demonstrated and could lead to cell dysfunction (Rosenthal 1977; Rubenstein 2000; Hendrickson, De Crécy-Lagard, & Schimmel, 2004; Rodgers, Hume, Dunlop, & Dean, 2004; Rubensten, 2008; Rogers & Shiozawa, 2008; Rodgers, 2014).

Several studies have evaluated the neurotoxicity of BMAA in mice, rats, and primate animal models. In these studies, BMAA was typically administered at high doses and over a short period of time (days to weeks) with mixed results: initial primate studies suggested BMAA could cause neurologic degeneration, but later rodent experiments showed no neurotoxicity with oral dosing of BMAA (Karamyan & Speth, 2008). Overall, *in vivo* studies to date demonstrate that BMAA can be acutely neurotoxic at very high doses (Spencer et al., 1987), can affect memory and learning with demonstratable immunohistochemical, microscopic, and proteomic changes consistent with neurodegeneration changes when exposure is during developmental stages (Karlsson, Roman, Berg, & Brittebo, 2011; Karlsson et al., 2014), can increase tau hyperphosprylation (Arif, Kazim, Grundke-Iqbal, Garruto, & Iqbal, 2014), and can induce specific phenotypes consistent with sporadic ALS (de Munck et al., 2013). BMAA was also shown in mice to be transferred through milk to suckling offspring (Andersson, Karlsson, Bergstrom, Brittebo, & Brandt, 2013).

Although few studies have investigated the effects of low concentrations of BMAA, the *in vitro* research of one team suggests that at low concentrations BMAA can cause endoplasmic reticulum stress and disrupt protein homeostasis (Okle, Stemmer, Deschl, & Dietrich, 2012). To date, there is no animal model that has studied chronic ingestion of BMAA, and the effect of chronic BMAA exposure remains unclear.

There are several caveats to animal model studies (Karamyan & Speth, 2008). Causality is likely multifactorial, and animal models may not account for other disease susceptibility factors, such as malnutrition. Because BMAA can substitute for L-serine in protein synthesis (Dunlop, Cox, Banack, & Rodgers, 2013), preexisting malnutrition and protein deficiency could result in the misincorporation of BMAA at a higher frequency. A similar mechanism of toxicity was described in the neurologic disease lathyrism, where a similar nonprotein

amino acid acquired in the diet through eating a drought-resistant legume (*Lathyrus sativus*) becomes neurotoxic, particularly in the setting of malnutrition (Haimanot et al., 1993; Spencer, 1994; Getahun, Lambein, Vanhoorne, & Van der Stuyft, 2003). Animal models have also not accounted for the potential of multiple environmental toxins acting synergistically, such as occurs between BMAA and methylmercury (Rush, Liu, & Lobner, 2012), or genetic susceptibilities that might make certain individuals more sensitive to BMAA neurotoxicity. Finally, it is unclear what particular populations of humans might be more susceptible to BMAA than others. There is no way to account for underlying genetic susceptibilities to the toxin, such as a genetic alteration that might increase the rate of transport of BMAA across the blood-brain barrier, increase the amount of BMAA misincorporated into protein, or alter other cellular mechanisms.

BEYOND GUAM

If there is the possibility that BMAA exposure can lead to neurodegeneration and ALS, how might this toxin be implicated outside of Guam? As is the case with other environmental toxins, one must consider that BMAA could be acquired through a number of mechanisms (Fig. 18–2).

Dietary exposure continues to be the most probable mechanism of exposure. Consumption of cyanobacteria has occurred in certain ethnic populations for hundreds of years; many of these cyanobacteria food sources have been demonstrated to contain BMAA (Johnson et al., 2008; Roney et al., 2009). Cyanobacteria or "blue-green algae" supplements have been produced commercially since the 1970s in the United States and could be a possible source of exposure. *Arthrospira* (*Spirulina*) spp. and *Aphanizomenon flos–aquae* are the cyanobacterial species most frequently cultivated for health food supplements (Dietrich, Fischer, Michel, & Hoeger, 2008). Although *Arthrospira* (*Spirulina*) is generally considered nontoxic, *A. flos-aquae* is known to produce anatoxin-a, saxitoxins, and BMAA (Cox et al., 2005; Dietrich et al., 2008). Most importantly, a variety of toxins have been documented in these health food supplements including microcystin that may be the consequence of additional cyanobacterial genera cohabitating collection sites (Dietrich et al., 2008). Concerns about possible synergistic effects between BMAA and other cyanotoxins have been noted but have not yet been sufficiently tested.

Cyanobacteria are ubiquitous in water bodies, and multiple species of cyanobacteria

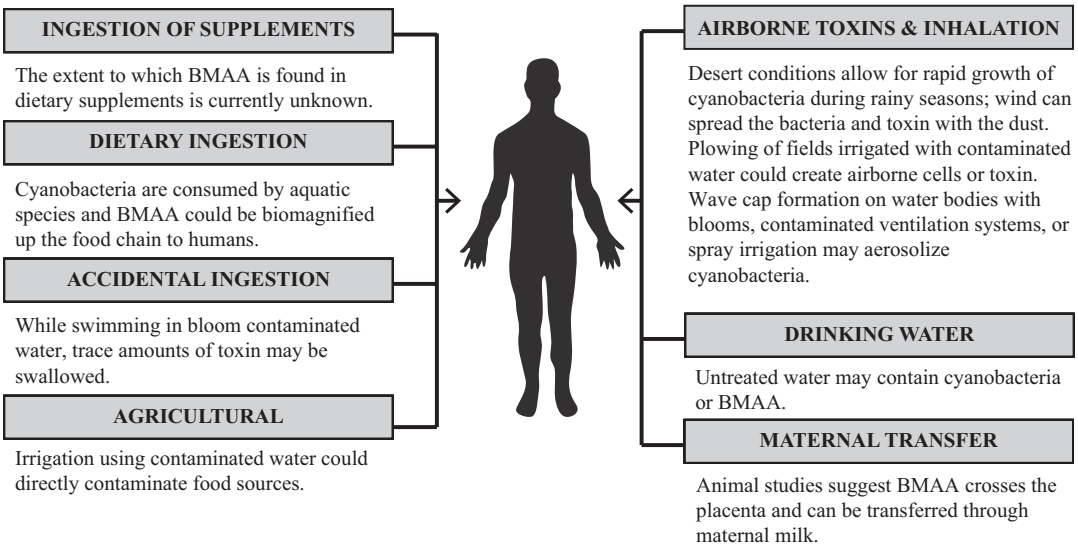

Figure 18–2. Potential routes of human exposure to BMAA.

are capable of BMAA production in aquatic environments (Cox et al., 2005; Banack, Johnson, Cheng, & Cox, 2007; Esterhuizen & Downing, 2008; Metcalf et al., 2008). The examination of other ecosystems has demonstrated the presence of BMAA in fish, sharks, and crustaceans in the United States, France, and Sweden (Brand, Pablo, Compton, Hammerschlag, & Mash, 2010; Jonasson et al., 2010; Mondo et al., 2012; Field et al., 2013; Masseret et al., 2013; Salomonsson, Hansson, & Bondesson, 2013). Epidemiologic studies of areas of high incidence of ALS have supported the possibility of BMAA being acquired through the consumption of seafood and freshwater aquatic animals. Investigation of the high incidence of ALS patients in Two Rivers, WI found that ALS patients ate fish from nearby Lake Michigan (a lake known to have cyanobacterial blooms) more frequently than control subjects (Sienko, Davis, Taylor, & Brooks, 1990). An increased incidence of ALS in the southern Finish Lakelands was identified in close proximity to the Baltic Sea, which suffers extensive cyanobacterial blooms (Sabel, Gatrell, Loytonen, Maasilta, & Jokelainen, 2000, Sabel et al., 2003). Bottom-dwelling crustaceans in the Baltic Sea have detectable concentrations of BMAA and are a human food source (Fig. 18–2). Another region of increased ALS incidence has been noted in the south of France, and BMAA has been identified in shellfish from Southern France's Thau Lagoon, a shallow coastal lagoon off the Mediterranean Sea that is frequently subject to cyanobacterial blooms (Corcia et al., 2003; Masseret et al., 2013). Epidemiologic studies can suggest associations but do not prove causation; further studies are needed to determine whether the exposure to BMAA is sufficient to cause neurodegeneration (Bradley et al., 2013).

Direct consumption of BMAA through agricultural plants irrigated by water contaminated by cyanotoxins is also possible. Several studies suggest that terrestrial plants can both bioaccumulate cyanotoxins and present toxins to humans from surface exposure following irrigation with water containing cyanobacteria (Codd, Metcalf, & Beattie, 1999; Corbel, Mougin, & Bouaïcha, 2014). It has also been directly demonstrated that some plants exposed to BMAA contaminated water quickly uptake BMAA (Esterhuizen Plugmacher, & Downing, 2001) including agricultural plants such as wheat, *Triticum aestivum* (Contardo-Jara, Schwanemann, & Pflugmacher, 2014). Since BMAA is produced by an array of cyanobacteria, irrigation of agricultural plants by cyanobacterially contaminated water is a potential source of human exposure.

There is also potential for persons living near cyanobacterial blooms to be exposed to BMAA and other cyanotoxins through aerosolization of cyanobacteria (Stommel, Field, & Caller, 2013). Laboratory studies have shown that cyanotoxins in water can be transferred to air via a bubble-bursting process suggesting the possibility of exposure to lung, gastrointestinal tract, and nasopharyngeal mucosa by wave action on water-bodies containing cyanobacterial blooms; recreational activities, such as water skiing; or through saunas and showers using cyanobacteria-contaminated water (Cheng, Zhou, Irvin, Kirkpatrick, & Backer, 2007; Sharma & Singh, 2010). There are many examples of toxin aerosolization leading to human illness, including brevetoxins (produced by dinoflagellates), which cause an asthma-like syndrome, and the marine organism *Pfiesteria piscicida*, which can produce delirium if inhaled (Morris & Pfiesteria, 1999; Fleming et al., 2007, 2009). Similarly, desert surfaces are stabilized by cyanobacteria-dominated cryptogamic crust, which has the potential to pose a human health risk through the inhalation of airborne toxins in desert dust (Powell, Chatziefthimiou, Banack, Cox, & Metcalf, 2014; Richer, Banack, Metcalf, & Cox, 2014). It has been hypothesized that cyanobacterial toxins in airborne desert dust could be responsible for the high rates of ALS among US veterans deployed in the 1990–1991 Persian Gulf War (Cox et al., 2009).

CONCLUSION

There is fairly convincing evidence to implicate BMAA as a causative agent of ALS/PDC on Guam for many reasons: the strength of association through identification of the toxin within the food chain in an environment with extremely high disease incidence, the identification of that toxin within brain tissue of patients who died from ALS/PDC, the temporal relationship between disappearance of

dietary BMAA and decrease in disease incidence, and the biologic possibility of BMAA to produce chronic neurodegeneration. Recent investigations into BMAA exposure routes outside Guam suggest multiple sources of potential exposure including toxins found in dietary supplements and aquatic food, and the inhalation of toxins through aerosolization or airborne particles. There are also limitations of the hypothesis implicating BMAA in the onset of sporadic ALS, most importantly the current lack of an animal model to demonstrate causality.

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Chapter 19

Animal Models of Adult Motor Neuron Disease

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BACKGROUND AND NEW POINTS

Animal models are useful tools to investigate underlying pathology and validate therapeutic strategies. Transgenic mouse models were based on superoxide dismutase (SOD1) mutations and reproduced many features of ALS, and were also used to screen candidate drugs.

The field of modeling has markedly expanded with the construct of invertebrate models and rat models. Each new gene mutation associated with amyotrophic lateral sclerosis (ALS) has led to new models. Although models allow for intricate genetic manipulations, elucidation of pathologic mechanisms in ALS remains elusive.

INTRODUCTION

Since the discovery of SOD1 mutations linked to ALS two decades ago (Rosen et al., 1993), additional genes have been discovered providing further opportunity to generate animal models linked to these ALS genes. This chapter summarizes findings from invertebrate and vertebrate animal models and points out the relevance of outcomes to human motor neuron disease. Although many ALS genes have been uncovered, the focus here is on animal models of SOD1, p150^{Glued}, TDP-43, fused in sarcoma/translocated in liposarcoma (FUS/TLS), and C9orf72. Select models from each of these are listed in Table 19–1.

Animal models are essential tools to clarify disease mechanism and to identify and validate therapeutic strategies. A variety of model organisms are available, each with advantages and limitations. Vertebrate models include nonhuman primates, rodents (mice and rats), and zebrafish and are critical for modeling human disease because of the extent of functional cross-species conservation. In addition, vertebrate models are required for evaluation of new treatment regiments as a prelude to clinical trials. However, in the context of high-throughput screenings to identify

Table 19–1 **Select Models From SOD1, DCTN1, TDP-43, FUS, and C9orf72**

	SOD1		DCTN1		TDP43		FUS		C9orf72	
	lof	gof	lof	gof	lof	gof	lof	gof	lof	gof
Drosophila		Watson et al., 2008	Lloyd et al., 2012		Wang et al., 2009 Feiguin et al., 2009 Fiesel et al., 2010 Lu et al., 2009	Lu et al., 2009 Li et al., 2010 Hanson et al., 2010 Elden et al., 2010 Estes et al., 2011	Wang et al., 2011	Lanson et al., 2011 Chen et al., 2011		Xu et al., 2013
Caenorhabditis elegans		Wang et al., 2009 Watson et al., 2008			Zhang et al., 2011 Zhang et al., 2012	Ash et al., 2010 Liachko et al., 2010 Zhang et al., 2011		Murakami et al., 2012	Therrien et al., 2013	
Zebrafish		Lemmens et al., 2007 Ramesh et al., 2010			Schmid, 2013 Hewamadduma et al., 2013 Armstrong et al., 2013b	Kabashi et al., 2010	Kabashi et al., 2011b Armstrong et al., 2013b	Kabashi et al., 2011a	Ciura et al., 2013	Lee et al., 2013
Mouse		Gurney et al., 1994 Wong et al., 1995 Bruijn et al., 1998 Jaarsma et al., 2008 Gong et al., 2000		Lai et al., 2007 Laird et al., 2008 Chevalier-Larsen et al., 2008	Kraemer et al., 2010 Chiang et al., 2010 Wu et al., 2012 Iguchi et al., 2013	Wegorzewska et al., 2009 Stallings et al., 2010 Xu et al., 2010, 2011 Wils et al., 2010 Shan et al., 2010 Tsai et al., 2010 Igaz et al., 2011 Cannon et al., 2012 Swarup et al., 2011	Hicks et al., 2000 Verbeeck et al., 2012	Verbeeck et al., 2012 Mitchell et al., 2013		
Rat		Howland et al., 2002 Nagai et al., 2001						Huang et al., 2011 Mitchell et al., 2013		

Note: gof = gain of function; lof = loss of function.

genetic interactions or pharmacologic therapies, murine or primate models are costly and time-consuming; hence, simple invertebrate models, including fruit fly (*Drosophila melanogaster*) and roundworm (*Caenorhabditis elegans*), are attractive alternatives.

INVERTEBRATE MODELS

Drosophila and *Caenorhabditis elegans* Models

Despite marked anatomic divergence, the basic molecular and cellular cascades mediating complex behaviors are remarkably conserved between invertebrates and mammals. In addition to rapid generation time, short life span, and low costs in growing and maintaining large colonies, both *C. elegans* and *Drosophila* have a compact genome size and limited genetic redundancy. Transgenic animals are relatively easy to produce in both systems. Tissue-specific and inducible promoters are widely available, allowing tissue and developmentally specific effects to be targeted.

SOD1

Since the discovery of mutations in the Cu/Zn SOD1 gene (Rosen et al., 1993), occurring in ~5–10% of autosomal-dominant cases of familial ALS (FALS), intensive research using animal models has been performed.

Expression of wild-type or disease-linked mutants of human SOD1 (A4V, G85R) selectively in motor neurons induces progressive climbing deficits, accompanied by defective neural circuit electrophysiology, accumulation of human SOD1 protein, and a stress response in surrounding glia (Watson, Lagow, Xu, Zhang, & Bonini, 2008). However, a major limitation of the *Drosophila* SOD1 model is that motor neuron survival and life span are unaffected.

In *C. elegans*, panneuronal expression of human G85R mutant SOD1, but not wild-type, causes severe locomotor defects (Wang et al., 2009). Animals expressing human SOD1$^{H46R/H48Q}$, a SOD1 double mutant that blocks copper binding, also display locomotor defects, although to a lesser extent. Correspondingly, only mutant proteins form aggregates, including soluble oligomers that have been linked to toxicity. The defects in movement seem to result from diminished numbers of synapses, presynaptic vesicles, and deficient cholinergic transmission. Nevertheless, similar to the *Drosophila* model, the transgenic worm model does not experience motor neuron loss or reduced life span. Expression of G93A mutant SOD1 selectively in GABAergic motor neurons leads to similar age-dependent paralysis associated with protein aggregation (Li, Huang, & Le, 2013). Notably, the SOD1-induced motor neuron dysfunction can be significantly ameliorated by upregulating autophagy levels in the motor neurons, which suggests that impaired autophagic flux may contribute to the ALS process.

Dynactin p150^{Glued}

The missense mutation G59S located in the microtubule-binding domain of dynactin p150^{Glued} subunit is linked to human late-onset motor neuron disease (Puls et al., 2003). The dynein/dynactin complex is highly conserved in *Drosophila*, and the p150 subunit, encoded by the *Glued (Gl)* gene, genetically interacts with dynein. A disease-associated mutation was introduced into the *Gl* locus using homologous recombination, and flies carrying this partial loss-of-function allele (Gl^{G38S}) develop neuromuscular junction terminal bouton swellings and adult-onset locomotor dysfunction (Lloyd et al., 2012).

TDP-43

The identification of mutations in *TARDBP*, a gene encoding TDP-43, which is a DNA/RNA binding protein linked to sporadic and FALS (Neumann et al., 2006; Kabashi et al., 2008; Sreedharan et al., 2008), stimulated research on how mutations in this gene cause motor neuron degeneration.

Two complimentary approaches have been used for the genetic characterization of TDP-43 proteinopathy: target disruption of the *Drosophila* TDP-43 ortholog (TBPH), and overexpression of TDP-43 mutants found in patients with ALS. All loss-of-function

models generated so far (Feiguin et al., 2009; Fiesel et al., 2010; Lu, Ferris, & Gao, 2009) are strongly in agreement that depletion of TBPH is deleterious to animal development. Two chromosomal deletions of portions of *TBPH* coding and regulatory regions that abolish TBPH protein expression result in a high percent of pupae failing to eclose (Feiguin et al., 2009). Animals that manage to reach adulthood display dramatic locomotive defects with spastic, uncoordinated, movements; incapacity to fly or walk normally; and reduced life span. The analysis of presynaptic terminals at neuromuscular junctions highlights a reduction of axonal branches and synaptic boutons present inside the muscles. In a further *TBPH* null line in which the entire *TBPH* coding sequence is deleted, homozygous TBPH null flies die at the second instar larvae stage (Fiesel et al., 2010). Another study introduces a premature stop codon at residue 367 (Q367X; Lu et al., 2009). Homozygosity of the null allele is semilethal, with a small percent of pupal flies surviving to adulthood. Null animals show significantly decreased dendritic branching in sensory neurons, similar to the reduction of axonal branches at neuromuscular junctions. The semilethality of homozygosity for TBPH deletion is confirmed by a ubiquitous *TBPH*-RNAi knockdown (Lu et al., 2009). Importantly, overexpression of human TDP-43 could efficiently rescue the phenotype caused by TBPH deficiency (Feigun et al., 2009; Lu et al., 2009), demonstrating that the functions of the human TDP-43 are evolutionary conserved.

Tissue-specific overexpression of either *Drosophila* orthologue or human TDP-43 in *Drosophila* has had varied outcomes; however, a consistent observation in different reports is that the ectopic TDP-43 expression leads to age-dependent toxicity (Li et al., 2010; Hanson, Kim, Wassarman, & Tibbetts, 2010; Elden et al., 2010). Expression of wild-type TDP-43 in the eyes, mushroom bodies, or motor neurons causes retinal degeneration, axonal loss and aggregate formation, and axon swelling and motor neuron death, respectively. In particular, motor neuron TDP-43 expression reduces locomotor activity of the third instar larvae, axonal branching, and synaptic boutons at neuromuscular junctions (Li et al., 2010). This latter observation differs from that seen by Lu et al. (2009), where increased dendritic branching is noted in sensory neurons. Thus, TDP-43 might exert a subtly different pathology between the motor and sensory neurons in *Drosophila*. Overexpression of disease-related TDP-43 mutants displayed variable levels of toxicity, probably depending on the exact level of overexpression with respect to wild-type protein (Elden et al., 2010; Estes et al., 2011). Most importantly, observations from these transgenic flies indicate that although TDP-43 mislocalization is not required for pathogenicity in these models, retaining the ability to bind RNA targets is crucial for TDP-43 toxicity (Voigt et al., 2010).

Knock-out of the ortholog of TARDBP in *C. elegans*, *tdp-1*, does not cause obvious defects (Ash et al., 2012; Liachko, Guthrie, & Kraemer, 2010), suggesting a divergent role between TDP-43 and TDP-1 in development. Transgenic studies in *C. elegans*, however, point toward a greater conservation in neuronal function. Expression of human wild-type and mutant TDP-43 panneuronally causes defects in motor function as indicated by an uncoordinated movement (Ash et al., 2010; Liachko et al., 2010).

FUS

ALS-causative mutations were found in the *FUS* gene (Kwiatkowski et al., 2009; Vance et al., 2009), which, like TDP-43, is a member of RNA-binding protein family.

Drosophila has a single FUS homolog with 53% amino acid identity to human FUS encoded by the *cabeza* (*caz*) gene. *Drosophila* mutants in which *caz* is disrupted exhibit aberrant development, defective adult locomotion, and reduced life span. These phenotypes can be rescued equivalently by transgenic wild-type *Drosophila* Caz or human FUS, but not FALS-associated FUS proteins (Wang, Brent, Tomlinson, Shneider, & McCabe, 2011).

Interestingly, targeted expression of mutant human FUS in the fly brain during development causes pupal lethality and larval locomotor defects. Conditional expression of mutant FUS in adult fly drastically reduces the life span and adult climbing abilities. Mutant FUS localized to both the cytoplasm and nucleus, whereas wild-type FUS localized only to the nucleus (Lanson et al., 2011). Furthermore, deletion of the nuclear export signal strongly suppressed toxicity, suggesting that cytoplasmic

localization is necessary for neurodegeneration (Lanson et al., 2011). When the expression is restricted in subpopulations of neurons like photoreceptors, mushroom bodies, or motor neurons, transgenic flies show progressive neural damages, including retinal degeneration, axonal loss in mushroom bodies, morphologic changes, and functional impairment in motor neurons (Chen et al., 2011).

Besides the *Drosophila* models there is also one *C. elegans* model expressing human wild-type or mutant FUS using a panneuronal promoter (Murakami et al., 2012). Expression of mutant FUS, but not wild-type FUS, causes cytoplasmic mislocalization associated with progressive motor dysfunction and reduced lifespan. The severity of the mutant phenotype in *C. elegans* closely parallels the disease presentation in humans with the same FUS mutations. Importantly, the mutant phenotype could not be rescued by overexpression of wild-type FUS, suggesting that FUS mutants exert toxic gain-of-function effects via accumulation of FUS in the cytoplasm, rather than titration of FUS from its physiologic location in the nucleus.

C9ORF72

A hexanucleotide repeat expansion in an intron of a previously uncharacterized gene, termed *C9ORF72*, is the cause of a major proportion of cases of FALS and frontotemporal dementia (Renton et al., 2011; DeJesus-Hernandez, et al., 2011). How the hexanucleotide repeat expansion causes neurodegeneration in ALS remains elusive. The finding that alternative transcripts of *C9orf72* are greatly reduced in patients supports the view that loss of C9orf72 is a major determinant leading to neurodegeneration in ALS. RNA foci, a feature common to other noncoding repeat expansion disorders, such as myotonic dystrophy and fragile-X associated syndrome, containing the expanded hexanucleotide repeats are observed in neurons in ALS and suggest the possibility of a toxic RNA mechanism via sequestration of RNA binding proteins that underlies neuronal degeneration. In addition, RNA translation (Mori et al., 2013; Ash et al., 2013; Zu et al., 2013) may be another mechanism by which the mutation leads to toxicity. In initial studies to examine whether loss of C9ORF72 function contributes to motor neuron degeneration in ALS, investigators generated a *C. elegans C9ORF72* orthologue (*alfa-1)* knock-out model. Null mutants displayed age-dependent motility defects, degeneration of GABAergic motor neurons, and paralysis (Therrien, Rouleau, Dion, & Parker, 2013). These results suggested the possibility that loss of C9ORF72 function contributes to motor neuron dysfunction in ALS. In contrast, *Drosophila* models overexpressing expanded G4C2 repeat in eye and motor neuron, respectively, exhibited age-dependent disruption of the fly eye and reduction in locomotion (Xu et al., 2013). Importantly, the G4C2 repeats can interact with specific RNA binding proteins, suggesting that RNA binding proteins may be sequestered in intranuclear RNA foci that would impact negatively on the function of motor neurons (Xu et al., 2013). Taken together, these initial results suggest that both loss- and gain-of-function mechanisms could contribute to the pathogenesis of C9ORF72-linked ALS.

VERTEBRATE MODELS

Zebrafish

The zebrafish is a small, rapidly breeding freshwater fish that is easy to grow in the laboratory. As a vertebrate, it shares many developmental and anatomic features with humans. The main advantages of studying motor neuron diseases in zebrafish are the rapid development of the spinal cord and the pattern of motor neuron branching accessible as early as 24 hours postfertilization. In addition, responses to touch and swimming can be monitored following hatching around 48 hours postfertilization. Moreover, the simplicity and effectiveness of manipulating gene expression makes the zebrafish an attractive model because more than 80% of the gene structure is available and shows 50–80% homology with most human sequences. When identified, the zebrafish homologs can be targeted for knockdown by injections of selective antisense morpholino oligonucleotides and by genomic editing approaches.

SOD1

To develop a SOD1 model in zebrafish, investigators overexpressed the zebrafish *Sod1*

gene harboring the G93A mutation. They showed that when accumulated to threefold of the endogenous Sod1 level, $Sod1^{G93R}$ caused a slow-progressing motor neuron disease characterized by increased motor abnormalities, muscle atrophy, motor neuron loss, and reduced survival (Ramesh, et al., 2010). In addition, age-dependent neuromuscular junction defects characterized by shorter and more punctate synaptic boutons were observed. Complementing this model, human wild-type SOD1, $SOD1^{G93A}$, $SOD1^{G37R}$, and $SOD1^{A4V}$ mRNAs were each injected into zebrafish embryos: mutant SOD1, but not wild-type SOD1, causes abnormal motor neuron axon branching and shortened axon length in a dose-dependent manner (Lemmens et al., 2007). These models are consistent with the idea of toxic gain of function by mutant SOD1 and provide a useful platform for identification and validation of therapeutic targets.

TDP-43

With discovery of TDP-43 mutations linked to ALS (Neumann et al., 2006; Kabashi et al., 2008; Sreedharan et al., 2008), the mechanism of how mutant *TARDBP* cause motor neuron degeneration was investigated by both gain- and loss-of function studies. By injecting human wild-type and ALS-associated mutant *TARDBP* mRNAs into zebrafish embryos, it was shown that overexpression of mutant, but less so of wild-type, human TARDBP caused a motor phenotype in zebrafish embryos characterized by shortening of motor axons, premature and excessive branching, and swimming deficits (Kabashi et al., 2010). Because wild-type TDP-43 can also be toxic, mutant TDP-43–specific effects in this model system are difficult to interpret.

Besides *tardbp*, the zebrafish has a paralogue, termed *tardbpl* (TAR DNA binding protein-like) gene. Several groups have independently generated *tardbp* and *tardbpl* knock-out and double ($tardbp^{-/-}$;$tardbpl^{-/-}$) knock-out zebrafish. In contrast to morpholino-induced transient knockdown of *tardbp* resulting in aberrant motor axons (Kabashi et al., 2010; Armstrong & Drapeau, 2013a), homozygous *tardbp* knock-out zebrafish exhibited no phenotype as a result of compensation by a unique splice variant of Tardbpl (Schmid et al., 2013; Hewamadduma et al., 2013). Such discrepancy is likely caused by off-target effects of morpholino used. Importantly, double-homozygous mutants ($tardbp^{-/-}$;$tardbpl^{-/}$) showed muscle degeneration, strongly reduced blood circulation, mispatterning of vessels, impaired spinal motor neuron axon outgrowth, and early death (Schmid et al., 2013). Similar results were described when elimination of both zebrafish TARDBP orthologs resulted in a severe motor phenotype with shortened motor axons, locomotion defects, and death at around 10 days postfertilization (Hewamadduma et al., 2013).

These studies suggest the possibility that loss of TDP-43 function plays a pathogenic role in TDP-43 proteinopathy in ALS and offer a useful model system to identify and validate therapeutic targets. Interestingly, upregulation of a muscle-specific actin binding protein (Filamin C) identified in zebrafish lacking Tardbp has been observed in cases of FTLD-TDP (Schmid et al., 2013).

FUS

A second ALS gene encoding another RNA binding protein gene, termed FUS, was identified (Kwiatkowski et al., 2009; Vance et al., 2009). Using genetic approaches similar to those used in studies of TDP-43, efforts were begun to investigate the mechanisms whereby mutant FUS causes motor neuron degeneration. Knockdown of zebrafish Fus generated a motor phenotype that could be rescued upon coexpression of wild-type human FUS (Kabashi et al., 2011a). In contrast, the two most frequent ALS–related FUS mutations, R521H and R521C, failed to rescue the knockdown phenotype. These results support the idea that loss of FUS function may contribute to degeneration of motor neurons in mutant FUS-linked ALS. However, the R521H mutation caused a toxic gain of function when expressed alone, similar to the phenotype observed on knockdown of zebrafish Fus (Kabashi et al., 2011a), indicating that caution is necessary when interpreting outcomes in overexpression studies of RNA binding proteins, such as FUS or TDP-43.

In parallel studies, it has been shown that expression of either mutant human FUS or knockdown of zebrafish fus resulted in impaired motor activity and reduced neuromuscular junction synaptic fidelity with altered quantal transmission. These impairments in neuronal function could be partially restored

in larvae depleted of fus expressing wild-type, but not mutant, human FUS. Although these results suggest that both a loss and gain of FUS function could play a mechanistic role in FUS-linked ALS (Armstrong & Drapeau, 2013b), additional experiments are required to clarify this issue.

C9ORF72

Animal models of GGGGCC repeat expansion in *C9orf72* have been developed in vertebrate models (Renton et al., 2011; DeJesus-Hernandez et al., 2011). To test whether loss of C9orf72 function participates in disease pathogenesis, investigators depleted the zebrafish orthologue of zC9orf72 in zebrafish and observed axonal degeneration of motor neurons (Ciura et al., 2013). Importantly, they demonstrated that such loss of motor neurons could be rescued by expressing human C9orf72 mRNA, highlighting the specificity of the induced phenotype. These results revealed a pathogenic consequence of decreased C9orf72 levels, supporting a loss-of-function mechanism of disease in C9orf72-linked ALS (Ciura et al., 2013). In contrast, investigators have used a gain of function study to assess whether the hexanucleotide expansion repeat plays a role in pathogenesis of disease. Zebrafish embryos injected with 383 or 723 GGGGCC repeats form intranuclear RNA foci, and animals carrying these expanded repeats undergo apoptotic cell death (Lee et al., 2013). These outcomes suggested that both loss- and gain-of-function mechanisms contribute to degeneration of motor neurons in C9orf72-linked cases. These model systems have the potential to identify and validate therapeutic targets.

Rodent Models

TRANSGENIC MODELS

Transgenic rodent models are genetically engineered model organisms created by microinjection of the ALS gene construct into fertilized eggs, and lines of animals are subsequently established and characterized. A cell type–specific or ubiquitous promoter is used to drive the transgene. The transgene usually encodes either the human normal or mutated protein. Because the site of integration of the transgene is random, multiple lines are usually characterized to rule out integration site-specific effect. Variation of transgenic models has also been developed. For example, chimeric approaches in which models host a mixture of normal and mutant expression cells are used to assess cell- versus non–cell-autonomous contributions in ALS.

CONSTITUTIVE AND CONDITIONAL GENE KNOCK-OUT/KNOCK-IN MODELS

In contrast to transgenic models, gene-targeting strategies via homologous recombination (Thomas & Capecchi, 1987) coupled with embryonic stem cells (cells that are capable of forming whole organism when introduced into foster mice; Martin, 1981; Evans & Kaufman, 1981) provided the opportunity to generate mice with any single gene (allele) of interest deleted (constitutive knock-out), the deletion of exons encoding a functional domain of the protein (constitutive functional knock-out), or to introduce a mutation (disease-associated) into a gene of interest (constitutive knock-in). When both alleles are disrupted (homozygotes) by intercrossing mice that harbor singly disrupted allele (heterozygotes), these constitutive knock-out mice provide a very useful tool to study the physiology or loss-of-function phenotypes of any gene of interest within the context of a whole organism.

The constitutive knock-out approach, however, suffers from two major limitations. First, if the gene of interest is essential for embryogenesis, it would preclude analysis of its physiology in the adult because homozygous knock-outs would die in utero. Second, the general deletion of the gene in all the cell types would preclude studies that assess whether specific cellular dysfunction is caused by loss of a gene in target cells (cell autonomous) or by loss of the gene in bystander cells (non–cell autonomous). The use of cre recombinase and loxP target site (Araki, Imaizumi, Okuyama, Oike, & Yamamura, 1997), for example, is one approach that will address these issues.

Cre recombinase is a phage-encoded enzyme that recognizes two identical fragments of DNA (loxP site) and mediates the recombination of the two loxP sites (Sternberg & Hamilton, 1981). Engineering a pair of

loxP sites, one on each side of any target gene sequence, in direct-repeat orientation allows the regulated deletion of the target sequence by selective expression or activation of cre recombinase in a time- or cell type–specific manner. The cell type–specific recombination is usually achieved by controlling cre recombinase expression driven by a specific promoter (Gu et al., 1994), whereas the temporal control is facilitated by expressing cre recombinase under the control of an inducible promoter (Kuhn, Schwenk, Aguet, & Rajewsky, 1995) or mutant estrogen receptor/cre recombinase chimeric protein that can mediate recombination in the presence of an estrogen agonist (Feil, Wagner, Metzger, & Chambon, 1997). Thus, both constitute and conditional knock-out mice are powerful tools that have been used to define gene function in development, physiology/behavior, or loss-of-function phenotype of genes linked to ALS.

SOD1

As indicated above, the identification of missense mutations in *SOD1* linked to FALS more than 20 years ago (Rosen et al., 1993) has led to the development of a variety of animal models including transgenic rodent models (Wong, 2012).

Mutant SOD1 transgenic mice recapitulate many features of ALS, including mitochondrial dysfunction, degeneration of motor axons, progressive motor neuron loss, denervation muscle atrophy, and paralysis (Gurney et al., 1994; Wong et al., 1995; Bruijn et al., 1998). Most of SOD1 transgenic rodent models use the SOD1 promoter to mimic its normal pattern of expression. In addition, models using neuronal-specific (Jaarsma, Teuling, Haasdijk, De Zeeuw, & Hoogenraad, 2008) or astrocytic-specific promoter (Gong, Parsadanian, Andreeva, Snider, & Elliott, 2000) have also been developed. Such transgenic mice have been important in initiating efforts to understand the non–cell-autonomous nature of ALS.

Transgenic SOD1 mouse models have variable ages of disease onset and rates of disease progression dependent on (1) SOD1 mutation, (2) transgene expression level, (3) gender, and (4) genetic background (Heiman-Patterson, 2011).

Although much of the rodent work has focused on mouse SOD1 transgenics, two human SOD1 mutations are modeled in rats: H46R SOD1 and G93A SOD1 (Howland et al., 2002; Nagai et al., 2001). Similar to the mouse, these transgenic animals have progressive degeneration of both upper and lower motor neurons and disease severity is directly proportional to mutant SOD1 expression. The G93A SOD1 mutation causes a more aggressive disease in rats than the H46R SOD1 mutation (Nagai et al., 2001). Unlike in mouse models, onset of muscle weakness occurs in either the forelimbs or the hind limbs; forelimb onset is an indicator of an aggressive disease course (Storkebaum et al., 2005).

CHIMERIC MODELS: CELL- AND NON–CELL-AUTONOMOUS ROLES OF MUTANT SOD1

A significant contribution of transgenic mouse studies was the finding of a role for nonneuronal cells in motor neuron disease (Boillee, Vande Velde, & Cleveland, 2006b). In chimeric mice that comprised mixtures of normal and mutant SOD1-expressing cells, toxicity to motor neurons was shown to require damage from mutant SOD1 acting within nonneuronal cells. Normal motor neurons in SOD1 mutant chimeras develop aspects of ALS-like pathology. Importantly, nonneuronal cells that do not express mutant SOD1 delay degeneration and significantly extend survival of mutant-expressing motor neurons (Clement et al., 2003).

To further clarify the contributions of nonneuronal cell types to disease, investigators used additional chimeric mouse model (Yamanaka et al., 2008b) and models in which mutant *SOD1* transgene can be selectively deleted either in microglia (Boillee et al., 2006a), astrocytes (Yamanaka et al., 2008a), or oligodendroglia precursors (Kang et al., 2013). Invariably, these models demonstrated that depletion of mutant SOD1 in nonneuronal cells attenuated disease and extended survival of mice, supporting the view that non–cell-autonomous mechanism is a major contributor to motor neuron loss in ALS.

DYNACTIN MODELS

Missense mutations in a dynactin gene (DCTN1) encoding the p150^{Glued} subunit of

dynactin have been linked to human motor neuron disease (Puls et al., 2003).

Mouse models have been generated with mutant p150^{Glued} (Lai et al., 2007; Laird et al., 2008). A mutant G59S p150^{Glued} knock-in mouse model in which the G59S point mutation is introduced into the mouse endogenous *Dctn1* allele has been made. The G59S mutation destabilizes p150^{Glued} and disrupts the function of dynein/dynactin complex, resulting in early embryonic lethality of homozygous knock-in mice, whereas heterozygous knock-in mice, which developed normally, start to display motor neuron disease–like phenotypes after 10 months of age. These mutant mice showed excessive accumulation of cytoskeletal and synaptic vesicle proteins at neuromuscular junctions, loss of spinal motor neurons, and astrogliosis (Lai et al., 2007). In contrast, transgenic mice that overexpress mutant G59S p150^{Glued} under the control of the neuron-specific Thy-1 promoter also display a motor neuron disease phenotype resulting in degeneration of motor axons, loss of motor neurons, muscle weakness, paralysis, and/or premature death (Laird et al., 2008; Chevalier-Larsen, Wallace, Pennise, & Holzbaur, 2008). Interestingly, there is evidence of autophagic cell death occurring in these mutant p150^{Glued} mice (Storkebaum et al., 2005).

TDP-43

The discovery of TDP-43 proteinopathy in ALS- frontotemporal dementia (Neumann et al., 2006) has led to attempts to model TDP-43–linked ALS in mice. Models with no TDP-43 replicate a robust age-dependent motor neuron disease observed in mutant SOD1 or dynactin mouse models. Similar to SOD1 mouse models, development of disease phenotypes in TDP-43 transgenic mice is highly dependent on the promoter used and the level of transgene expression.

Transgenic TDP-43 models mouse lines have been developed expressing human TDP-43 under the control of various promoters, including mouse Prion (Wegorzewska, Bell, Cairns, Miller, & Baloh, 2009; Stallings, Puttaparthi, Luther, Burns, & Elliott, 2010; Xu et al., 2010, 2011), Thy1.2 (Wils et al., 2010; Shan, Chiang, Price, & Wong, 2010), or CamKII (Tsai et al., 2010; Igaz et al., 2011) promoter. These mice lacked lower motor neuron loss, axonal degeneration, and pathologic hallmarks of TDP-43 proteinopathies were reported. The first published transgenic TDP-43 mouse model (Wegorzewska et al., 2009) accumulated the mutant protein to approximately three-fold the level of the endogenous Tdp-43 and exhibited abnormal gait by 13 weeks of age and paralysis and death by 22 weeks of age. This rapid disease course was accompanied by a loss of upper motor neurons but a relatively mild loss of lower motor neurons, with signs of axonal degeneration. However, the cause of death is likely intestinal dysfunction (Guo et al., 2012; Esmaeili, Panahi, Yadav, Hennings, & Kiaei, 2013).

Several groups have also documented similar findings of rapid early death of mutant TDP-43 mice (Wils et al., 2010; Tsai et al., 2010) or rats (Zhou et al., 2010; Huang et al., 2012) expressing relative high levels of mutant protein. Because high level of expression of wild-type human TDP-43 was shown to be highly toxic in rodents (Shan et al., 2010; Igaz et al., 2011; Cannon et al., 2012), a likely interpretation of outcomes from these mutant TDP-43 rodents is that toxicity arises from simply overexpressing the human TDP-43 protein rather than a toxic gain of adverse property by the mutant protein.

To avoid such high levels of TDP-43 expression, work has focused on developing lines of animals expressing modest levels of human TDP-43. Wild-type or mutant (G348C and A315T) TDP-43 mice models have been generated using a large human *TDP-43* genomic fragment such that the levels of transgenic products were only two- to three-fold of endogenous Tdp-43 (Swarup et al., 2011). These transgenic mice developed age-related pathologic and biochemical changes including ubiquitinated TDP-43–positive inclusions, TDP-43 cleavage fragments, intermediate filament abnormalities, axonopathy, and neuroinflammation. In addition, these lines of mice exhibited impaired learning and memory as well as motor dysfunction in an age-dependent manner (Swarup et al., 2011). However, there was no evidence of age-dependent motor neuron loss or paralysis in these lines.

In parallel, transgenic wild-type and mutant TDP-43 mice have been developed that express modest levels of transgenic products under the murine PrP promoter (Arnold et al., 2013). The ALS-causing mutants (TDP-43^{Q331K}

and TDP-43^{M337V}), but not wild-type human TDP-43, showed age-dependent, progressive motor axon degeneration and motor neuron loss. However, these lines of mice do not exhibit a paralytic disease. Although these transgenic lines expressing modest levels of TDP-43 are promising tools for identification and validation of therapeutic targets, they point to the possibility of development of transgenic lines that will lead to a robust disease phenotype that are observed in mutant SOD1 models.

Tardbp knock-out mouse models have been generated. In contrast to gain of function studies, investigators used gene-targeting approaches to test whether loss of TDP-43 function contributes to motor neuron degeneration in TDP-43 proteinopathy-associated ALS. Initial efforts focused on development of constitutive TDP-43 knock-out mice. Homozygous knock-out mice died during early embryogenesis showing that TDP-43 is a critical RNA binding protein in the developing organism (Kraemer et al., 2010; Sephton et al., 2010; Wu et al., 2010). However, heterozygous Tdp-43 knock-out mice developed signs of motor disturbance with age-dependent deficits in grip strength and cage-hanging tests compared with wild-type littermates but there is no evidence of neuropathology consistent with motor neuron disease (Kraemer et al., 2010).

An alternative approach to examine the effects of reduced TDP-43 expression is to use a conditional knock-out strategy to bypass embryonic development. Chiang and colleagues (2010) found that heterozygous deletion of *Tardbp* did not alter TDP-43 protein or mRNA levels, supporting the notion that TDP-43 is tightly regulated. Interestingly, conditional deletion of both *Tardbp* alleles in adult mice caused rapid loss of body fat and mice died rapidly after Tdp-43 is ubiquitously depleted (Chiang et al., 2010). To assess the impact of depletion of Tdp-43 in motor neurons during aging, cell type–specific deletion of *Tardbp* alleles in adult mice using Cre recombinase approach is necessary. Using the Hb9-Cre driver thought to possess efficient excision in motor neurons, Wu, Cheng, and Shen (2012) reported that mice with homozygous deletion of *Tardbp* in spinal motor neurons exhibited motor neuron loss, reactive astrocytosis, microglia activation, and accumulation of polyubiquitinated proteins. Using a different driver, ChAT-Cre driver, Iguchi et al. (2013) showed that the knock-out of Tdp-43 in postnatal motor neurons led to progressive weight loss and motor impairment by the age of 60 weeks. These animals exhibited atrophy of spinal motor neurons, degeneration of large motor axons, and denervation muscle atrophy (Iguchi et al., 2013). These results are consistent with the view that loss of TDP-43 function contributes to pathogenesis of TDP-43 proteinopathy in ALS.

FUS

Even before the discovery of missense mutations in *FUS* linked to ALS (Kwiatkowski et al., 2009; Vance et al., 2009), constitutive *FUS* knock-out mice were produced to investigate the effects of FUS deficiency (Hicks et al., 2000). FUS null mice died within 16 hours of birth and exhibited evidence of abnormal lymphocytes and chromosomal instability. Conditional knock-out of *FUS* in the adult nervous system will be useful to examine whether loss of FUS function may contribute to the pathogenesis of FUS-linked ALS.

Taking a complementary approach, investigators used a transgenic approach to assess the mechanism whereby ALS-linked mutant FUS led to motor neuron degeneration. Initial efforts showed that expression of human wild-type FUS, mutant R521C, and Δ14 in brains of mice exhibited FUS cytoplasmic aggregates in neurons; however, these mice do not show robust evidence of motor neuron disease (Verbeeck et al., 2012).

Rat models overexpressing either mutant (R521C) or wild-type FUS have been made. Mutant FUS transgenic rats developed progressive paralysis. Neuropathologically, mutant FUS rats showed ubiquitin aggregation, gliosis, degeneration of motor axons, and loss of neurons in the cortex and hippocampus. Although transgenic rats that overexpressed the wild-type human FUS were asymptomatic at young ages, they showed a deficit in spatial learning and memory and a significant loss of cortical and hippocampal neurons at advanced ages (Huang et al., 2011). Similarly, overexpression of wild-type FUS under the mouse Prion promoter revealed that although no effect was observed in heterozygous FUS mice, homozygous FUS mice displayed an aggressive neurodegenerative phenotype with early death at the age of 12 weeks (Mitchell, McGoldrick, Vance,

& Hortobagyi, 2013). These results indicated that strong accumulation of wild-type FUS is highly toxic and makes it difficult to interpret the effect of mutant FUS in these animal models. In the future, generation of lines of animals that modestly accumulation the FUS protein would be useful for mechanistic studies.

CONCLUSION

Over the past decade, marked advances have occurred in the development of invertebrate and vertebrate animal models of genes implicated in human motor neuron diseases, particularly ALS and ALS–frontotemporal dementia. These animal models, in aggregate, begin to teach us important lessons regarding disease mechanisms and point to potential pathways that may be amendable to therapeutic strategies. To date, outcomes from studies in SOD1 models are consistent with the view that mutant SOD1 confers a toxic property in a cell- and non–cell-autonomous manner. However, the precise toxicity elicited by mutant SOD1 remains elusive. In the case of mutant dynactin p150^{Glued}, results from mouse and drosophila work suggest that disease arises through a dominant-negative mechanism. In contrast to SOD1 and dynactin p150^{Glued}, overexpression of TDP-43 or FUS is highly toxic in model systems and often confounds the interpretation of outcomes from studies of the corresponding mutant protein. At present, it remains unknown as to how mutations in TDP-43 or FUS cause motor neuron degeneration in ALS. However, TDP-43 loss-of-function studies in fly, zebrafish, and mouse model systems suggest that depletion of TDP-43 in neurons could be one mechanism whereby TDP-43 proteinopathy leads to motor neuron degeneration. Understanding the molecular basis of ALS-linked genes is crucial in the identification and validation of therapeutic targets for this illness. Using the TDP-43 models, investigators have begun to work toward these goals. For example, treatment with autophagy inducer attenuated a mouse model of TDP-43 (Wang et al., 2012) and a compound that inhibits eIF2a phosphorylation attenuates toxicity in a drosophila TDP-43 model (Kim et al., 2014). Rigorous target validation studies using animal model systems are necessary to ensure effective translation of neurobiologic insights into the clinic to benefit patients. In the future, it is anticipated that current existing animal models coupled with a new set of model systems will be instrumental for not only providing greater understanding of disease mechanisms, but also identification and validation of mechanism-based therapeutic targets for development of effective treatment strategies for ALS.

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Chapter 20

Multidisciplinary Clinics and Practice Parameters and Guidelines

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Clinical care of patients with motor neuron disease (MND) has advanced with the advent of multidisciplinary clinics, now considered the standard of care. Formal practice parameter/guidelines for multidisciplinary care have been put forward, but are based largely on clinical experience, because studies of care of patients with MND are lacking.

The clinical care challenge for the provider is compassionate delivery of the diagnosis of amyotrophic lateral sclerosis (ALS), and for the clinic team is helping the patient make informed and timely decisions at various time points in the course of the disease. Since the publication of practice parameters/guidelines, updates have been published. Data support an increase in longevity and improvement in quality of life for the patient and caregiver when care is optimized.

INTRODUCTION

All forms of MND are progressive and affect many motor modalities, including speech, nutrition, activities of daily living, mobility, and eventually respiration, and also behavioral issues. Associated are a number of troublesome symptoms that patients may experience. With disease progression there is concurrently a growing burden on the caregiver. Maximal care of patients with MND requires consideration of several domains, including physical, psychological, and social issues. These can best be managed in a clinic where patients and caregivers can be attended to by a multitude of providers.

PRACTICE PARAMETERS AND GUIDELINES AND MULTIDISCIPLINARY CLINICS

Efforts sponsored by the American Academy of Neurology (AAN) resulted in a practice parameter in 1999 and an update in 2009 that focus on evidence-based data for ALS care (Miller et al., 1999, 2009). With similar goals, the European Federation of Neurological Societies (EFNS) performed a systematic review of the literature to establish evidence-based guidelines for patients with ALS and caregivers in 2005 and an update in 2012 (Andersen et al., 2005, 2012).

Domains singled out in the practice parameters and guidelines are (1) breaking the news or giving the diagnosis, (2) management of common symptoms, (3) role of multidisciplinary clinics, (4) management of adequate nutrition, (5) management of respiratory symptoms, (6) management of cognitive and behavioral impairment, (7) enhancement of communication, and (8) palliation and end-of-life care.

Within these domains are challenges working with an inexorably progressive disease: (1) need for adequate information delivered in a compassionate manner to enable patient and family to make decisions, (2) allowing for patient self-determination and autonomy, and (3) discussion of advanced directives and end-of-life care introduced in a timely manner.

The initial practice parameter laid out the main domains, and both the practice parameter and guidelines reviewed evidence-based data to support management and interventions. Another purpose of both efforts is to identify areas for future research.

CLINIC PROVIDERS

Clinical care has focused on ALS, but also applies to all forms of MND. Optimum care involves a spectrum of issues that can best be addressed by a matched spectrum of providers in a multidisciplinary clinic, and both the AAN practice parameter and EFNS guidelines recommend care in such clinics (Miller et al., 2009; Andersen et al., 2012). Clinics typically include occupational therapists, physical therapists, speech therapists, respiratory therapists, nutritionists, social workers, and nurses, in addition to neurologists (Table 20–1; Corr, Frost, Traynor, & Hardiman, 1998). Recent additions to clinics include clinical pharmacists (Jefferies & Bromberg, 2012). Other providers that frequently interact with patients are occasioned by particular conditions and issues, and include pulmonologists, sleep medicine specialists, gastroenterologists, otolaryngologists, assistive technologists, and psychologists or psychiatrists (Table 20–2). The ideal clinic allows a patient to see the full spectrum of providers (Table 20–1) during each visit, taking away from each provider that which is beneficial. Clinic visits are typically every 3 months, and more frequent if clinically necessary. One difficulty for patients is visit length, which are 3 hours or longer. The spectrum of providers is usually available in regional ALS or MND specialized clinics. However, smaller general neurology clinics can function as "virtual multidisciplinary clinics" by outside consultations or referrals to the full spectrum of providers as needed.

IMPACT OF PRACTICE PARAMETERS AND GUIDELINES

Assessments have been made to determine the level of care provided to the patient with ALS prior to, but in terms of, the initial practice parameter based on a review of care data collected from the ALS CARE Database between 1996 and 1999 (the year the practice parameter was published; Bradley et al., 2001). The ALS CARE Database existed from 1996 to 2006 and included 91 clinics (mostly specialized ALS centers) that enrolled more than 2,500 patients and enumerated demographic data and data on patient care issues (Miller et al., 2000). The assessment of care is based on 2,393 patient records. Eighty-two percent believed that they had been given sufficient information about ALS. With respect to symptoms and their management discussed in the practice parameter only a small number of patients were treated: 54% for sialorrhea, 41% for reported depression, and 28% for dyspnea. Among the 373 who had died, 90% had advanced directives, but only 47% had hospice services. During the terminal stage

Table 20–1 **Core Providers in Multidisciplinary ALS Clinics**

Discipline	Activities
Speech therapist	Assess swallowing: • Provide strategies; coordinate with nutritionist Assess speech: • Provide strategies • Coordinate acquisition of augmentative (computer) devices
Occupational therapist	Assess daily activities: • Provide equipment (utensils, splints)
Physical therapist	Assess mobility and safety: • Provide equipment (canes, walkers) • Coordinate acquisition of braces, wheelchairs, lifts
Respiratory therapist	Assess respiratory function: • Perform pulmonary function tests • Demonstrate noninvasive ventilation, cough-assist devices • Coordinate acquisition of respiratory equipment; pulmonologist
Nutritionist	Assess weight loss: • Design appropriate diet (weight loss/gain; with feeding tube) • Provide strategies, discuss feeding tube • Coordinate acquisition feeding tube; gastroenterologist
Social worker	Assess psychological and home environment of patient and family: • Provide coping strategies • Offer counselling
Nurse	Oversee clinical recommendations: • Patient follow-up
Clinical pharmacist	Counsel medications • Provide information on dosing and side effects • Suggest pharmacologic symptom management • Manage follow-up laboratory studies

Table 20–2 **Ancillary Providers Available to the Clinic**

Discipline	Activities
Pulmonologist	Assess respiratory function: • Noninvasive and tracheal ventilator management
Sleep medicine	Assess nocturnal ventilation • Noninvasive ventilator management • Polysomnogram needs assessment and interpretation
Gastroenterologist	Assess nutritional issues: • Gastric feeding tube placement and management
Otolaryngologist	Assess speech, swallowing, and respiratory function: • Tracheostomy placement and management
Assistive technologist	Assess communication and mobility function: • Computer-based communication devices assessment • Adaptive mobility devices assessment
Psychologist/psychiatrist	Assess psychosocial issues: • Mood issues assessment • Coping strategies management
Home care	Assess home living situation: • Home safety needs determination • Equipment and modifications assessment
Hospice	Assess personal care needs: • Home aid and care provision • Ensure comfortable death
Spiritual care	Assess spiritual needs

for the 373 who had died, 2.5–17% had symptoms of breathing difficulties, anxiety, pain, and chocking during the terminal stage that apparently were not adequately managed.

One beneficial measure is patient survival, and from two prospective comparisons, patients attending a multidisciplinary clinic lived significantly longer with mean survival increased by 7.5–10 months (Traynor, Alexander, Corr, Frost, & Hardiman, 2003; Zoccolella et al., 2007). In contrast, another study showed no survival benefit (Zoccolella et al., 2007).

CLINIC CHALLENGES

There are many challenges to managing MND, for patients, caregivers, and clinic staff. The first is giving the diagnosis, the second is providing a positive face in the setting of inexorable loss of function, the third is managing key points related to nutrition and respiration, the fourth is addressing and managing end-of-life issues, and a final one is bereavement support. These challenges result in parallel stresses for clinic staff managing these issues.

Delivering the Diagnosis

A key issue is delivering the diagnosis in an appropriate manner that is compassionate, provides sufficient information, and provides reassurance for long-term support. This is termed "breaking the news." Recommendations are available in the practice parameters, and addressing the challenges has been summarized as a protocol abbreviated SPIKES (Box 20.1; Miller et al., 1999, 2009; Chio & Borasio, 2004; Andersen et al., 2012). It is recommended that there be an open discussion of the possibility of MND during the diagnostic evaluation. Collusion with family members to spare the patient is discouraged, but there may be social and cultural factors to be taken into

Box 20.1 Algorithm for Giving the Diagnosis of MND (based on SPIKES algorithm)

S: Setting of delivering the diagnosis and provider's listening skills
- Focused interview with open time limit
- Provider's ability to listen to the patient's response and tailor discussion accordingly

P: Patient's perception of the condition and its seriousness
- Understanding how much a patient knows and their perception of disease seriousness
- Correcting erroneous information

I: Invitation from the patient to be given the information
- Awareness of how much patient is able to receive
- Awareness of possibility of patient defense mechanisms

K: Being knowledgeable about the condition and facility for explaining medical facts
- Providing succinct explanations
- Providing written information for after-visit patient review

E: Able to recognize patient's emotions and provide empathy as the patient responds
- Being comfortable with marked patient emotional responses

S: Have a management strategy and provide a summary
- Offering the services of a multidisciplinary clinic and a between-visit contact person
- Final summary to assess for misunderstandings

Modified from Chio and Borasio (2004).

account. An initial explanation of the form of MND should be complete, but brief, because there maybe limits as to how much a patient can absorb. An especially challenging issue is explaining the inexorable progression, and at the same time offering hope. Having available written material for the patient to review outside of clinic is helpful.

The attitude and practice pattern of the physician making the diagnosis is important in how the news is presented and received by the patient and family (Chio & Borasio, 2004). Factors include fear of being the messenger of bad news, not having answers to patient's questions, lack of sufficient time to give the diagnosis, and lack of training and experience for this activity. Giving the diagnosis also opens for the physician fears of one's own mortality. Some of these may explain why private practice neurologists frequently transfer the task to multidisciplinary clinic neurologists by recommending a confirmatory or second opinion.

A survey of how the diagnosis of ALS was received included 94 patient-caregiver pairs, 50 unpaired patients, and 19 unpaired caregivers (McCluskey, Casarett, & Siderowf, 2004). The diagnosis was given by a neurologist in 91%, by an ALS specialist in 8%, and by physiatrists or surgeons in less than 1%. Patient perceptions of physician's performance was poor in 16%, below average in 9%, average in 31%, good in 19%, and excellent in 25%. Many deficiencies were noted: only 50% of physicians mentioned the availability of multidisciplinary clinics and national organizations that focus on ALS (ALS Association and the Muscular Dystrophy Association in the United States); and 35% did not mention that ALS is a terminal disorder. There was a positive association with patient satisfaction and more time spent giving the diagnosis (average, 32 min; range, 1–180 min).

The SPIKES algorithm includes elements of reading a patient's emotions and projecting empathy. A study of neurology resident performance during a mock session giving the diagnosis to a standardized patient revealed that communicating empathetically was a major issue (Schellenberg, Schofield, Fang, & Johnston, 2014). The exercise was believed by the residents to be useful, and a recommendation was for more training in residency for giving difficult diagnoses.

Providing Hope

Providing the element of hope in the setting of an invariably progressive disorder is very challenging for providers. Hope can be projected by providers to the patient in the form of a positive attitude. Another form is a clinic's participation in formal clinical trials with the underlying hope for a successful drug or intervention. Hope can be shared among patients through patent-oriented Internet web sites. Patients frequently generate an element of hope for themselves when they choose alternative or complementary medications. Approximately 50% of patients with ALS in Europe and the United States partake in complementary medicine, at a large cost, most of which is not covered by insurance (Wasner, Klier, & Borasio, 2001; Vardeny & Bromberg, 2005). An extreme example of patient hope is their participation in unproven stem cell trials that are not conducted under the auspices of regulatory agencies, and have very high cost and some element of medical risk (Bedlack, 2011).

Managing Key Time Points

Patients vary as to areas of weakness and patterns and rates of progression. Standards of care are available that include guidelines for offering and managing treatment and interventions based on a timeline or level of disability (Miller et al., 2009; Andersen et al., 2012). Key points include offering riluzole at the time of diagnosis, managing nutrition and respiration interventions and durable medical equipment along the course, discussing end-of-life issues and hospice late in the course, and bereavement after the patient dies. Discussion of interventions likely need to be broached several times to optimize patient acceptance, at the same time keeping in mind that patient refusal reflects patient autonomy.

Addressing End of Life

Introducing end-of-life issues to the patient is as challenging as giving the diagnosis. There are guidelines in the practice parameters and guidelines for initiating the discussion based on the level of respiratory symptoms and insufficiency.

Bereavement

Bereavement takes several forms: one for the patient and family as loss of function and abilities are mourned, and traditionally for the family at time of death. For the former, empathy is important during clinic visits. The latter takes several forms: one is contacting the family at time of death to give recognition and closure for the family and clinic staff, and the other is formal bereavement counseling. From a survey of 32 ALS multidisciplinary clinics, 66% of neurologists and 94% of clinic managers (most commonly nurses) contacted the family at time of death, and 25% of neurologists and 55% of managers attended some funerals (Bromberg, Schenkenberg, & Brownell, 2011).

CLINIC STRESS

Participating as providers in multidisciplinary ALS clinics, in which the number of new or second ALS diagnoses can reach 250 per year, can be stressful. A survey of 32 clinics indicated that there was stress at all time points, from diagnosis to death, but varied markedly among neurologists and clinic managers (most commonly nurses; Bromberg et al., 2011). Stress was experienced and tended to be lower among more experienced providers (neurologists and managers). Despite such stress, there was a high level of work satisfaction and little evidence for "burn out" leading to leaving the position.

CLINIC BENEFITS

Attendance at a multidisciplinary MND clinic compared with a general neurologic clinic has been shown to be beneficial. Potential positive factors for the multidisciplinary clinic include more rapid attention to adequate nutrition and respiratory function, more timely offering of ambulatory aids, greater use of riluzole, and better support that reduces psychosocial stress (Van den Berg et al., 2005).

CLINICAL CARE QUALITY IMPROVEMENT

One goal of the AAN ALS practice parameter and EFNS guidelines is to encourage and increase compliance with the recommendations. The AAN has developed an ALS quality improvement measurement tool that can also be used in quality initiatives (Miller et al., 2013). From an evidenced-based literature review, 11 recommendations were based on clinical importance and with links to desired outcomes; they were open for public review and comments, and then finalized (Table 20–3).

Table 20–3 **Quality Improvement Measures Chosen to Improve ALS Patient Care**

Quality Measure	Evaluation and Monitoring
1. Multidisciplinary care plan—developed or updated	Care plan developed and updated annually
2. Disease-modifying drugs offered and discussed	Drugs discussed/offered and reviewed annually
3. Cognitive and behavioral impairment screen	Screened and repeated annually
4. Symptom management therapy offered and discussed	Therapy discussed/offered
5. Respiratory insufficiency assessed and tested	Insufficiency assessed/tested every 3 months
6. Noninvasive ventilation discussed	Discussed and updated annually
7. Screened for impaired nutrition	Screened every 3 months
8. Nutritional support offered	Support offered
9. Communication support offered	Communication support offered and updated annually
10. End-of-life planning	Assistance offered and updated annually
11. Querying falls	Queried of past year

They are suitable for evaluation and monitoring for quality of care. Modified from Miller et al. (2013).

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Chapter 21

Allied Health and Other Providers

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Beyond the neurologist and nurse, a wide range of providers including physicians and providers from allied health fields are important for orchestrating the full range of care for patients with motor neuron disease (MND) and caregivers and to manage the effects of progressive weakness and associated consequences. Pulmonologists and gastroenterologists are essential for ventilator issues and placement of feeding tubes, respectively. Physical, occupational, and respiratory therapists have leading roles, but also dietitians, social workers, pharmacists, wheelchair and orthotic specialists, and assistive technologists have key positions in the multidisciplinary clinic.

Despite the importance of these providers for MND care, there are few publications on how to use them effectively and on outcome measures of their efficacy.

INTRODUCTION

The progression of weakness in MND affects a great range of physical and emotional functions for the patient, caregiver, and family. To manage these issues effectively requires an equally wide range of physicians and allied health providers (Box 21.1). The range of issues can best be managed in a multidisciplinary clinic where a core group is available at every clinic visit, and a peripheral group that can be consulted for challenging or uncommon issues.

Some providers have obvious roles, but because the forms of MND are rare, training and experience with this group of patients is not common, and therapeutic advice may not always be appropriate in the setting of MND.

Box 21.1 List of Physician and Allied Health Providers That can be Supportive to Patients With MND and Caregivers (alphabetical order)

Physician Providers

Interventional radiology
Gastroenterology
Psychiatry
Pulmonology
Sleep medicine
Surgery

Allied Health Providers

Assistive technology
Clinical pharmacy
Nursing
Nutrition
Occupational therapy
Orthototics
Physical therapy
Psychology
Respiratory therapy
Speech therapy
Social work
Wheelchair fitting

These concerns are not helped by gaps in the literature.

PULMONOLOGY

There are many review articles on noninvasive and invasive ventilation use in MND, but few articles on specific particulars of respiratory management from the pulmonologist's perspective (Bach, 2002; Winck et al., 2004; Bach, Bianchi, Aufiero, 2004). An explanation for the relative lack of directed articles is that pulmonologists generally work with respiratory therapists and thus care recommendations and consensus guidelines to help with respiratory insufficiency represent joint efforts.

Initial assessment of pulmonary function and initiation of cough-assist devices and noninvasive ventilation in the clinic is usually managed by the respiratory therapist. Pulmonologists are generally available for pulmonary complications, such as pulmonary infections, and if a patient is hospitalized and requires temporary invasive ventilation. One key role for the pulmonologist is in discussing tracheal ventilation, and if selected by the patient, managing training for home care and periodic follow-up (Bromberg, 2001). A second key role is withdrawal of invasive ventilation when a patient requests it (LeBon & Fisher, 2011).

GASTROENTEROLOGY, INTERVENTIONAL RADIOLOGY, AND SURGERY

Adequate nutrition and nutritional support prolongs survival in MND. When nutrition is inadequate, support can be managed by gastric

feeding tubes, which can be placed by gastroenterologists, interventional radiologists, or surgeons. Complications of tube placement increase when pulmonary function is low, and placement is encouraged before forced vital capacity is less than 50% of predicted. However, endoscopic feeding tube placement by a gastroenterologist or interventional radiologists can be accomplished in these situations by use of noninvasive ventilation during the procedure (Gregory, Siderowf, Golaszewski, & McCluskey, 2002; Czell, Bauer, Binek, Schoch, & Weber, 2013). Feeding tube placement by a surgeon is performed under general anesthesia.

PSYCHIATRY AND PSYCHOLOGY

Psychiatric disorders are rare in amyotrophic lateral sclerosis (ALS) in the absence of frontotemporal involvement, but behavioral disorders are more common in the setting of ALS with frontotemporal syndrome or dementia (Strong, 2008). When patients with ALS have psychiatric disorders consideration should also be given to a premorbid condition. Behavioral disorders are common in patients with behavioral variant frontotemporal lobe dysfunction, but there are few studies to guide treatment or management of behavioral disorders (Merrilees, Klapper, Murphy, Lomen-Hoerth, & Miller, 2010; Seltman & Matthews, 2012). Patients respond to anxiolytic, antidepressant, and antipsychotic medications. Psychiatrists are usually called on in difficult situations when their broad treatment experience may be helpful. Depression in ALS is not common, and responds to antidepressant medications.

MND itself can tax all aspects of mental health of the patient, caregiver, and family members. Counseling can be effective. Psychologists can have a role in counseling. Psychologists may not be familiar with the progressive aspects of MND if they are not working in the clinic (Pagnini, Rossi, Lunetta, Banfi, & Corbo, 2010).

PHYSICAL, OCCUPATIONAL, AND RESPIRATORY THERAPY

All patients with MND lose function because of muscle weakness, compounded by spasticity.

Physical and Occupational Therapists

Physical and occupational therapists provide a wide range of services at all stages of MND (Table 21–1). The primary goals of physical and occupational therapists are to maintain as much independence for activities of daily living and mobility; secondary goals are to recommend and manage exercise to reduce complications caused by immobility and help prevent or manage pain; and tertiary goals are to assess for adaptive equipment and home modifications (Lewis & Rushanan, 2007). Assessments by physical and occupational therapists are

Table 21–1 **Range of Activities Provided by Physical, Occupational, and Respiratory Therapy**

Adaptive devices	Large-diameter utensils/writing instruments/keys/knobs
	Special drinking cups
	Button hooks
	Aids to downing socks and shoes
	Long-reach grabbers
	Electronically controlled switches
Transfer aids	Raised toilet seats
	Arm rails by toilet
	Pivot disks
	Transfer belts
	Transfer boards
	Mechanical lifts (chairs, toilet seats)
Splints and braces	Finger/wrist splints
	Ankle-foot-orthosis
Neck supports	Collars
Wheelchairs	Evaluation and fitting
	Manual/power
Home modifications	Home safety evaluations
	Structural changes bathroom/bedroom
	Lift devices (stair lifts/elevators)
Driving evaluations	Evaluations
	Modifications
Pulmonary interventions	Oral suction machines
	Insuflators/exsuflators
	Noninvasive ventilators
	Invasive ventilators
Communications	Dry write/erase boards
	Letter/picture boards
	Computer tablets
	Computers (desk/laptop)
	Eye-gaze controlled computer

Modified from Lewis and Rushanan (2007).

efficiently conducted during multidisciplinary clinic visits where the range of assistive technology devices can be assessed and offered (Casey, 2011). Physical needs of patients intersect with caregiver abilities to easily and safely provide and fulfill them, and both parties must be queried for safety, particularly for transfers and lifting activities involved in activities of daily living (Lewis & Rushanan, 2007).

Respiratory Therapists

All patients with MND lose diaphragm strength and experience a variety of respiratory symptoms, including shortness of breath with speech and activities, orthopnea, and respiratory failure. Respiratory therapists ask about respiratory and sleep symptoms; perform pulmonary function tests; and introduce and guide use of respiratory interventions, such as breath stacking exercises, cough-assist devices, and noninvasive ventilation including fitting of the interface and monitoring use (Table 21–1). One clinic's experience with noninvasive ventilation use showed a four-fold increase when the services of a respiratory therapist were available compared with before (Kareus, Kagebein, & Rudnicki, 2008).

NURSING

The nurses' role is broad, and varies among clinical settings—multidisciplinary clinic, private clinic, and hospice care. The nurse manages the flow in a multidisciplinary clinic and is frequently the person who fields calls and questions from the patient and caregiver between clinic visits (Clarke & Levine, 2011). In a private neurology clinic, the nurse can recommend referrals to other physicians and allied health providers (Davis & Lou, 2011). When patients are in hospice the nurse guides end-of-life issues.

NUTRITION

Optimizing nutrition can be viewed as a continuum, starting with adjustments to oral feeding when dysphagia causes reduced intake, to encouraging placement of a gastric feeding tube when oral intake is insufficient, and adjusting enteral intake and managing complications after a feeding tube is placed. Other areas are answering queries about supplements (Muscaritoli et al., 2012).

SPEECH THERAPY

Speech is an essential component of personal interactions that include communicating basic needs and clarifying them, maintaining social intercourse and personal intimacy, and sharing new information. Speech is impaired to varying degrees in 80% of patients with MND. Changes progress slowly but steadily and patients can become essentially mute. A number of augmentative and alternative communication devices are available (Table 21–1) and speech therapists are important in assessing and recommending appropriate devices.

Patient usage of augmentative and alternative communication devices is highly variable, and specific recommendations can be challenging. One study reviewed modes of communication across a wide spectrum and concluded that it is important to assess past and current preferred means of communication between patient and caregiver before making recommendations for specific devices (Fried-Oken et al., 2006). A patient's actual use of augmentative and alternative communication devices may be less than predicted by the speech therapist (Gruis, Wren, & Huggins, 2011), and many patients rely on speech to communicate even when markedly impaired. Under these conditions, patients and primary listeners adapt with equal perceptions of communication effectiveness between the patient and listener (Ball, Beukelman, & Pattee, 2004). However, speaking strategies (voice amplification) for the patient are limited in distracting social settings.

GENETIC COUNSELING

The prospect of familial ALS has a profound impact on the family. Most patients with ALS enquire about the possibility of passing ALS on to offspring. Until recently, the question could be addressed by asking about the patient's

family history for ALS, and if negative, the answer of "highly unlikely" could be given with relatively high confidence. With the findings of 12 associated genes, and in particular the c9orf72 mutation and its pattern within a family of individuals having either frontotemporal lobe dementia or ALS combined with the issue of hexanucleotide repeat length, the answer to the question of passing ALS on to offspring has reduced confidence.

Genetic testing for the full spectrum of known mutations is readily available, but a negative result does not exclude a genetic cause because ~40% of families with ALS have undiscovered gene mutations. The expanded genetic knowledge raises challenging clinical questions for the patient and family, and these include whether to test, reasons for the choice, whether to communicate results to siblings and offspring, and how to report complicated genetic information. The latter issue is especially important because basic genetic information has been found to be limited among patients with ALS (Fanos, Gelinas, & Miller, 2004).

Guidelines for genetic testing have been offered (Chio et al., 2014). They include testing patients with first- or second-degree relatives with ALS or frontotemporal lobe dementia, but consideration should be given to particular family circumstances. Given the complexities of ALS genetics and the relative lack of genetic knowledge among patients, genetic counseling is highly recommended when testing is considered (Chio et al., 2013).

From the patient perspective, 25 were queried as to their thoughts about genetic testing, and 60% would agree to being tested, and 30% believed it was appropriate to test their children (Fanos et al., 2004). Another study of 20 patients from families with known mutations associated with high genetic penetrance showed that 70% chose to have the results of their testing revealed (Fanos et al., 2011). Reasons for wanting to know were reduced anxiety and the ability to plan; reasons for not wanting to know were being overvigilant of symptoms and if tested negative feeling guilty that a sibling was positive. Basic understanding of patterns and penetration were lacking for some subjects. The psychological impact varied among patients, both positive and negative. The mode of test reporting varied in that some patients wished the results be given in person and others by telephone.

ORTHOTICS

Bracing at the ankle with an ankle-foot orthosis can be helpful when there is ankle dorsiflexion weakness, although weakness from lower motor neuron loss may be helped more by bracing than weakness from upper motor neuron loss (spasticity). There are several different orthotic devices made from a variety of materials and the experience of the orthotist is essential for choosing a functional device.

WHEELCHAIR SEATING

Most patients with MND benefit from a wheelchair to allow travel over greater distances with less fatigue and less risk of falling. Wheelchairs must be fitted to the patient, and for power wheelchairs, features and accessories must be selected to match the patient's physical needs and home environment (Trail, Nelson, Van, Appel, & Lai, 2001).

A survey of 32 patients with ALS about the prescription process, use, and satisfaction with power wheelchairs reinforces the scope of the process to achieve satisfaction (Ward et al., 2010). The wheelchair-seating specialist is usually a vendor who works with a physical or occupational therapist to write the prescription. Insurance companies and governmental agencies (Medicare in the United States) have specific requirements that must be met.

DRIVING EVALUATIONS

The effect of reduced motor control (weakness and spasticity) and reduced executive decision making from frontotemporal lobe dysfunction on driving ability of patients with MND is an area with no literature. Driving evaluations can be arranged through physical or occupational therapists with special training or through departments of motor vehicles.

CLERGY

Religiosity as measured by the Idler Index of Religiosity (two items assessing public and two items assessing private religiousness) has been

found to be correlated with quality of life of the patient with ALS measured by the McGill questionnaire with disease progression (Walsh, Bremer, Felgoise, & Simmons, 2003) and also of the caregiver (Calvo et al., 2011). Despite the importance of religiosity there are no publications on the direct role of clergy with patients with ALS.

ASSISTIVE TECHNOLOGY

The ability to aid a patient with daily activities has expanded and therapists are the principal people to assess and offer devices (Table 21–1; Casey, 2011). Although such aids are routinely offered when therapists believe they would help, patients do not use all items that are offered. A survey queried the use of 33 devices (included in Table 21–1) by 63 patients with ALS (Gruis et al., 2011). Devices used often or always by greater than or equal to 20% of patients included arm rails by the toilet, raised toilet seat, shower chair, shower bars, slip-on shoes, ankle-foot orthoses, transfer boards, speaker phone, and electronic wheelchair controls. Devices commonly offered that were rated as less useful included buttonhooks, dressing sticks with hooks, and long-handed reaching tools. Interestingly, electronic speaking devices, although widely prescribed, were rarely used by most.

TIME LINE OF DURABLE EQUIPMENT NEEDS

The progressive nature of MND means that items of durable medical equipment will be required in a timely fashion and selection of features, especially for power wheelchairs, must be made with future needs taken into consideration. One study of patients with ALS showed that by 50% of a patient's disease course most patients need a wheelchair, and other ambulatory aids at shorter intervals (Fig. 21–1; Bromberg, Brownell, Forshew, & Swenson, 2010). A similar study of patients with primary lateral sclerosis showed the same progressive need for equipment for ambulation (Peters & Floeter, 2009). What is not considered in the time line is the time period a patient needs to accept a recommendation for an item of durable medical equipment: from experience, a patient may think about a wheelchair over one or more clinic visits that span 3 or more months. Thus, when a patient agrees with the device timely delivery is very important.

EXERCISE

A frequent question posed by patients with ALS is whether physical therapy exercise programs, designed and supervised by physical therapists, can restore lost muscle power or slow the progression of weakness. Exercise may result in increased strength of exercised muscle groups but does not slow progression or reduce fatigue. Maintaining an exercise program is difficult for patients.

The role of exercise in ALS has been addressed by several studies. One study of patients with El Escorial Criteria definite or probable ALS completed a 6-month program in which 8 were randomized to exercise and stretching and 10 subjects to stretching only. The exercise group had thrice weekly and individualized moderate level, endurance-type exercise for limbs and trunk and were based on general health, neurologic status, and fitness level; the stretching program was daily to twice daily for both groups (Bello-Haas et al., 2007). At 6 months, ALS Functional Rating Scale scores were significantly reduced and quantitative leg strength score increased in the resistive exercise group, but there was no difference in level of fatigue. Dropout over the 6 months was high, and for a variety of reasons. In another study, 14 patients with definite or probable ALS were randomized to individualized resistive exercise compared with 11 who continued with daily activities (Drory, Goltsman, Reznik, Mosek, & Korczyn, 2001). At 3 months there was a significant slowing in the ALS Functional Rating Scale score in the exercise group, and at 6 months the trend remained but not to a significant degree. Strength showed a positive trend in the exercise group. Dropout was also high with insufficient numbers of subjects for valid comparisons.

ALS is a progress disorder and the degree of loss of muscle strength and how it affects walking has been studied. A group of 118 subjects

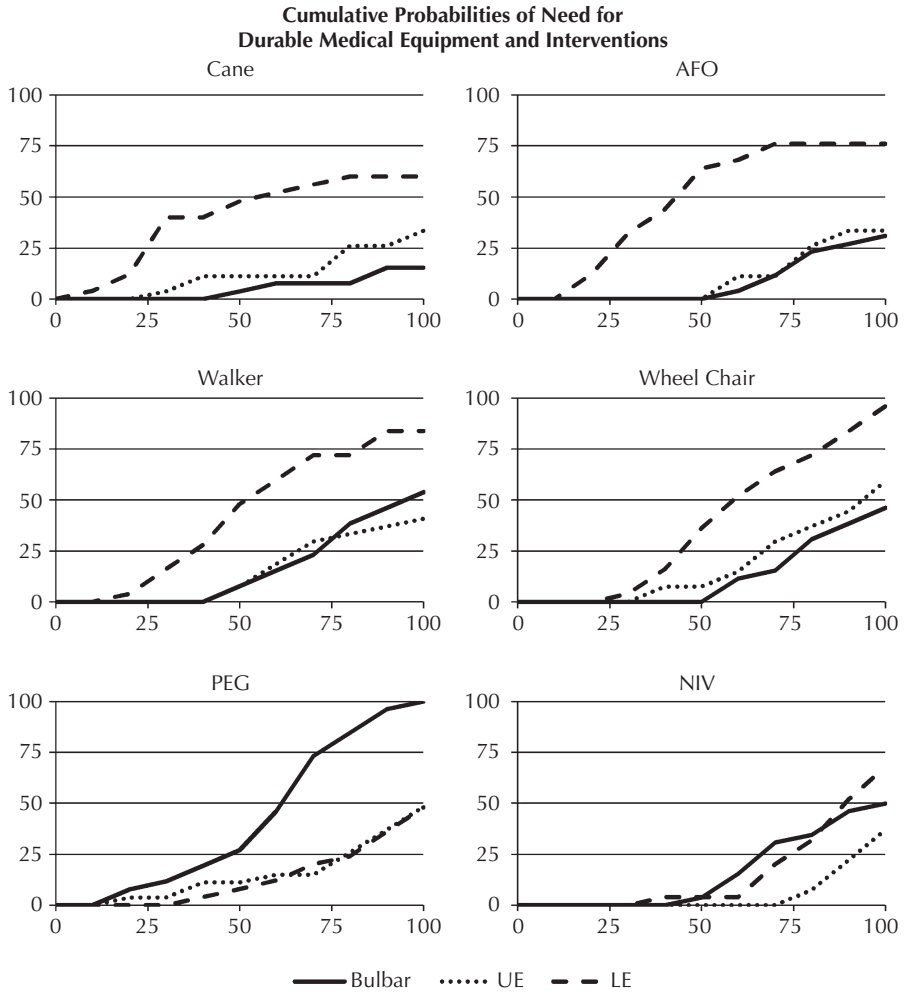

Figure 21–1. Time line of cumulative needs for durable equipment. X axis: percent of disease (ALS) duration. Y axis: percentage of patients needing durable item. AFO = ankle-foot orthosis; LE = lower extremity; NIV = noninvasive ventilation; PEG = percutaneous endoscopic gastrostomy; UE = upper extremity. (From Bromberg et al., 2010, with permission.)

with ALS had serial quantitative assessment of leg strength from major muscle groups and expressed as normalized summed leg strength (Tufts Quantitative Neurologic Examination), and Tufts Quantitative Neurologic Examination scores were compared with their walking ability. When global leg strength declined to 54% patients lost the ability to ambulate independently (without aids) in the community, when it declined to 37% they lost the ability to walk outside, and when it reached 19% they lost the ability to ambulate. Among leg muscle groups, strength of knee flexion was, somewhat surprisingly, more important in maintaining ambulation than strength of knee extensor or distal muscles (Slavin, Jette, Andres, & Munsat, 1998).

Physical symptoms of fatigue, muscle cramps, muscle spasms, spasticity, and musculoskeletal pain are assessed by physical therapists. Although many interventions are individualized for patients by the therapist, efficacy data from randomized controlled trials are important and have been assessed formally in Cochrane Reviews.

General recommendations include consultation with a physical therapist and planning for gradual increase in intensity and frequency of exercise over time (Anziska & Sternberg, 2013). Aerobic exercise is favored over resistive

exercise. Nonambulatory patients can participate with hand-held ergometers. Signs of excessive exercise activity include muscle pain that does not resolve within a day, and the intensity of exercise should be lessened accordingly. Patients often ask about the value of neuromuscular electrical stimulation in ALS: however, muscles are activated by stimulation of motor nerve fibers and not muscle fibers, and with disease progression the number of achievable fibers lessens (discussed in the section on diaphragm pacing in the chapter on respiration).

Exercise for patients with ALS may be limited because of respiratory insufficiency. To compensate for this, a trial of eight patients with ALS exercising on a treadmill with the assistance of bilevel noninvasive ventilation to maintain oxygen saturations showed that such patients can exercise without harmful effects and forced vital capacity values were maintained compared with a control group not exercising (Pinto et al., 1999).

Diaphragm strength is the limiting factor in MND and the question of the use of diaphragm exercises arises. In one study the diaphragm was exercised by exaggerated breathing efforts, but no change in forced vital capacity was found over 3 months (Nardin et al., 2008). Only eight patients were recruited and there was difficulty in mastering the technique.

Another approach to diaphragm exercise is use of a resistive inhalation device. A study of 26 patients with ALS with normal respiratory function was divided into two groups: one group participated in resistive inhalation exercises for 8 months, whereas the other group did not for 4 months and then participated in the exercise program for 4 months (Pinto, Swash, & de Carvalho, 2012). There were no significant differences between the two groups.

Another frequently asked question is the role of supplements on strength. One study of 28 patients with ALS assessed quantitative strength of 10 muscle groups before and 6 months after taking creatine, 20 g daily for 7 days and 3 g daily thereafter (Mazzini et al., 2001). Maximal strength improved statically only in two muscle groups (knee extensors and elbow flexors) and only in some subjects. Overall, strength declined in a linear manner over 6 months. Fatigue was also reduced in these muscle groups.

Possible Harmful Effects of Exercise

A remote history of relatively intense exercise has been questioned as a possible factor in the later development of ALS. Epidemiologic studies suggest that premorbid lean body mass and athleticism may be more common among patients with ALS (Scarmeas, Shih, Stern, Ottman, & Rowland, 2002). However, other studies have not confirmed the association (Veldink et al., 2005). A review of 23 studies showed that half reported a possible association and half did not (Veldink et al., 2005). Epidemiologic methods were questioned for many of the studies.

FALLS

Falls are a common occurrence with patients with MND, because of spasticity (ALS and primary lateral sclerosis) and leg weakness (ALS and progressive muscular atrophy). Spasticity affects the ability to correct for being off-balance with rapid contraction of muscles, and weakness can affect proximal or distal leg muscles. Clinical assessment of spasticity and weakness is not predictive of the likelihood of falling. The time it takes to stand up from a chair, walk three meters, turn around, and sit down again (Timed Up and Go test) has been shown to correlate with falls (Montes et al., 2007).

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Chapter 22

Respiratory Assessment and Management

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Respiratory failure is the proximate cause of death with amyotrophic lateral sclerosis (ALS) and progressive muscular atrophy (PMA). Symptoms of failure progress in a predictable manner but early on may be subtle and require frequent monitoring. Interventions with noninvasive ventilation (NIV) are helpful and may prolong survival, but optimum time to recommend use has not been determined. Fulltime ventilation can prolong life beyond the natural course and a decision about fulltime ventilation is the most important one a patient has to make.

Several pulmonary tests are available to assess respiratory status, but the most sensitive metric has not been determined. The optimum time to initiate NIV in the course of ALS is

undetermined. Comfortable use of NIV may be challenging for some patients and more of an issue for those with bulbar muscle weakness and frontotemporal lobe dysfunction. Cough-assist devices can aid pulmonary hygiene. Electrical stimulation of the diaphragm may prolong survival and enhance diaphragm muscle function, but definitive data are lacking.

INTRODUCTION

Respiratory failure occurs with ALS and PMA and to a less predictable degree with primary lateral sclerosis. Assessing respiratory symptoms and pulmonary function testing are the first steps, leading to recommendations initially for improving pulmonary hygiene and later for respiratory interventions. Because respiratory failure is the proximate cause of death with ALS and PMA, counseling of the patient and family is essential at multiple time points to give the patient autonomous choices for breathing interventions. Guidelines for care of patients with ALS suggest thresholds for conversations with patients when breathing becomes symptomatic or forced vital capacity (FVC) is 50% of predicted (Clinical indications, 1999; Miller et al., 2009; Andersen et al., 2012).

RESPIRATORY ANATOMY AND PHYSIOLOGY

Inspiration results from activation of the diaphragm, scalene muscles, the external intercostal muscles, levator costae (origin, transverse processes of thoracic vertebrae; insertion, rib below), and the parasternal intercostal muscles (small muscles close to the border of the sternum and chondrocostal junctions). In the setting of quiet inspiration, activation of the diaphragm moves about 45% of the air volume, and external intercostal muscles about 25% of the air. Quiet expiration is passive from elastic recoil of the rib cage.

With greater respiratory drive from respiratory insufficiency, accessory inspiratory muscles are activated and include the sternocleidomastoid, serratus anterior, pectoralis group, upper trapezius, various spinal muscles, and abdominal muscles. With active or forceful expiration, such as with a cough, internal intercostal and abdominal muscles are activated. An effective cough requires glottic control (closure), which becomes compromised with marked bulbar weakness.

The diaphragm consists of two hemidiaphragm muscles, each innervated by a phrenic nerve that is derived from third to fifth cervical roots. Chest wall muscles are innervated by nerves derived from second to sixth thoracic roots. Although respiratory failure results primarily from lower motor neuron (LMN) loss, there is also likely a component of upper motor neuron (UMN) failure from neuron loss in brainstem regions associated with generation of rhythmic respiratory drive. Although little is known about the effects of the UMN component in ALS, the diaphragm motor evoked potential to transcranial magnetic stimulation was found to be absent or reduced in up to half of patients (Shimizu et al., 2010). Of note, some of these patients had no respiratory symptoms.

ALS PROGRESSION AND RESPIRATORY FAILURE

The pattern of LMN loss as it relates to the phrenic nerve is obviously important in patient care. It is interesting that clinically, respiratory function may be reduced but is generally better than limb or bulbar function at late stages of ALS and PMA; that is, patients may be bedbound from limb weakness but have relatively comfortable respiration. The late onset of respiratory involvement has been confirmed in a series of 150 patients with ALS followed at 3-month intervals until respiratory symptoms developed (Fujimura-Kiyono et al., 2011). Regions of involvement were determined from the ALS Functional Rating Scale-Revised score: respiratory symptoms developed in only 3.3% of patients when the second body region was affected, in 32.6% when the third region was involved, and in 57% when the fourth region was involved.

There is also likely a degree of neural compensation that preserves respiratory capacity in the setting of progressive phrenic nerve loss (Nichols et al., 2013). Mechanisms considered include (1) greater UMN respiratory drive from brainstem plasticity, (2) increase in the size of the phrenic nerve motor units,

and (3) increased use of accessory inspiratory muscles.

SLEEP-DISORDERED BREATHING

Sleep-disordered breathing represents alterations of the respiratory pattern with pauses that reduce the quantity of ventilation during sleep. Obstructive sleep apnea is common in the general population, and is likely also a factor in some patients with ALS. However, there are greater concerns for hypoventilation during rapid eye movement (REM) sleep in ALS, supported by a variety of findings. One study of 21 patients with definite ALS who had polysomnography showed rare apnea and hypopnea episodes, but among those with reduced diaphragm function some experienced markedly reduced or no REM sleep, and some had activation of the sternocleidomastoid muscles during REM sleep, interpreted as a compensator mechanism because only the diaphragm and extraocular eye muscles are usually active during REM sleep (Arnulf et al., 2000).

EARLY DIAPHRAGM DYSFUNCTION

Respiratory function can be impaired at several sites, centrally with reduced neural drive, in the airways with obstruction, and at the diaphragm with weakness and fatigue. It is important that sleep-disordered breathing may occur with good respiratory metrics. A study of 11 patients with ALS who had no medical respiratory disease, normal respiratory tests (FVC, maximal inspiratory and expiratory pressures [MIP and MEP]), normal P_{CO_2}, and normal diaphragm response to electrical stimulation underwent polysomnography because they had saturations to 90% on nocturnal oximetry studies (Atalaia, De Carvalho, Evangelista, & Pinto, 2007). Sleep-disordered breathing was detected in eight patients, which included apnea and hypopnea occurring in REM or non-REM sleep. In another study of 261 unselected patients with ALS followed with periodic nocturnal oximetry, 13 (5%) had desaturations despite normal diaphragm function (normal evoked diaphragm response and no evidence for needle electromyogram abnormalities), and their FVCs were 84–128% of predicted and MIP between 2 and 93 cm H_2O (de Carvalho, Costa, Pinto, & Pinto, 2009). These patients were characterized clinically as having marked UMN signs. Thus, nocturnal oximetry may detect sleep-disordered breathing not predictable from other respiratory measurements in the clinic.

RESPIRATORY METRICS

Several respiratory function tests are available in the clinic, at home, or in a sleep laboratory (Box 22.1; Gruis and Lechtzin, 2012). Common clinical tests include FVC (both sitting and supine, and rapid and slow), MIP and MEP, and maximal nasal sniff pressure. Oxygen saturation can be measured in the clinic and by nocturnal oximetry in the patient's home during sleep. Carbon dioxide levels can be measured in the clinic noninvasively by capnography (Kim et al., 2011). A full or abbreviated nocturnal polysomnogram provides information about hypoxia and hypercarbia, and central and peripheral (obstruction) factors during different stages of sleep. The diaphragm muscle response (amplitude) to electrical stimulation can be measured in the electrodiagnostic laboratory. Diaphragm excursion can be measured radiographically or by ultrasound and diaphragm thickness by ultrasound (Hiwatani, Sakata, & Miwa, 2013).

Methods of Testing

FVC and MIP and MEP values are affected by testing variability from a variety of factors, and the optimal testing strategies have not been determined (Box 22.1; Pinto, Geraldes, Vaz, Pinto, & de Carvalho, 2009).

Respiratory Survey Questionnaires and Instruments

Several survey questionnaires or instruments developed for other sleep-related breathing disorders have been used to assess respiratory symptoms in ALS. They assess indirectly

Box 22.1 Pulmonary Tests, Testing Factors, and Testing Strategies

Pulmonary tests

Forced vital capacity (rapid exhalation): seated or supine
Slow vital capacity (slow exhalation)
Maximal inspiratory or expiratory pressure
Nasal sniff pressure
Day and nocturnal oximetry
Day carbon dioxide levels
Diaphragm muscle response (compound muscle action potential) to phrenic nerve stimulation
Diaphragm excursion: fluoroscopy or ultrasound
Diaphragm thickness: ultrasound
Polysomnogram

Pulmonary testing factors

- Reference values
  Volume in liters or pressure in centimeters of H_2O
  Reference values from normative data vary among sources: percent of predicted
- Patient learning
  Higher values with practice
- Patient fatigue
  Lower values with respiratory muscle fatigue
- Patient coordination
  Variable values because of incoordination of bulbar function from UMN loss
- Facial muscle weakness
  Inability to achieve lip seal around tube (use of mask to circumvent weakness)
- Pseudobulbar affect
  Ease of laughing

Pulmonary testing strategies

Average of 5–10 tests
Better of two technically good responses
Highest of three tests within 5% variability
Learning effect: best of 15–20 tests

respiratory function, as a means to identify early changes, including those that may occur during sleep and whose impact may not be obvious (Box 22.2). Questionnaire sensitivity assessment is usually based on correlations with pulmonary function measurements, which in turn are static daytime measurements and whose ability to assess respiratory function is uncertain (Bourke, McColl, Shaw, & Gibson, 2004). Periodic patient and family querying for a variety of symptoms (Box 22.2), supported by pulmonary function test values, likely provide as much clinically useful information as survey questionnaires or instruments.

NIV

The most common form of NIV used with ALS and patients with PMA is bilevel ventilation.

Box 22.2 Respiratory Symptoms, Sleep Survey Instruments, and Respiratory Threshold Values for NIV

Respiratory symptoms

Dyspnea
Tachypnea
Orthopnea
Shortened spoken sentence length caused by dyspnea
Use of accessory muscles at rest
Disturbed sleep caused by nocturnal arousals
Morning headaches
Excessive daytime fatigue and sleepiness

Sleep survey questionnaires/instruments

Epworth Sleepiness Scale
Sleep Apnea Quality of Live Index

Threshold respiratory test values for NIV

Forced vital capacity of <80–50% of predicted
Maximal negative inspiratory force less than −60 cm H_2O
Sniff pressure <40 cm H_2O
Nocturnal saturations of <88% for 5 consecutive minutes
Arterial P_{CO_2} > 45 mm Hg

There are many factors involved with the success of bilevel NIV, and there is an art to working with patients to improve patient tolerance and hours of usage (Gruis & Lechtzin, 2012). One factor that is difficult to codify is the clinical effort used to initiate NIV in terms of personnel to monitor and adjust the interface and pressure settings and follow-up, which in the extreme is the initiation of NIV in a hospital setting lasting several day and nights (Volanti et al., 2011).

Bilevel Ventilation

With bilevel ventilation there are two pressure levels, which can be adjusted separately (inspiratory and expiratory airway pressures), and the difference between the two is the level of pressure support. The NIV machine can function in two modes, spontaneous and spontaneous-timed. Both modes start with the expiratory pressure; the machine increases to the inspiratory pressure and flow when it senses a change initiated by the patient's inhalation, and the inspiratory flow lasts 3 seconds or when inspiratory flow slows, indicating the end of the patient's inspiration. In the spontaneous mode, the patient's breathing rate sets the machine's respiratory rate. In the spontaneous-timed mode, a back- up rate of machine-triggered inspiratory pressure and flow and pressure can be set. The spontaneous-timed mode can be used when respiratory muscles are too weak to trigger the inspiration flow. There are autotitrate NIV machine models that adjust pressures and flows to help achieve target volumes.

RESPIRATORY THRESHOLD FOR NIV

The physiologic threshold for recommending NIV has not been determined and likely is

highly dependent upon the individual patient's symptoms and respiratory testing values (Gruis & Lechtzin, 2012). However, there are general guidelines that include symptoms and signs, and some threshold respiratory test values are linked to national and health insurance regulations (Box 22.2; Clinical indications, 1999; Miller et al., 2009; Andersen et al., 2012).

PREDICTORS OF NIV USE

The goal of 4 hours per night use of NIV is an arbitrary number. Adherence varies across studies, and two external variables are the level of personnel available for telephone follow-up encouragement and pressure and interface adjustments; and the location of where NIV is started, at home or in a hospital setting with greater patient attention. Intrinsic variables include the degree of bulbar weakness and frontotemporal lobe dysfunction (Volanti et al., 2011).

Patients with ALS with bulbar weakness are reported to have greater difficulty with NIV, attributed to dysphagia and difficulty with secretion management. A study of 71 patients with ALS found that mild to moderate bulbar symptoms (based on a 0–8 scale) were factors for poor NIV tolerance (Lo Coco et al., 2006). In another study of 50 patients, adherence was six time more likely in limb-onset than in bulbar-onset patients (Gruis, Brown, Schoennemann, Zebarah, & Feldman, 2005).

Patients with ALS with frontotemporal lobe dysfunction are also reported to have greater difficulty with NIV (Olney et al., 2005). In a study of 81 patients with ALS, those that had frontotemporal lobe dysfunction had shorter survival and were less compliant with NIV. Of note, there were more patients with bulbar weakness in the frontotemporal lobe dysfunction group.

EARLY VERSUS LATE USE OF NIV

FVC guidelines for institution of NIV vary among clinics from 50% to 80% of predicted, although there are other factors and test values that influence this threshold. There are no studies to determine if starting NIV when respiratory function is normal is better than at a symptomatic time. One study of patients with ALS who received NIV and used it for more than 4 hours per night focused on two groups: an early use group of 25 patients whose FVC was more than 65% (average, 73.4%), and a late-use group of 67 patients whose values were less than 65% (average, 48.3%) at initiation of NIV (Lechtzin et al., 2007). There were no significant clinical differences between the two groups, but the average MIP was 54 cm H_2O for the early use group and 28 cm H_2O for the late-use group. Tracheal-free survival was significantly prolonged among the early use group (2.7 years) compared with the late-use group (1.7 years).

EFFICACY OF NIV

The efficacy of NIV can be viewed as correction of nocturnal desaturations. It can be a challenge to achieve such efficacy. In one study of 82 patients with ALS, 40 were adequately ventilated and 42 were not (Gonzalez-Bermejo et al., 2013). Air leakage around the interface was the main source of persistent nocturnal desaturations (53%) and obstructive events another source (26%).

LONG-TERM EFFECTS OF NIV

NIV represents an intervention in the management of ALS and there have been a large number of studies, dating from the mid-1990s, looking for positive effects. There are many NIV variables, including at what level of respiratory compromise intervention is recommended (FVC percentage) and ability of the patient to use the device (hours per night or day), and what is measured metric (survival, quality of sleep, and quality of life). Most studies are observational and none fully controlled (sham ventilation).

Survival and Quality of Life

A review of the literature to 2005 found four retrospective, seven prospective, and one randomized controlled studies, providing class II to IV evidence (Piepers et al., 2006). Overall,

for survival, four studies reported longer survival for patients who tolerated NIV compared with those who did not and survival was longer for those without bulbar symptoms; for effect on pulmonary function, two of six studies showed no impact of NIV and one showed a slower decline in FVC among those who tolerated the intervention; for quality of life, all five studies found it to be positive; for respiratory symptoms, reductions were found in the three studies that queried them.

A recent Cochrane review found only two randomized studies (Radunovic, Annane, Rafiq, & Mustfa, 2013). One study of 13 patients with ALS who had nocturnal desaturations of less than 90% were randomized to two groups: seven to "early NIV initiation" with mean FVC of 77%; and six "standard NIV initiation" with FVC less than 50% of predicted (Jackson, Lovitt, Gowda, Anderson, & Miller, 2006). The study focused on clinical and pulmonary functional associations between the symptomatic groups with no long-term follow-up. Another study randomized 22 patients to NIV and 19 to standard care and followed them until death (Bourke et al., 2006). NIV use was higher in patients with good bulbar function (mean, 9.3 hours/day) compared with those with poor function (mean, 3.8 hours/day), and the former group used NIV many months longer with progression of respiratory decline. Quality of life was measured by the Short Form-36 health-related instrument and two sleep-related instruments. Those on NIV and who had better bulbar function showed greater elements of quality and for longer periods of time compared with those not on NIV or those on NIV but with poor bulbar function. Patients on NIV and who had good bulbar function had longer survival than those not using NIV, but there was no survival advantage with NIV for those with marked bulbar function.

Greater use of NIV has been singled out as the main identifiable factor in improving survival in a single center as assessed among 2,037 patients with ALS over 2002–2009 (Gordon et al., 2012). The proportion of NIV use was 16% during the period 2002–2004 and 51% during the period 2006–2008).

Prolonged daily use of NIV (>20 hours/day) to maintain oxygen saturations greater than 94% is feasible, and is aided by NIV machines with spontaneous-timed mode capabilities. In one study, the combination of assisted cough and spontaneous-timed mode ventilators, which were used for more than 20 hours/day was effective in supporting comfortable respiration for long periods (mean, 11 ± 18 months), and was believed to be effective in lieu of tracheal ventilation (Bach, Bianchi, & Aufiero, 2004). However, patients with marked bulbar weakness were frequently unable to maintain adequate saturations and required tracheostomy (mean, 22 ± 25 days) or died (mean, 48 ± 61 days).

Sleep

The effects of NIV on sleep have been assessed in 12 patients with ALS with home polysomnograms, one before and one after NIV (Katzberg et al., 2013). NIV resulted in improved oxygen saturations (7% non-REM, 6.7% REM sleep) and time at less than 90% saturation improved from 30% to 19% but NIV did not have an effect on apnea or hypopnea indices, sleep efficiency, or arousal index of sleep stage distribution.

Another study of 19 subjects with ALS who were accustomed to NIV and who had polysomnography studies showed frequent asynchrony between the ventilator and patient breathing (Atkenson et al., 2011). Asynchrony may be caused by air leaks from mask or mouth or inappropriate pressure setting, leading to nontriggered and double-triggered breathing efforts, which in turn increase the work of breathing and disrupt sleep. No ALS clinical features were found to predict asynchrony.

PREDICTORS OF NONINVASIVE USE

Use of NIV varies among patients and predictors of compliance have been sought. Patients with bulbar-onset ALS may have greater difficulties with NIV, but data are equivocal (Gruis et al., 2005; Jackson et al., 2006).

SUPPLEMENTAL OXYGEN

When oxygen saturations are low there is a tendency for physicians to order supplementary oxygen. If there is a component of pulmonary

or cardiac disease supplemental oxygen may be appropriate. However, older literature indicates that supplemental oxygen in the setting of hypercarbia could reduce respiratory drive. Little data are available on possible respiratory depression with the use of supplemental oxygen in the patient with ALS, but caution is recommended.

ELECTRICAL PHRENIC NERVE AND DIAPHRAGM STIMULATION

The diaphragm is activated from medullary respiratory neurons via phrenic nerves and is affected by both UMN and LMN loss. During sleep there may be reduced UMN drive leading to disordered respiration and sleep. The degree of LMN loss is the ultimate variable for diaphragm strength, and partial denervation may result in alterations of diaphragm muscle fiber type. In this setting, electrical activation of the diaphragm has the possibility of maximizing respiration during sleep, optimizing diaphragm strength, and prolonging survival.

Phrenic Nerve Stimulation

The diaphragm can be activated by electrically stimulating the phrenic nerve. There are two techniques distinguished by types of stimulating electrodes and their locations: one activates the phrenic nerves in the neck using a variety of electrode configurations (cuff, ribbon electrodes), and the other activates the nerves as they enter the diaphragm (hook electrodes; Amirjani, Kiernan, McKenzie, Butler, & Gandevia, 2012). Both techniques were originally developed for quadriplegic patients who have intact phrenic nerves. For patients with ALS, there are limited data on the use of electrodes activating the phrenic nerves in the neck, and recent interest is with electrodes activating the nerves as they enter the diaphragm, also called diaphragm pacing.

Diaphragm Pacing

Electrical stimulation of the diaphragm, or more accurately, electrical stimulation of the phrenic nerve as it enters the diaphragm muscle, has been available to patients with ALS (Onders et al., 2009). Hook electrodes are implanted in the underside of each hemidiaphragm muscle during a laparoscopic procedure, with site of optimum diaphragm activation determined during the procedure. Diaphragm pacing was originally developed for quadriplegics who have intact phrenic nerves, but the Food and Drug Administration granted Humanitarian Device Exemption for ALS in 2011. The stimulating electrode and ground wires are tunneled to the outside and stimulation is via an external battery-powered device. The procedure is well tolerated with minimal surgical morbidity and rare mortality (Amirjani et al., 2012), and a gastric feeding tube is commonly placed during laparoscopy. Stimulation is typically at night in conjunction with NIV and the electrical shocks are mildly uncomfortable but readily tolerated.

Diaphragm pacing is intended to be used in patients with ALS with early and moderate respiratory failure and in conjunction with NIV. Inclusion criteria included at time of surgery are an FVC between 45% and 85% of predicted and demonstration that the diaphragm can be activated either by electrical stimulation of the phrenic nerve or movement demonstrated with fluoroscopic sniff test.

Putative positive effects of full diaphragm activation by electrical phrenic nerve stimulation include conditioning of muscle fiber types (fast twitch to slow twitch type), reduction of atelectasis, increase in compliance, better synchrony with NIV, and possibly longer survival (Onders et al., 2009). From a study of 14 patients with ALS who underwent polysomnography before and after 4 months of diaphragm conditioning there were positive effects on sleep efficiency, arousal index, and neck muscle activity despite progressive decrease in respiratory metrics (Gonzalez-Bermejo, Morelot-Panzini, Salachas, Redolfi, & Straus, 2012).

Long-term effects of diaphragm pacing have been reported from case series with comparisons with historic controls, but several randomized trials are underway. Because diaphragm pacing requires a surgical procedure under general anesthesia there are notes of caution until the results of randomized studies are available with patients with ALS (Scherer & Bedlack, 2012; Mahajan, Bach, Saporito, & Perez, 2012). It is important for the patient

to understand that over time there is greater phrenic nerve loss and reduced ability to electrically activate the diaphragm.

LONG-TERM MECHANICAL VENTILATION

Although respiratory failure is the most common proximate cause of death for patients with ALS and patients with PMA, respiration can be managed for long periods with positive pressure mechanical ventilation. Various terms are used, including long-term mechanical ventilation (LTMV) and tracheal ventilation. Ideally, a patient should be given the option to actively choose LTMV, but many patients are placed on a ventilator under emergency medical conditions. This speaks to the need for periodic discussions about respiratory failure and patient choices.

An important factor in patient selection for LTMV is the attitude of the neurologist or pulmonologist toward the intervention. From a survey of neurologists in the United States and Japan, 79% of US neurologists reported seldom or never suggesting or encouraging LTMV compared with 32% in Japan (Rabkin et al., 2013). This resulted in 84% of US neurologists reporting that less than 10% of their patients were on LTMV compared with 32% of Japanese neurologists. Interestingly, most (70%) neurologists from both countries would opt against LTMV if they had ALS.

The characteristics of who choses mechanical ventilation have been studied in a prospective study on a group of patient who were followed toward the end of their natural course of ALS (FVC <50% of predicted; Rabkin et al., 2006). Among 72 patients, 14 choose mechanical ventilation and 58 died from ALS without mechanical ventilation. The 19% who choose ventilation is higher than reported in other studies (except for Japan, where it is 45%). Statistically significant factors favoring future choice of mechanical ventilation were young age (mean, 51 vs. 65 years), having children age younger than 21 years, and more optimism about the future. Nonsignificant factors were level of functional impairment, degree of bulbar dysfunction, degree of cognitive impairment, and importance of religious faith. Among the 14 patients who were placed on mechanical ventilation and followed for 3–55 months (mean, 25 months) most continued to derive satisfaction and pleasure with their daily lives despite progression of weakness. Communication became more difficult for all, with a range of use strategies for communication tactics from computer use, to "yes-no" answers, to being locked-in without the ability to communicate. Several of these patients were placed in nursing homes. Caregiver burden increased with the use of LTMV, but satisfaction remained high.

The clinical features of patients using LTMV have been studied (Spataro, Bono, Marchese, & La Bella, 2012). From one center, 87 (31%) of 279 patients with ALS chose LTMV and there were more males than females (1.7:1). Most (94%) were cared for at home. Survival was significantly longer compared with patients who did not chose LTMV (median, 47 compared with 31 months), but influenced by 25% of patients who died within 1 month, and most of these were associated with the need for emergency tracheostomy. Nutrition by gastric feeding tube occurred in 66%.

PATIENT AUTONOMY FOR RESPIRATORY DECISIONS

The challenges patients face when they deliberate over choices for respiratory interventions should not be underestimated by providers. One important factor in the decision-making process brought out from interviews is that patient fears focus more on the manner of death than fear of death itself (Lemoignan & Ells, 2010). A related issue is the ability of the patient to assimilate detailed information about ventilator choices and consequences if not chosen. Thus, it is important to periodically review respiratory options. There is also likely an adaptation process by the patient, first to choosing (or not) an intervention and then to becoming comfortable with any form of ventilation. This factor is especially important in moving from NIV to LTMV.

COUGH AUGMENTATION

An effective cough is essential to clear respiratory secretions and aspirated material.

A threshold adequate cough, measured in liters per minute, is more than 160 L/s, compared with normal flows of 6 L/s. An effective cough also requires control of the glottis, and thus can be more impaired in patients with bulbar weakness.

Several respiratory interventions can be offered before or in conjunction with the need for assisted ventilation.

Air or Breath Stacking

Air or breath stacking is a technique where small volumes of air are consecutively held by glottis closure until no more air can be retained, and can be performed manually by the patient or with the aid of a mask with a one-way valve and a resuscitation bag. A study in patients with Duchenne dystrophy showed that breath stacking results in greater volumes and higher peak cough flows (Kang & Bach, 2000).

Insufflation and Exsufflation Devices

This is a device that provides positive pressure via a mouthpiece during inhalation preceding a cough, followed by negative pressure during the cough. Insufflation-exsufflation devices are helpful to increase peak cough flow when glottal weakness prevents the ability to generate sufficient pressure (Chatwin et al., 2003; Bach, 2003).

An assessment of the efficacy of a variety of cough interventions among 28 patients with ALS, measured by an increase in peak cough flow and patient's subjective feelings of clearance, included manual techniques (coaching, abdominal thrust) and instrumented techniques (abdominal thrust augmenting deep breath enhanced by a resuscitation bag, abdominal thrust augmenting end-inspiratory breath augmented by NIV, and assisted cough by insufflation-exsufflation; Senent et al., 2011). All techniques increased peak cough flow from low baseline values in patients with and without bulbar symptoms, but the insufflation-exsufflation device provided the greatest flow (up to five times the unassisted cough).

INSPIRATORY MUSCLE EXERCISE

Respiratory muscles can be exercised with resistive breathing (inhaling through a device with adjustable pressure settings). One study of patients with ALS with normal respiratory function randomized to resistive breathing exercise compared with another group randomized to breathing without resistance (Pinto, Swash, & de Carvalho, 2012). Despite exercising, there was no slowing in the rate of change of respiratory measures.

HIGH-FREQUENCY CHEST WALL OSCILLATORY TREATMENT

High-frequency chest wall oscillatory treatment is effective in assisting mucus clearance in hypersecretion disorders, such as cystic fibrosis. In ALS, small, randomized controlled trials have compared the addition of this treatment to standard NIV. Twenty-two patients with ALS were randomized to high-frequency chest wall oscillatory treatment twice daily for 12 weeks compared with 24 patients who did not receive treatment (Lange et al., 2006). There were improvements in the feeling of breathlessness, but not in objective respiratory measures. Another study looked at long-term survival (Chaisson, Walsh, Simmons, & Vender, 2006). Among eight patients with ALS receiving NIV, four were randomized to receive high-frequency chest wall oscillatory treatment for 15 minutes twice a day. There was no difference in survival between the two groups.

ASPIRATION PNEUMONIA

The combination of dysphagia and respiratory insufficiency increases the risk of aspiration pneumonia. An incidence study from Olmstead County, MN revealed 4 cases of aspiration pneumonia among 40 patients with ALS (Sorenson, Crum, & Stevens, 2007). Dysphagia and respiratory compromise were common features, and three had gastric feeding tubes. Residence in a nursing home was a risk factor (three of the five). Aspiration

pneumonia was a late event and although death was within 6 months, overall survival was not affected.

CAUSE OF DEATH IN ALS AND END-OF-LIFE CARE

The proximate cause of death in ALS includes several factors. There are differences between clinical and pathologic causes of death. In a study of 100 patients with ALS assessed by autopsy, the following causes were noted: clinical diagnoses were hypoxia (47%), bronchopneumonia (20%), sudden death (12%), acute respiratory failure (9%), heart failure (7%), pulmonary embolus (4%), and bulbar deterioration (1%); pathologic diagnoses were agonal bronchopneumonia (55%), aspiration pneumonia (16%), heart failure (10%), pulmonary embolus (6%), hypoxia (3%), hemoperitoneum (2%), and unknown (8%; Corcia et al., 2008). Thus, hypoxia was a rare cause, and bronchopulmonary factors a common cause.

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Chapter 23

Nutritional Assessment and Management

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Nutritional status is a concern in amyotrophic lateral sclerosis (ALS), and can be difficult to assess because of predictable weight loss from muscle atrophy caused by lower motor neuron (LMN) degeneration. Contributing issues are loss from reduce food intake related to swallowing difficulties and upper extremity weakness and fatigue reducing food delivery. Gastric feeding tubes can be used to manage nutrition.

There are data supporting an association between high normal weight and enhanced ALS survival. Elevated serum lipids may also be a positive factor for survival. Metabolic needs may be elevated because of a hypermetabolic state in some patients with ALS. There are different methods of feeding tube placement, and although the procedure is recommended before respiratory function is compromised, it can be accomplished safely using bilevel ventilation during the procedure when respiratory function is low.

INTRODUCTION

Weight, or body mass index (BMI; weight [kg]/height [m^2]), are important metrics in ALS. Reductions can be caused by loss of muscle mass from denervation, loss of appetite, dysphagia and fatigue of swallowing, weakness and fatigue of arm function for feeding, and a hypermetabolic nutritional state. However, there are major issues to measuring losses in body mass compartments to determine factors particular to an ALS patient, and to measuring energy expenditure for nutritional guidance. The importance is that there are data supporting greater longevity with higher BMI and with elevated lipids. Nutritional supplementation can be managed with the aid of gastric feeding tubes.

PREMORBID WEIGHT AND RISK OF DEVELOPING ALS

Epidemiologic data support an increased risk of developing ALS associated with low

premorbid BMI. From a cancer and nutrition study in Europe that included 518,108 individuals followed for 13 years, there was a statistically significant association between low BMI (underweight BMI <18.5 kg/m^2; normal-weight 18.5– 25 kg/m^2) at enrollment in the study and the risk of dying from ALS for both genders (Gallo et al., 2013). Among women there was a three-fold increased risk with underweight compared with normal-weight women; among men there was a trend for reduced risk compared with normal-weight men, but note was made that there were insufficient numbers of underweight men, possibly accounting for statistical differences between genders. Another study of 1,100,910 subjects followed from 14 to 28 years showed that low BMI was also associated with developing ALS, with a step-wise decrease with increasing BMI values (O'Reilly et al., 2013). Pathophysiologic explanations are conjectural, and may relate to hypermetabolic states in some individuals.

WEIGHT LOSS OVER THE COURSE OF ALS

Weight loss occurs in about 50% of patients with ALS (Korner et al., 2013). Several factors can be involved, as enumerated previously, but weight loss can also occur among patients with ALS who have normal swallowing.

Weight or BMI loss from reduced muscle mass caused by LMN degeneration (neurogenic muscle atrophy) is an obvious disease-based pathologic cause, but it is difficult to separate reduced muscle mass from loss of fat and water.

Reduced appetite is described among patients with ALS, and from a study of 51 patients given a questionnaire about appetite, 47% described severe loss of appetite that increased to 67% over 6 months and was not correlated with dysphagia (Holm et al., 2013). This was associated with a 5% weight loss among patients with reduced appetite compared with 2% loss in those without impaired appetite.

EFFECT OF WEIGHT LOSS

An effect of loss of BMI on survival has been demonstrated. A study of 285 patients fulfilling El Escorial Criteria for definite or probable ALS who were seen quarterly showed that patients who had greater BMI losses had significantly more rapid rates of progression and shorter survival. A significant association was found between negative change in BMI over time and bulbar site of onset (Jawaid et al., 2010). It is not clear if negative changes in BMI on survival reflect greater loss of muscle mass from denervation or a hypermetabolic state or other factors.

NUTRITIONAL ASSESSMENT

The ability to assess nutritional status is important in managing nutrition. A variety of metrics to assess nutritional status are available, but factors unique to ALS influence interpretation of commonly used metrics. Change in weight or BMI as a measure of caloric expenditure is complicated by predicted weight loss from muscle atrophy caused by LMN degeneration and weight gain caused by pedal edema from leg weakness and immobility (Kasarkis et al., 2014). Nutritional needs in ALS are also difficult to determine because caloric needs can be reduced due to less energy expenditure from muscle weakness, but also increased from muscle stiffness from spasticity, and some patients are in a hypermetabolic state (Desport, Torny, Lacoste, Preux, & Couratier, 2005).

Total daily energy expenditure (TDEE) can be estimated by a number of methods, including determination of the basal or resting metabolic rate, use of several empiric equations based on body metrics and gender, or directly measured by doubly labeled water. Basal metabolic rate is assessed immediately on wakening after 8 hours sleep and 12 hours of fasting, and is performed in a hospital or laboratory setting. Resting metabolic rate is a less stringent and a more practical proxy for basal metabolic rate, and is measured after several hours of rest and fast and no recent exercise. Both measure oxygen taken up and carbon dioxide given off, using a variety of indirect calorimetry instruments, and calculations (with assumptions) are made to arrive at an estimate of calories (kilocalorie) used. Empiric equations using body metrics (height, weight, BMI) and gender have

been generated to simplify approximations to energy needs (e.g., Harris-Benedict and other equations). The most comprehensive method is to use doubly labeled water, which permits assessment of actual energy used over a long time period (days) and can include energy used during daily activities.

A study of TDEE in 10 bedridden ALS subjects on full time tracheal ventilation using the doubly labeled water method showed values that differed from conventional estimates using equations (Harris-Benedict, Dietary Reference Intake, etc; Ichihara et al., 2012). Another study assessed TDEE in 80 ambulatory ALS subjects by the doubly labeled water method and measured energy expended over 10 days in the patient's home environment (Kasarskis et al., 2014). Data were modeled against a large number of variables with the result that for patients with ALS the following equation accurately predicts TDEE:

- TDEE (kcal/day) = Harris-Benedict resting metabolic rate + (55.96 × ALS Functional Rating Scale [ALSFRS]-6)–168
- For men:
  TDEE (kcal/day) = 66 + (13.7 × weight in kg) + (5 × height in cm) – (6.76 × age in years) + (55.96 × ALSFRS-6) – 168
- For women:
  TDEE (kcal/day) = 655 + (9.6 × weight in kg) + (1.8 × height in cm) – (4.7 × age in years) + (55.96 × ALSFRS-6) – 168.

To facilitate calculations, a web-based calculator has been developed:

https://mednet.mc.uky.edu/aslcalculator/

METABOLIC RATES

Metabolic needs in ALS are complex, and likely vary among patients. An important finding is that patients may be in a hypermetabolic state. In a study of 61 patients with ALS in whom resting metabolic energy expenditure was measured at 6-month intervals for up to 2 years (or until death or incapacitation), nearly 50% were in a hypermetabolic state (Bouteloup et al., 2009). Most patients did not change their metabolic requirements over time, but among the 20% who did the changes were from hypermetabolic to normometabolic or vice versa. No clinical features of ALS or clinical metrics were identified that clearly correlated with or predicted those in the hypermetabolic state. A study of 11 patients with ALS from 10 families with history of ALS (none with superoxide dismutase mutations) found that all patients with a family history had higher resting metabolic rates compared with 52% of patients with sporadic ALS (Funalot, Desport, Sturtz, Camu, & Couratier, 2009).

Reduced nutrition has been shown to be a negative factor for the development of ALS and a negative prognostic factor for those with ALS. In a large population study of more than 500,000 subjects from 10 Western European countries, underweight subjects (BMI <18.5) were at increased risk for developing ALS, especially for women (Gallo et al., 2013). In a study of 55 patients with ALS in whom malnutrition was assessed by a BMI less than 18.5 malnutrition was diagnosed in 16% and survival was significantly shorter in the malnourished group (Funalot et al., 2009).

SEROLOGIC NUTRITIONAL COMPONENTS

Lipid level has been reported to be elevated in patients with ALS compared with control subjects, and higher levels are associated with better survival in a study of 369 French patients with ALS (Dupuis et al., 2008), but not confirmed in a study of 658 Italian patients with ALS (Chio et al., 2009). The effect of the use of statins among patients with ALS has been investigated, and although there were early reports of increased rate of functional decline (ALSFRS-R; Zinman, Sadeghi, Gawel, Patton, & Kiss, 2008), a meta-analysis of case controlled studies investigating the role of lipids revealed only two case controlled studies and one retrospective cohort study (Zheng, Sheng, & Shang, 2013). No significant differences were found in the incidence of ALS and statin use and none with prolonged survival.

Serologic factors have been investigated in ALS, showing increases in ferritin (Nadjar et al., 2012) and decreased uric acid levels (Zoccolella et al., 2011). Urine factors have also

been investigated, with normal urine values of arsenic, lead, mercury, cadmium, thallium, cobalt, and aluminum (Qureshi, Brown Jr, Rogers, & Cudkowicz, 2008).

NUTRITIONAL INTERVENTIONS

Adequate fluid and nutrition can be maintained by dietary supplementation, and when not effective, by gastric feeding tubes.

Nutritional Supplements

In the setting of ALS, progressive weight loss usually has a component of dysphagia, which includes fatigability from swallowing efforts. Nutritional supplements are directed to high-calorie products that are easy to swallow. Although any thick liquid facilitates swallowing, high calories per unit volume are achieved most easily with commercial feeding products that have 1–1.5 kcal/ml and include a balanced formula.

In line with higher lipids as possible favorable factors, there is interest in supplementation with high calories from fats, but with small numbers of subjects and for short periods of time (Paganoni & Wills, 2013). One study of 16 patients with El Escorial Criteria definite ALS were randomized to receive a control diet or one containing 70% mild protein and 30% starch, and were followed for 16 weeks (Silva et al., 2010). A large number of body and nutritional measurements were made, and the control group lost on average 1 kg, whereas the treatment group gained 1 kg (changes in both groups statistically significant).

Gastric Feeding Tubes

The American Academy of Neurology practice parameter and the European Federation of Neurological Sciences guidelines outline clinical trigger points to discuss and recommend feeding tube placement (Miller et al., 1999; Andersen et al., 2005). The timing of feeding tube placement has two issues to consider: safety of the procedure and optimizing nutrition. The general recommendation is to perform gastric feeding tube placement before the forced vital capacity is less than 50% of predicted to reduce the incidence of side effects, but data are minimal for this limiting number. Gastric feeding tube placement by the percutaneous endoscopic gastroscopy (PEG) technique can be performed with low forced vital capacity values when concurrent noninvasive ventilation is used. A prospective series of 30 patients with ALS with forced vital capacity values of 35% (± 18%), 25 of whom were using noninvasive ventilation regularly, included 27 who had feeding tubes successfully placed (Sancho et al., 2010).

Timing for nutritional needs includes a number of factors, many of which are interrelated:

- Degree of bulbar dysfunction (degree of dysphagia)
- Weight loss (approximately 10% loss from premorbid weight)
- Fatigue of eating
- Prolonged feeding time
- Upper extremity weakness affecting food delivery

Gastric feeding tubes can be placed by several techniques:

- Endoscopy (PEG)
- Fluoroscopy (radiologic inserted gastrostomy [RIG])
- Open surgery

PEG and RIG are performed under conscious sedation and the open surgical procedure under general anesthesia. A variety of physiologic patient factors can influence which technique to choose (Stavroulakis, Walsh, Shaw, & McDermott, 2013). A comparison of success rate and complications among 100 patients with ALS receiving gastric feeding tubes showed placement failure rate of 9 of 57 (16%) with PEG, with subsequent successful placement by RIG, and 1 of 51 (2%) with RIG (Allen et al., 2013). Postoperative aspiration was more frequent with the PEG technique.

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Chapter 24

Communication Assessment and Management

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Speech dysfunction occurs in more than 80% of patients with motor neuron disease (MND) and communication becomes challenging for patients and caregivers. A variety of communication aids are available, from simple to complex electronic devices, and the variety has expanded as computer devices have evolved.

It is important to offer augmentative and alternative communication devices with early speech changes and to review the spectrum of devices as further speech changes occur. Patient choice for devices varies, and professional support with ongoing patient training is essential to optimize use, especially with eye-tracking systems. The challenges related to frontotemporal dysfunction on the ability to use communication devices has not been determined. Brain-computer interface devices are attractive but not well developed for amyotrophic lateral sclerosis (ALS).

INTRODUCTION

Speech is an intrinsic element of human interaction and under normal circumstances is effortless. It is used for needs and wants, information transfer, social closeness, and social etiquette (Murphy, 2004). A progressive speech disturbance occurs in more than 80% of patients with MND and ranges from mild impediments to anarthria. It may occur early with bulbar-onset ALS, or late in the course with limb onset. Both upper motor neuron (UMN) and lower motor neuron (LMN) loss contributes to dysarthria, and thus speech disturbances occur across the spectrum of MND (Table 24–1). Communication can perhaps be viewed from two perspectives: one for basic body needs and comfort, and the other for communication of abstract thoughts and feelings. Both are essential, and it might be envisioned that the latter is especially important to express feelings related to an inexorably progressive disorder. Complicating the latter perspective are elements of frontotemporal lobe

Table 24–1 **Speech Features Dependent on Loss of Upper or Lower Motor Neurons, but Most Patients Have a Degree of Loss of Both Neurons**

Speech Components	Upper Motor Neuron Loss	Lower Motor Neuron Loss
Quality	Strained/strangled	Breathy/hoarse
Resonance	Hypernasality	Nasal air emissions
Pitch	Low pitch	Pitch breaks
Prosody	Short phrasing	Slow speech
Intensity	Variable	Decreased
Respiration	Reduced breath control	Reduced breath support

Adapted from Brownlee and Palovcak (2007).

dysfunction that is characterized by reduced verbal output, which occurs in 50% of patients.

SPEECH PRODUCTION AND DYSARTHRIA

Dysarthria can be of the flaccid type caused by LMN loss (tongue atrophy, nasal emission of air, slurred speech) or spastic type caused by UMN loss (no tongue atrophy, slow tongue movements, strained and slow speech), but most patients exhibit a mixed flaccid-spastic pattern that can change over time with changing degrees of LMN and UMN losses (Table 24–1; Tomik & Guiloff, 2010). Other cranial nerves may be involved, such as lower facial muscles for lip control (cranial nerve VII) and the vagus nerve (cranial nerve X) for palate elevation, vocal cord movements, and laryngeal movements. Respiratory control is another factor affecting speech in MND, and patients may adjust sentence length based on reduced respiratory reserve.

SPEECH ASSESSMENT SCALES

Speech assessment scales are available to assess specific aspects of speech (Ball, Willis, Beukelman, & Pattee, 2001). However, changes in speech in ALS are readily detected, first by the patient, and shortly thereafter by the family. Patients initially describe the need to concentrate more and to work harder to speak. Speech fatigue is factor in clarity, and there may be days of good speech intermixed with days of poor speech. When verbal output falls from a normal rate of 200–250 words per minute to 100–125 words per minute intelligibility is reduced, and intelligibility falls rapidly with further slowing. The site of ALS onset is less a reliable predictor of when and severity of altered speech, but patients with bulbar onset experience impaired speech earlier than those with limb onset.

SPEECH EXERCISES

Speech exercises are generally not effective in improving altered speech from ALS (Beukelman, Fager, & Nordness, 2011; Korner et al., 2013). However, recommendations for compensatory communication strategies are helpful.

COMPENSATORY SPEECH STRATEGIES

Suggestions to improve verbal communication include the following (Brownlee & Palovcak, 2007):

1. Communication strategies: negotiate for having the listener try to complete sentences to reduce the burden on the patient, or have the patient complete them if they wish; negotiate to have the listener repeat the last word comprehended to prevent fatigue from repeating.
2. Limit distractions and optimize setting: communicate face-to-face; sit away from distractions (isolated from other conversations) and reduce extraneous sound distractions (television, radios).
3. Communication timing: for communicating personal issues schedule times early before

the patient tires from communicating common issues; for social communication in groups minimize communication earlier in the day or schedule a rest period.
4. Speech rate: slow speech rate to reduce slurring.

Patients and caregivers generally rate communication effectiveness similarly (Ball, Beukelman, & Pattee, 2004). When intelligibility is poor there is frequently marked frustration for both parties.

PALATAL LIFT AND AUGMENTATION

Intraoral prosthetic devices have been used to augment speech in patients with ALS. A palatal lift, which raises the soft palate to reduce nasal emission and improve resonance, or combined with a palatal augmentation device, which reduces the space between the dorsum of the tongue and the hard palate, have been evaluated in a series of 25 patients with ALS (Esposito, Mitsumoto, & Shanks, 2000). Twenty-one patients found benefit, but two found it uncomfortable and did not use the device. Among the 19 who initially used a lift, 10 progressed to needing an augmentation component. Duration of use was affected by disease progression, and most benefited for 6 months, but three benefited for 2 years. The greatest effect was with patients who had predominant UMN dysfunction.

AUGMENTATIVE AND ALTERNATIVE COMMUNICATION

Augmentative and alternative communication is defined as any mode of communication other than speech. Augmentative refers to supplementing impaired speech, and alternative refers to reliance on another method of speech. A range of devices is available (Box 24.1) and patients may benefit from different devices over time as speech changes: augmentation for simple communication, and alternatives in the setting of speech fatigue to alternative devices as speech fails. It is advantageous to offer augmentative and alternative communication devices early in the progression of speech deterioration to maximize benefit. For high-technology devices, there is a high cost and a time period for insurance authorization, and also a time period for training.

Patient acceptance of augmentative and alternative communication varies (Beukelman et al., 2011). Success is influenced by patient physical limitations (degree of arm and hand weakness for typing and using a mouse), cognitive status (frontotemporal lobe dysfunction), environment (willingness of speech pathologist and caregiver to help), and motivation (both patient and caregiver). Eye-tracking systems have the potential to be the most useful for communication but require extensive training and ongoing support from both communication specialists and family members (Binger et al., 2012).

IMPACT OF COMMUNICATION DEVICES

Health-related quality of life of patients with ALS with dysarthria is enhanced by the use of communication devices (Korner et al., 2013). Use of devices varies among patients. Needs for and subsequent use of communication devices depends on many factors, including the degree of dysarthria and situational needs. Among 15 patients with ALS, a key feature to reduce tension and frustration between patient and caregiver was the development of communication strategies (Murphy, 2004). These include analysis by the patient and caregiver of specific blocks to understanding a word or concept and use of nonverbal signs. A study of 26 patients with ALS and 34 associated caregivers used a "communication device use checklist" to assess usage (Fried-Oken et al., 2006). Devices included a range of moderate-technology devices (text-to-speech). The highest uses were for getting needs met, clarifying needs, and giving instructions; lowest uses were for casual conversations. Another study of patients with ALS using an eye-gaze system showed that they used it to good effect, but note is made that the survey was among patients actively using the system (Caligari, Godi, Guglielmetti, Franchignoni, & Nardone, 2013). Another survey found a low percentage of patients with ALS who used communication devices, and rated them as "medium" for usefulness (Gruis, Wren, & Huggins, 2011).

Box 24.1 Augmentative and Alternative Communication Interventions and Devices

- Speech therapy and exercises
  Not considered effective
- Speech environment strategies
  Effective at reducing competing sounds
- Patient and family coping
  Natural process; successful
  Family familiarity with speech
  Gesturing
- Palatal lift
  Limited data
- Low technology
  Alphabet and picture boards
  Alerting systems
- Medium technology
  Microphone-amplifiers
  Voice recognition software
  Touch screen and mouse typing
- High technology
  Eye-gaze tying
  Brain-computer interface

It is likely that the successful impact of communication devices depends on training and ongoing support. Thus, it is important in the clinic to help the patient and caregiver to acknowledge communication frustrations and introduce to them the idea of communication strategies, which evolve over time from simple to technical.

BRAIN-COMPUTER INTERFACE

Brain-computer interfaces take information directly from the brain, which obviates the need for movements from the subject, and can be used to spell messages, operate computers, and control the environment (Wolpaw, 2013). A survey of 61 patients with ALS was conducted to determine what features in a brain-computer interface would be desirable (Huggins, Wren, & Gruis, 2011). Among the features queried, 84% expressed preference for an electrode cap but 72% were interested in surgically implanted electrodes; training sessions ideally were to be short and setup time to use the instrument low at 30 minutes; speed of letter selection was idealized at 15–25+ per minute with an accuracy of 90%.

Brain activity is commonly monitored by encephalography using scalp electrodes in standardized positions, and the most common method is based on event-related potentials, although other methods are available (Cipresso et al., 2012). The P300 evoked potential occurs 200–700 milliseconds after a stimulus: a grid of characters on a screen is presented and then the desired character is located by the subject, a P300 potential is generated, and several trials are necessary to obtain an averaged potential out of background encephalography noise. Accuracy is high at 90%.

Although most studies demonstrate positive use of brain-computer interfaces in ALS, one

study assessed the ability to communicate over time (Silvoni et al., 2013). A small number of patients were studied yearly for up to 3 years and there was no loss of ability to communicate using activation of P300-based cursor control of icons with progression of ALS as measured by the ALS Functional Rating Scale-Revised.

FRONTOTEMPORAL LOBE DYSFUNCTION EFFECT ON COMMUNICATION

Cognitive impairment in ALS as it relates to language dysfunction is complex and its complexity has not been fully sorted out (Bak & Hodges, 2004). Verbal fluency is impaired as a component of frontotemporal lobe dysfunction, but its impact on the ability for social communication is not known, and likely varies with the degree of frontotemporal lobe and bulbar dysfunction. Early writing errors have been noted (Ichikawa et al., 2008). Comprehension is much less impaired.

Impaired communication cannot be solely attributed to executive dysfunction because negative scores with executive dysfunction (naming, sentence completing tests, spatial anticipation test, card sorting test) overlap with scores of language dysfunction (confrontational naming, comprehension, verb processing) by 44% among patients with ALS (Taylor et al., 2013). The impact of cognitive impairment on the use of brain-computer interfaces is complex and has not been fully studied (Cipresso et al., 2012).

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Chapter 25

Psychological and Psychiatric Assessment and Management

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Studies of psychological and psychiatric symptoms in amyotrophic lateral sclerosis (ALS) focused on the incidence of depressive symptoms, which are relatively low. With the awareness that a frontotemporal syndrome and dementia can be present in 50% of patients with ALS, there is recognition that behavioral symptoms can have an impact on the caregiver. However, there are few studies of how to manage them.

INTRODUCTION

Given the inexorable progression of motor neuron disease, depression was a consideration that could affect quality of life for the patient and caregiver, and could be treated. The finding that frontotemporal syndrome and dementia are present in up to 50% of patients with ALS, and commonly includes behavioral symptoms, expands the psychological and psychiatric spectrum. Furthermore, certain forms of familial ALS can include family members with only psychiatric disturbances. At this point, investigations are descriptive with little information on treatment.

DEPRESSION

A review of the literature to 2007 on the psychological health of patients with ALS revealed the following (Averill, Kasarskis, & Segerstrom, 2007). Depression, based on reports from structured interviews, was estimated at an incidence of 5.5% among patients with ALS, and the level of depression was estimated from standardized tests (Beck Depression Inventory, Hospital Anxiety and Depression Scale) and was found to be in the minimal range. The feeling of hopelessness, defined as a belief that the future holds no possibility for positive events, emotions, or outcomes, was reported to have a wide range (absent to severe), and moderate to severe hopelessness was expressed by 20–30% of patients. The progressive nature of ALS suggests an accumulative psychological burden. However, most studies did not find a progression of depression or hopelessness over time. Overall, this literature review supports the notion that the features of ALS do not in general determine the psychological state of

the patient, and a positive meaning to life can be found by the patient.

Management and treatment of depression in ALS is by counseling and medications. For some patients with ALS, fear of the unknown future is a factor in reduced mood, and frank discussions about progression and manner of death can be helpful. Standard use of antidepressant medications may be helpful.

BEHAVIORAL SYMPTOMS

Changes in behavior are a common component of frontotemporal dysfunction (behavioral variant) in ALS (Strong, 2008), and also primary lateral sclerosis (Grace et al., 2011). They include alternations in personality and social conduct. They can be categorized as apathetic type with apathy, inertia, and loss of volition; stereotypic type with ritualistic behavior and conformity to routines; and disinhibition type with overactivity, disinhibition, and distractibility. Symptoms are progressive over time. Patients may not fulfill formal criteria for frontotemporal dementia.

Management and treatment of behavioral symptoms can be difficult in the setting of ALS. This fact results in great stress on the caregiver (Lillo, Mioshi, & Hodges., 2012; Houseman et al., 2013). Physical exercise for the patient can be beneficial but difficult in the setting of weakness. Pharmacologic management focuses on neurotransmitter involvement: serotonin selective uptake inhibitors may help with disinhibition, impulsivity, and repetitive behaviors. Patients may be more susceptible to extrapyramidal side effects of antipsychotic medications and use of medications with less D_2 receptor antagonism is preferable (Pressman & Miller, 2014). Without specific medication trials, caregiver awareness that behavioral symptoms are under patient control may be helpful in reducing some of the stress (Merrilees, Klapper, Murphy, Lomen-Hoerth, & Miller, 2010).

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Chapter 26

Symptoms and Management

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Amyotrophic lateral sclerosis (ALS) is primarily a disease involving loss of upper motor neurons (UMNs) and lower motor neuron (LMNs), resulting in weakness and spasticity as the main motor symptoms. However, there are secondary symptoms related to these features and to consequences of infirmity. There is also recognition of behavioral issues related to frontotemporal lobe dysfunction.

With few randomized controlled trials involving patients with ALS for symptoms management, recommendations are almost entirely based on experience with similar symptoms in other diseases.

INTRODUCTION

Patients with ALS describe a range of symptoms, but robust patient surveys on the number, frequency, and severity of symptoms are not available. One study of treatment efficacy set forth 14 common symptoms in ALS based on clinical experience, but additional symptoms are encountered (Table 26–1; Forshew & Bromberg, 2003). Another approach to assess frequency and severity is to query patient experiences from PatientsLikeMe, a voluntary online Internet-based ALS patient forum (PatientsLikeMe.com). From such a query, 33–51% of patients experience most of the symptoms listed in Table 26–1 (Nakamura, Bromberg, Bhargava, Wicks, & Zeng-Treitler, 2012).

Symptoms described by patients with ALS caused by infirmity are not unique to ALS. With few randomized trials for symptom management in ALS, most information on efficacy is from trials conducted in other diseases or from clinical experience. Treatment efficacy has been assessed from both the clinician's and the patient's perspectives (Forshew & Bromberg, 2003; Nakamura et al., 2012), and

Table 26–1 **List of Common Symptoms and Treatment Options**

Symptom	Treatment
Behavioral disturbance	Understanding
	SSRIs (common dosages)
Pseudobulbar affect	Amitriptyline (10–80 mg)
Severe 5%; moderate 19%; mild 37%	Nuedexta (dextromethorphan, 20 mg/quinidine, 10 mg)
Yawning	
Severe 4%; moderate 19%; mild 37%	
Anxiety	Reassurance
Severe 5%; moderate 19%; mild 38%	Benzodiazepines: lorazepam (1–2 mg), valium (2–5 mg)
	SSRIs (common dosages)
Depression	SSRIs (common dosages)
Severe 5%; moderate 18%; mild 38%	
Weakness	Energy conservation
	Walking aids (sticks, walkers), DME (wheelchairs)
Shortness of breath	Noninvasive ventilation
	Lorazepam (1–2 mg), morphine sulfate (1 mg +)
Fatigue	Energy conservation
Severe 15%; moderate 40%; mild 34%	Walking aids (sticks, walkers), DME (wheelchairs)
Fasciculations	Gabapentin (300 mg +)
Severe 9%; moderate 35%; mild 42%	
Spasticity, spasms	Baclofen (20–80 mg)
Severe 13%; moderate 34%; mild 37%	Tizanidine (4 mg +)
	Benzodiazepines (5 mg +)
Muscle cramps	Muscle stretching
	Quinine sulfate (325 mg)
	Baclofen (20–80 mg)
Sialorrhea	Amitriptyline (25–50 mg)
Severe 10%; moderate 19%; mild 32%	Glycopyrrolate (1–2 mg)
	Scopolamine (patch)
	Botulinum toxin (500 units)
	External beam radiation (20 Gy)
Thick phlegm	Guaifenesin (200 mg +)
Laryngospasm	Counseling
	Benzodiazepines: lorazepam (1–2 mg), valium (2–5 mg)
Pain	Nonsteroidal anti-inflammatory drugs (common doses)
Severe 6%; moderate 21%; mild 33%	Opioid drugs
	Acetaminophen (325 mg +)
	Physical therapy
Disrupted sleep	Sleep hygiene
Severe 5%; moderate 19%; mild 31%	Noninvasive ventilation
	Trazodone (25–50 mg)
	Zolpedim (5–10 mg)
Constipation	Fiber
Severe 5%; moderate 19%; mild 38%	Hydration
	Stool softeners
Urinary and bowel urgency	Oxybutynin (5 –15 mg)
Dependent limb symptoms	Limb elevation
	Compression gloves and hose
Deep venous thrombosis	Aspirin (325 mg)
	Warfarin

Percentages of severity for some symptoms from PatientsLikeMe self-reporting (approximately 3,200 patients). Order of treatment modalities from PatientsLikeMe self-reporting and from the literature.
DME = durable medical equipment; SSRI = selective serotonin reuptake inhibitor.

of note, estimates of drug efficacy made by clinicians are more often positive and higher in magnitude than estimates made by patients.

BEHAVIORAL DISTURBANCE

Changes in ALS patient behavior are common and are related to the frontotemporal lobe syndrome and dementia. Symptoms include irritability, executive deficits, apathy, loss of insight, disinhibition, and aggressive behavior.

From one study, 81 ALS caregivers reported symptoms of reduced motivation (81%), apathy (41%), and symptoms consistent with dementia (11%; Lillo, Mioshi, Zoing, Kiernan, & Hodges, 2011). No drug treatment trials for behavioral changes are available for ALS, and drugs suggested for specific symptoms are by categories (selective serotonin reuptake inhibitors, atypical antipsychotics, *N*-methyl-D-aspartate agonists). Another approach suggested for the caregiver is an understanding and acceptance of behavioral issues (Merrilees, Klapper, Murphy, Lomen-Hoerth, & Miller, 2010; Seltman & Matthews, 2012).

PSEUDOBULBAR AFFECT

Pseudobulbar affect is common in patients with ALS and can be a source of embarrassment for both patient and caregiver. The presence of pseudobulbar affect and quantitative measure of its magnitude can be assessed by a survey instrument (Center for Neurologic Study-Lability Scale; Moore, Gresham, Bromberg, Kasarkis, & Smith, 1997).

Using this instrument, a combination of dextromethorphan, 20 mg, and quinidine, 10 mg orally twice daily, has been shown to be effective at reducing laughing and crying (Brooks et al., 2004; Pioro et al., 2010). Dextromethorphan is the active drug and quinidine reduces its rapid metabolism. Side effects are dizziness and nausea, which may limit tolerability but there are no cardiac issues. The drug combination has been approved by the Food and Drug Administration for pseudobulbar affect, under the brand name Nuedexta.

Amitriptyline has also been used for pseudobulbar affect, supported by empiric data and a single older study using a dose range of 30–100 mg (mean, 64 mg) with good effect in 20 of 22 patients after 6 weeks (Szczudlik, Slowik, & Tomik, 1995). From PatientsLikeMe data, 21% of patients believed amitriptyline was effective (Nakamura et al., 2012).

EXCESSIVE YAWNING

Patients with ALS often describe excessive and forced yawning. Based on a survey from PatientsLikeMe, 60% reported mild-moderate yawning and 9% severe yawning (Wicks, 2007). The mechanism is believed to represent UMN pathology. Most patients do not seek treatment, but patients have responded to thioridazine (Williams, 2000).

ANXIETY AND DEPRESSION

Anxiety in ALS may derive from concerns about the future, concerns about dying, and shortness of breath. Addressing questions and concerns about specific issues may help relieve symptoms.

There are no controlled trials of anxiolytic drugs in ALS. Pharmacologic treatment should be individualized, based on patient circumstances with consideration for premorbid anxiety and whether a short-term versus a long-term antianxiety medication is appropriate. In the setting of respiratory compromise from ALS there is some concern for oversedation. From PatientsLikeMe data, 27% of patients believed lorazepam was effective (Nakamura et al., 2012).

Depression in ALS occurs with low frequency (15–20%), and consideration should be given to circumstances related to ALS, and also to premorbid mood conditions. There are no controlled trials of antidepressant drugs in ALS. Selective serotonin reuptake inhibitors are appropriate. From PatientsLikeMe data, 17% of patients believed sertraline was effective and 9% believed fluoxetine was effective (Nakamura et al., 2012).

WEAKNESS

Weakness in ALS is primarily caused by loss of LMNs, but spasticity and stiffness from

UMN loss also contributes. The drug tirasemtiv enhances the sensitivity of sarcomers to calcium and increases force generation, and is currently being tested in a pilot study in patients with ALS (Shefner, Wolff, & Meng, 2013; Shefner, Watson, Meng, & Wolff, 2013). Because the drug improves muscle strength, it could result in a degree of global improvement in function. Data from several small and short trials (3-week duration) were pooled: with oral doses up to 500 mg there were trends toward improved ALS Functional Rating Scale (ALSFRS) scores and handgrip endurance (Shefner, Wolff, & Meng, 2013). Dizziness was the most common side effect. Interestingly, when tirasemtiv was taken in combination with riluzole, serum level of riluzole rose by a factor of 2.5.

SHORTNESS OF BREATH

Shortness of breath eventually occurs in most patients with ALS because of progressive respiratory failure. The American Academy of Neurology (AAN) Practice Parameter Guideline offers a respiratory symptom management algorithm that focuses on periodic assessment of respiratory function by pulmonary function tests and inquiry of symptoms. When acute and reversible factors, such as secretion management and pneumonia, have been assessed, long-term management is by use of noninvasive ventilation (Miller et al., 1999).

There are situations where shortness of breath is transient, lasting minutes and not associated with exertion or accountable by other factors, that are likely caused by anxiety. Reassurance can be helpful. Directing cool air from a fan onto the patient can also help. Low doses of lorazepam (0.5–2 mg sublingually) can be effective.

Shortness of breath caused by respiratory insufficiency that cannot be managed by noninvasive mechanical ventilation, or if the patient cannot tolerate the device, or wishes not to use it, needs to be managed by medications. The AAN Practice Parameter Guideline suggests lorazepam, 0.5–2 mg sublingually, or morphine, 2.5–5 mg sublingually (Miller et al., 1999). Shortness of breath during the terminal stages of ALS should be managed by hospice and palliative care.

FATIGUE

Fatigue is a common symptom expressed by patients with ALS and may have multiple contributing factors, including changes in psychological drive, changes in central drive caused by premotor cortex changes, loss of UMNs resulting in changes in LMN activation patterns, loss of LMNs, and changes in excitation-contraction coupling (Thomas & Zijdewind, 2006; Lou, 2008). It is not possible to distinguish among these factors for an individual patient.

In one study of 223 patients surveyed at 3-month intervals, fatigue, as measured by the Fatigue Severity Scale, was found in the clinically significant range in 44% at first visit and in an additional 22% at 3 months later (McElhiney, Rabkin, Gordon, Goetz, & Mitsumoto, 2009). Fatigue was correlated with greater weakness, as measured by lower ALSFRS-R scores, whereas depression was less prevalent and did not increase over time. Other factors include reduced mood and side effects of medications, and poor sleep caused by nocturnal cramps and nocturia (Lo Coco & La Bella, 2012).

A randomized placebo trial of modafinil with an open-label extension showed that with doses up to 300 mg daily there was a significant improvement in the Clinical Global Impression Score and in energy and stamina on visual analogue scales (Rabkin, Gordon, McElhiney, Rabkin, & Chew, 2009).

FASCICULATIONS

Fasciculations are almost ubiquitous, and a key feature in making the diagnosis of ALS. They are sufficiently troublesome in about 5% of patients from PatientsLikeMe to receive therapy (Nakamura et al., 2012). No studies are available on treatment of fasciculation potentials, but from the same PatientsLikeMe survey, 8% of patients believed that gabapentin was helpful.

SPASTICITY AND SPASMS

Spasticity of movements (velocity-dependent increase in muscle tone) in ALS and primary

lateral sclerosis is caused by loss of UMNs leading to several changes that include increased stretch-induced activity from muscle spindles, decreased spinal inhibitory pathways, and increased LMN excitability (although not confirmed in humans; Gracies, 2005). This leads to difficulties in arresting inappropriate muscle activity and leads to limb stiffness during movements. Stiffness affects the legs with poor balance during walking and greater risk of falls.

Spasms reflect uncontrolled and exaggerated responses to peripheral stimuli (cutaneous or stretch of limb muscles) leading to uncontrolled extension or flexion of the legs. In addition to interfering with ambulation and patient care, spasticity and spasms can be painful.

Treatment of spasticity and spasms in ALS includes a spectrum of nonpharmacologic efforts and drugs. A Cochrane Review included assessment of physical therapy, nonpharmacologic applications, prescription and nonprescription medications, nerve and muscle chemoneurolysis, surgical interventions, and alternative therapies (Ashworth, Satkunam, & Deforge, 2012). Only one exercise program compared the effects on spasticity assessed by the Ashworth scale (Drory, Goltsman, Reznik, Mosek, & Korczyn, 2001). Twenty-five patients with ALS were randomized to an exercise program or usual activity, and the Ashworth scale was significantly reduced in the exercise group at 3 months, but not at 6 months. Drop-out was high at 6 months for both groups.

Several drugs are available for spasticity, including baclofen, tizanidine, dantrolene, and benzodiazepines, but there are no controlled trials to guide choice in ALS. A treatment principle is to start with a low dose and increase slowly to reduce or prevent side effects, most commonly fatigue and occasionally weakness caused by reduced limb stiffness from loss of spasticity. A PatientsLikeMe survey indicated that 29% of patients treated by baclofen believed it was effective, whereas 5% of patients believed tizanidine was effective (Nakamura et al., 2012). Intrathecal delivery of baclofen is an approach to delivering the drug at appropriate doses to the spinal cord without systemic side effects. A review of intrathecal baclofen in eight patients with ALS indicated good pain relief in half, but there was no mention of its effects on ambulation (McClelland et al., 2008). A positive response to an intrathecal test dose predicted response after pump implantation, and could also be used to assess and predict its effect on ambulation.

CRAMPS

Muscle cramps, including nocturnal cramps in leg muscles but also with other muscles in the setting of routine activities, such as neck muscles with yawning, abdominal muscles with bending over, and hand and forearm muscles with dressing, are common among patients with ALS, occurring in 35% of patients in a PatientsLikeMe survey (Nakamura et al., 2012). Nocturnal cramps can be severe and frequent and disrupt sleep.

A Cochrane Database review of drug trials in ALS found only one trial with cramp frequency as a primary endpoint and 13 studies as a secondary endpoint, and no study showed a favorable response (Baldinger, Katzberg, & Weber, 2012). Drugs studied included tetrahydrocannablinol (study with cramps as primary endpoint), vitamin E, baclofen, riluzole, L-threonine, xaliproden, indinavir, and memantine (studies with cramps as secondary endpoint).

In a global review of quinine sulfate for cramps, two Class I studies showed a positive effect, whereas several Class II and III studies showed both efficacy and no efficacy (Katzberg, Kahn, & So, 2010). Of note, in 2005 the Food and Drug Administration considered cramps an off-label indication for quinine due to side effects. Among ALS PatientsLikeMe 32% believed that quinine was effective and 27% that baclofen was effective (Nakamura et al., 2012; Katzberg et al., 2010). Preventative stretching exercises have not been adequately assessed (Katzberg et al., 2010).

SIALORRHEA

Sialorrhea in ALS reflects an increased amount of saliva in the mouth caused by reduced clearance from inefficient swallowing (Scully, Limeres, Gleeson, Tomas, & Diz, 2009). Approximately one to one and a half quarts of saliva are normally produced, and clearance requires frequent swallowing, about 600 swallows in a 24-hour period. The submandibular and sublingual glands are responsible for 70%

of saliva production in the unstimulated state, whereas in the stimulated state (chewing) saliva production may increase five-fold and the larger amount is from parotid gland. Thus, accumulation of saliva is expected with even mild dysarthria.

There are no clinical trials of oral drugs to reduce saliva production, but the AAN Practice Parameter has an algorithm based on clinical experience (Miller et al., 1999). Oral drugs commonly used include amitriptyline (12.5–75 mg), glycopyrrolate (1–4 mg), scopolamine patch, and atropine (oral drops 1%). Among ALS PatientsLikeMe, 29% believed amitriptyline was effective and 18% believed glycopyrrolate was effective (Nakamura et al., 2012).

Botulinum toxin has been formally studied in small ALS trials, and a review of 13 studies showed the following (Stokholm, Bisgard, & Vilholm, 2013). Botulinum types A and B were equally effective, but type B is associated with shorter onset latency. Toxin is delivered to parotid and submandibular glands by anatomic landmarks or by ultrasound guidance. Dosages vary among toxin types and brands and are diluted compared with treatment of dystonia. Treatment is believed by patients to be effective, supported by quantitative measurements of saliva. Treatment is temporary, and repeat injections are at 3- to 5-month intervals or on a personalized schedule. Side effects are mild, with some patients reporting thicker saliva and some a dry mouth, and good oral hygiene is emphasized.

External beam radiation to the salivary glands has been used in patients who have not had a good response to pharmacologic or botulinum treatment. The pattern of salivary glands radiated varies; most include the submandibular glands with variable inclusion of the parotic glands, and one study radiated a single side. Doses varied but mean doses were 20 Gy delivered in five fractions (Guy et al., 2011; Kasarskis, Hodskins, & St Clair, 2011; Bourry et al., 2013). Efficacy was judged to be high by patients.

THICK PHLEGM

Patients with ALS frequently describe thick phlegm deep in their throat, but there are no studies on phlegm in ALS. Phlegm represents mucus, likely largely secreted by respiratory epithelial cells (Rubin, 2010). Mucin can form gel-like glycoproteins. Thus, it likely that thick phlegm in patients with ALS represents normal secretions that collect because of inefficient swallowing and a weak cough. Treatment has been empiric, and includes guaifeneson. Assistive cough devices may also be helpful.

LARYNGEAL SPASMS

Laryngospasm represents a feeling that air cannot be moved with respiration and is associated with inspiratory stridor. It is caused by spasm of the laryngeal sphincters (there are three sphincters: aryepiglottic fold, ventricular fold [false vocal cords], and the true vocal folds). It is not clear which are involved in laryngospasm in ALS, but muscle activity does not seem to be caused by spasms from UMN loss because it is more common among patients with spinal bulbar muscle atrophy (Kennedy disease), which has no UMN involvement, than among patients with ALS who have UMN involvement (Sperfeld, Hanemann, Ludolph, & Kassubek, 2005). The cause of laryngeal sphincter tightness is not known but gastroesophageal reflex has been noted. Laryngospasm, although frightening, lasts a brief period of time and is not life threatening, and an understanding of this is important to impart to patients.

PAIN

ALS is not considered an intrinsically painful disorder. However, several patient surveys show that more than 50% of patients with ALS experience pain, occurring at all stages (Chio et al., 2012; Rivera et al., 2013). Causes of pain are not clear and likely varied, but common factors are pain related to immobility and not being able to shift position, joint and muscle contractures related to immobility and not being able to activate a full range of motion, muscle soreness from relative overactivity of weak muscles, and muscle cramps and spasms. The distribution of pain is primarily of the musculoskeletal category and affects most commonly shoulders, neck, and legs.

A Cochrane Database review found no randomized or quasirandomized trials for pain

in ALS (Brettschneider, Kurent, & Ludolph, 2013). The available data on pain management in ALS was reviewed and collated. The musculoskeletal nature of pain in ALS leads to treatment with nonsteroidal anti-inflammatory drugs as the first line (44%), then opioids (40%) and acetaminophen (19%). Conclusions about doses and relative efficacy were not readily extractable from the data.

DISRUPTED SLEEP

Issues with sleep are common among patients with ALS, and from a survey of 91 patients, 57% meet experienced poor sleep on the Pittsburgh Sleep Quality Index (Lo Coco & La Bella, 2012). An interview revealed nocturia occurring in 63%, nocturnal cramps in 45%, difficulty turning in bed in 39%, and snoring in 24%. Restless leg syndrome has also been identified as more common among patients with ALS (25%) than a control group (8%; Lo Coco, Piccoli, & La Bella, 2010).

Treatment includes assessing manageable issues (nocturia) and medications for treatable associated causes (muscle cramps, restless leg syndrome). Medications directed toward sleep have not been assessed formally, but from a PatientsLikeMe survey, amitriptyline was judged to be effective by 34% and zolpidem by 16% (Nakamura et al., 2012). Sleep hygiene is important to emphasize because with immobility patients may nap in the daytime more that realized, taking away from nocturnal sleep time.

CONSTIPATION

Constipation is a common complaint, and in a general review of systems among healthy individuals, 16% overall and 33% among individuals older than age 60 years respond positively (Bharucha, Dorn, & Lembo, 2013). Furthermore, patients may rely on personal definitions of constipation, but the formal definition includes infrequent bowel movements (frequency of bowel movements in the general population varies markedly), hard-to-pass stool, sensations of incomplete evacuation, and straining (Lembo & Camilleri, 2003). There are no objective data on the frequency of constipation among patients with ALS, but one telephone survey of 16 patients unable to attend clinic reported a frequency of "constipation and inability to clear lung secretions" in 61%, increasing to 72% over 6 months (Mannino et al., 2007).

Treatment regimens are not specific for patients with ALS. It is not clear that reduced fluid intake and reduced physical activity *per se* contribute to constipation (Meshkinpour et al., 1998). A graded approach can be used and based on responses to increased fiber intake (foods; psylliym, 25 g daily), osmotic agents (milk of magnesia; polyethylene glycol, 17 g daily), and stimulant laxatives (bisacodyl, senna; Lembo & Camilleri, 2003).

BLADDER AND BOWEL INCONTINENCE

Bladder and bowel incontinence and other symptoms of dysfunction in ALS have received little attention. It is known that the LMNs that innervate the external rectal sphincter (nucleus of Onuf) are mostly spared in ALS and frank incontinence is not common, especially when LMNs from the same spinal segments to leg muscles are markedly reduced (Kihira, Yoshida, Yoshimasu, Wakayama, & Yase, 1997). It is likely that LMNs innervating the bladder sphincter are also spared, because bladder incontinence is not common. A survey of 54 patients with ALS for urinary symptoms indicated that 14% experienced incontinence, and 23% postmicturition dribble (Lopes de Carvalho, Motta, Battaglia, & Brichetto, 2011). Other symptoms included 18% urgency, 27% increased frequency, 50% nocturia, and 60% feeling of incomplete emptying.

There are no objective data on treatment of bowel symptoms in ALS, but reports from PatientsLikeMe indicate that oxybutynin is judged effective by 46% of patients (Nakamura et al., 2012).

URINARY DISORDERS

Urinary symptoms are described by patients with ALS, and a study of 54 patients meeting

definite or probable ALS were queried for urinary symptoms (Lopes de Carvalho et al., 2011). Among the 41% with symptoms experienced after the onset of ALS symptoms, a feeling of incomplete emptying was reported by 59%, nocturia by 50%, increased frequency by 27%, urgency by 18%, and incontinence by 14%. Although postvoid residual volumes were lower among asymptomatic patients, 22% had residual volumes greater than 50 ml (cut-off value for normal voiding). There were significant linear correlations between postvoid residual volumes and the ALSFRS-R (negative) and Ashworth spasticity (positive) scores. Treatment and management were not addressed in detail, but antispasticity for urgency and intermittent catheterization for residual volumes were mentioned.

DEPENDENT LIMB SYMPTOMS

Severe limb weakness leads to immobility and dependent limb postures. This causes hands and distal legs to become cold to the touch, very red in appearance (including a purple hue), swollen, and occasionally painful. Although many of these symptoms (rubor, tumor, and dolor) are associated with inflammation or vascular insufficiency, they are a normal response in ALS to reduced blood flow to atrophic distal limb muscles (coldness), lack of lymph fluid movement (swelling), and cutaneous capillary engorgement (redness).

Treatment is to elevate the limb or wear pressure hose or gloves, but the symptoms are not of medical concern and reassurance is appropriate.

DEEP VENOUS THROMBOSIS

All forms of motor neuron disease are associated with reduced mobility and the possibility of developing deep venous thrombosis as a consequence. In a study of 438 patients with ALS over a 4-year period, 3% experienced venous thrombosis (Elman, Siderowf, Houseman, Kelley, & McCluskey, 2005). Lack of ambulation was believed to be a major risk factor (five times more common than in ambulatory patients). The incidence of thrombosis in ALS was lower than in patients with stroke and spinal cord injuries but five times higher than in the general older population. Thrombosis prophylaxis was not believed to be necessary.

SKIN BREAKDOWN

Skin breakdown leading to pressure ulcers seems to be less common in patients with ALS than expected given the accentuation of boney prominences caused by loss of muscle combined with reduced subcutaneous fat from weight loss in the setting of immobility. Charcot noted this paradox in patients with ALS compared with those with other debilitating diseases. This does not mean that patients with ALS are immune to pressure ulcers, and a review of patients admitted to a single hospital revealed a 5% rate of skin breakdown among patients with ALS, which was similar to patients with other neurologic diseases, although there were unusual factors in the cases (Hayashi et al., 2007).

SWEATING

Patients occasionally describe excess sweating. In a quantitative study of sweat production at distal arm and leg sites, 39 patients with El Escorial Criteria definite to probable ALS were compared with 39 control subjects (Beck et al., 2002). Subjects with ALS who were relatively early in the course of their disease had higher rates of sweat production than control subjects in the hands (but lower in the feet), but those later in the course had lower rates. It is not clear if the findings early in the course explain the occasional patient with marked sweating about the head or burning of the eyes.

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Chapter 27

Intimacy, Sexuality, and Pregnancy

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Intimacy and sexuality are essential human issues. Most patients with motor neuron disease (MND) are cared for by spouses. The progressive physical dependence for care can be rewarding for the patient and caregiver, or taxing and affect intimacy. Premorbid coping skills and the spousal relationship, combined with ongoing patient physical limitations and emotional comfort with their changing bodies, affects intimacy and sexuality.

Despite its importance, little is written about intimate interactions between patients with MND and caregivers. Sexual activity occurs with patients with amyotrophic lateral sclerosis (ALS), including those on long-term mechanical ventilation. The topic is not often brought up at clinic visits, leaving patients and caregivers uncertain as to whom to discuss issues with. Women with ALS have conceived and given birth without significant obstetric issues.

INTRODUCTION

The progressive need in MND for more help with personal care, most often delivered by the spouse, suggests a possible parallel increase in intimacy that can be viewed as a positive aspect of the disease. However, balancing possible positivity are issues for the patient, related to how they feel about changes in their body, and for the caregiver, related to fatigue from physical and temporal demands on their time and difficulties related to possible changes in behavior of the patient. Furthermore, physical intimacy in a relationship naturally changes over time, and most patients with ALS are older. Other factors are past coping skills of the individuals and past interactions as a couple.

INTIMACY

MND affect all aspects of intimacy and sexuality for both the patient and caregiver. The issue of intimacy and sexuality in the setting of MND has not been studied extensively, and there is little discussion of issues and how to handle them in clinic.

A broad survey of intimacy using the Morris Intimacy Scale, which includes questions on affection, cohesion, expressiveness, compatibility, conflict resolution, sexuality, autonomy and identity, and global satisfaction with the relationship, was given to 50 patients with ALS

and their spouses (Atkins, Brown, Leigh, & Goldstein, 2010). Morris Intimacy Scale scores were similar when estimated for the time before the onset of ALS, with small drops in scores over approximately 2 years of serial evaluations. Note is made that 80% of the patients with ALS were male, and the caregivers' scores were lower, but not significantly so, at the last evaluation.

SEXUALITY

Sexuality and sexual activity are tightly linked to intimacy. Although the frequency of sexual activities varies among healthy couples and over time in the relationship, and most ALS couples are older and have been married for a long time, there are likely additional changes related to MND.

In a survey of 26 couples (three partners and seven single patients), one of whom had ALS, sexuality was found to be an important issue for both individuals in the relationships (Wasner, Bold, Vollmer, & Borasio, 2004). Over the course of ALS, ranging from 3 to 218 months (mean, 30 months), there was a decline in physical function among the patients as measured by the ALS Functional Rating Scale score, which included weak patients (mean score, 28; range, 3–46). Physical weakness was the most common factor linked to sexual problems. There was a decline in interest in sexuality for both individuals, from about 75% being initially "very interested" before ALS onset to 44% at time of the survey. Satisfaction with their sex life declined for both individuals, from an initial 73% in the patients and 66% in the caregivers, to 44% for both at the end of the survey. It was noted that 60% of patients and 44% of caregivers felt an improvement in their overall relationship since disease onset, and 20% of patients and 11% of caregivers also noted an improvement in their sexual relationships. There was a smaller decline in having sexual intercourse at least once a month, from 94% of patients and 100% of caregivers, to 76% of patients and 79% of caregivers. It is also noteworthy that despite obvious physical and mechanical issues associated with invasive ventilation, five of six patients reported having sexual intercourse at least once per month. Issues associated with sexuality for the patient were fear of rejection, fear of not satisfying their partner, their own passivity, and decreased libido; for the caregiver the issues were their partner's attitude, their attitude, changes in the patient's body, and their decreased libido.

From the same study, only 13% reported having been asked about sexual issues by their physicians (type of physician not stated). Related to this issue was uncertainty among the individuals as to who was the appropriate provider to consult.

Sexual activity has been queried among patients using ventilation (Kaub-Wittemer, Steinbuchel, Wasner, Laier-Groeneveld, & Borasio, 2003). Sexual activity was more important for patients using noninvasive ventilation (56%) and long-term mechanical ventilation (43%), than for the caregivers (41% and 20%, respectively). Sexual activity was higher among couples in the setting of noninvasive ventilation (31%) than in the setting of long-term mechanical ventilation (14%).

PREGNANCY WITH ALS

Pregnancy implies sexual activity plus issues with carrying and delivering the child. Several female patients with ALS who conceived and delivered babies have been reported (Chio et al., 2003; Sarafov et al., 2009; Sobrino-Bonilla, 2004). Issues with and methods of delivery depended on overall strength and respiratory status of the mother, and delivery was vaginal when constitutionally strong and by cesarean section when impaired. No untoward effects on the fetus were reported.

INAPPROPRIATE SEXUAL BEHAVIOR

Inappropriate sexual behavior and inappropriate aspects of sexual drive (timing and unwillingness of the partner) can be part of the frontotemporal lobe syndrome experienced by the patient, but are uncommon elements with frontotemporal lobe syndrome features in ALS (Strong et al., 2009).

One case report describes demands for frequent sexual intercourse by a husband with a

recent diagnosis of ALS and symptoms of frontotemporal lobe syndrome that responded to sertraline (Anneser, Jox, & Borasio, 2007).

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Chapter 28

Quality of Life

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BACKGROUND AND NEW POINTS

Assessing quality of life and attempting to improve it is especially important in the setting of an inexorably progressive disorder. However, quality of life is difficult to define and measure. Early efforts were linked to health-related quality of life instruments.

Several amyotrophic lateral sclerosis (ALS)–specific quality of life instruments have been developed that include existential elements that focus on sense of purpose and meaning in life. Using these instruments, quality of life for patients with ALS has been found to be relatively stable over the course of the disease and independent of the progressive loss of motor function. Quality of life of the caregiver has been measured and found to be somewhat lower than that of the patient.

INTRODUCTION

Quality of life is an essential element for all people. It is hard to define and measure, and there are many scales and instruments available. Quality of life is important for the patient with motor neuron disease (MND), and also for the caregiver, because there are major functional and daily changes for both. At time of diagnosis, patients are concerned about these changes, and commonly ask how disease progression will affect their quality of life.

TYPES OF INSTRUMENTS

Efforts to assess quality of life in MND can be based on the type of instrument used (Bromberg, 2008). Instruments include (1) health-related, (2) disease-specific, (3) open-format or existential-based, and (4) single-item questions. In addition, there are instruments addressing specific areas, such as depression, anxiety, meaning in life and purpose in life (existential items), coping mechanisms, and other areas (Table 28–1). Health-related and disease-specific quality of life instruments are multidimensional because they include several domains addressing physical, social, health, functional, and emotional

Table 28–1 **Commonly Used Quality of Life Instruments in Assessing Patients With ALS**

Health-related	
• Short Form-36	Ware & Sherbourne, 1992
Disease-specific	
• ALS Assessment Questionnaire-40	Jenkinson et al., 1999
• ALS-Specific Quality of Live	Simmons et al., 2006
Open or existential	
• Schedule for the Evaluation of the Individual Quality of Life-Direct Weighting	Clarke et al., 2001
• McGill Quality of Life Questionnaire	Cohen et al., 1995
• ALS-Specific Quality of Life	Simmons et al., 2006
Single item	
• McGill Single Item	Cohen et al., 1995
Item-specific	
• Beck Depression Inventory-II	Beck, 1996
• Caregiver Burden Inventory	Novak & Guest, 1989
• Idler Index of Religiosity	Idler, 1987

issues. Open-format quality of life instruments allow the subject to define and assess elements that make up their perceived quality of life. Single-item questions represent a global assessment. Instruments addressing specific areas are frequently used to supplement or validate more general instruments.

A point to bear in mind when assessing quality of life data related to the various forms of MND is that patient strength and function inexorably decline, and instruments that include physical and functional questions show lower scores over time for those areas or domains, and thus these questions tend to drive summary scores to lower ratings. In contrast, open-format and single-item questions are not necessarily directly influenced by physical and functional losses and have a higher likelihood of providing more realistic estimates of quality of life from the subject's perspective. Of note, although health-related instruments are accurately designated as such, publications that use these instruments frequently refer to them in general terms as "QoL" instruments and do not always distinguish their health-related nature.

QUALITY OF LIFE FOR THE PATIENT AND CAREGIVER

Concern for quality of life in the setting of a disease that is progressive, without treatment, and that shortens life is an important issue for both the patient with MND and family. When estimating future quality of life patients usually conclude that it will be poor.

Patient Quality of Life

The important finding for ALS from comparisons using a variety of open instruments (Schedule for the Evaluation of the Individual Quality of Life-Direct Weighting, McGill Quality of Life Questionnaire; Table 28–1) is that quality of life is relatively preserved despite continued loss of function as measured by the ALS Functional Rating Scale-Revised in both cross-sectional and longitudinal studies (Robbins, Simmons, Bremer, Walsh, & Fischer, 2001; Neudert, Wasner, & Borasio, 2001; Bromberg & Forshew, 2002). However, another study that used the single-item portion of the McGill instrument, a 10-cm visual analogue scale marked to indicate current quality of life (higher numbers indicate better quality of life), showed a slow statistically significant decline over 13 months from 7.49 to 5.89 cm (Lou, Moore, Gordon, & Miller, 2010).

Patients with ALS who go on to use full-time ventilation also experience a good quality of life despite progression of weakness (Gelinas, O'Connor, & Miller, 1998). In one study comparing patients using tracheal ventilation (most with ALS, but also patients with brainstem strokes) with those with ALS and strokes who are using noninvasive ventilation or need no ventilation, neither quality of life (using the McGill Quality of Life instrument) nor depression were significantly different among the groups (Rosen et al., 1993). Another study confirmed that patients with tracheal ventilation maintain their McGill scores (Rousseau, Pietra, Blaya, & Catala, 2011).

Hopelessness and Suicidal Ideation

It remains likely that there is a negative affect on quality of life early in ALS because when

given the diagnosis and informed about the prognosis of ALS patients frequently expressed an element of hopelessness. In support are findings that state anxiety (an unpleasant emotional arousal in the face of threatening issues) is higher at the time of diagnosis and lower at a later point in time (Vignola et al., 2008). However, longitudinal studies of depression show no increase including evaluation in the final 3 months of life (Rabkin et al., 2005).

More than half of patients in one study said that they might under some circumstances consider taking a prescription to end their life (Ganzini, Johnston, McFarland, Tolle, & Lee, 1998). Hopelessness was cited as the main factor, depression a minimal factor, and religion a positive supportive factor. In a follow-up study after these patient had died, one-third had expressed to their spouses an interest in assisted suicide in the last month of life, again largely attributed to feelings of hopelessness (Ganzini, Johnston, & Silveira, 2002). Toward the end there is the concept of "quality of death" (Bromberg, 2008). Factors that contributed to poor quality of death include difficulty with communication, dyspnea, insomnia, pain, and generalized discomfort. It is important to emphasize that most patients with ALS pass away peacefully in their sleep and symptoms of discomfort can be managed.

Response Shift

The explanation for lack of change in quality of life is a "response shift," whereby individuals reset or reframe their expectations as circumstances change (Bromberg, 2008). The finding of good psychosocial adjustment in the setting of a terminal disease is not unique to the particulars of the disease and has been found among patients with cancer (Lule et al., 2012). This information is important to present to patients to reduce some of their fears. The response shift is a natural phenomenon, and a common example that occurs for everyone as natural "aging" when priorities important at a young age become less important at an older age with no loss of satisfaction. Another related finding is that when "psychological health" subscales from the ALS-Specific Quality of Life (Table 28–1) are compared with functional scales in a group of patients with ALS there is no change over time despite progressive loss of function (Cupp et al., 2011).

Caregiver Quality of Life

The lives of patients with MND and caregivers are linked in many ways, both physically and emotionally. Thus, it is not surprising that quality of life of both parties is interdependent. Of note, most caregivers are spouses, and given the male predominance of ALS, most are female.

An advantage of open quality of life instruments is that the same instrument can be administered to both the patient and caregiver. When the same instrument (Schedule for the Evaluation of the Individual Quality of Life-Direct Weighting) is given to spousal pairs, caregivers score lower in quality of life than patients (Bromberg & Forshew, 2002), but another study showed similar scores for both (Lo Coco et al., 2005). Data support a linkage between a patient's higher existential scores and their caregiver's sense of well-being (Pagnini et al., 2011). Conversely, behavioral symptoms from frontotemporal syndrome, apathy in particular, are associated with lower quality of life of the caregiver. Of note, as judged by the caregivers, almost 50% of patients with ALS had elements of frontotemporal lobe symptoms (apathy most common, followed by executive decision-making and disinhibition), making this an important element for caregivers (Chio et al., 2010).

Depression among caregivers is higher than among patients (Rabkin, Albert, Rowland, & Mitsumoto, 2009). From a survey of 71 patient spousal pairs tested with the Beck Depression Inventory, 10% of patients and 13% of caregivers were depressed. A longitudinal study supports greater depression in caregivers compared with patients over 9 months (Gauthier et al., 2007).

Invasive ventilation requires greater caregiver effort. The results of a survey of 52 patients with ALS on invasive ventilation showed a good quality for the patient and a lower level of quality of life for the caregiver (Kaub-Wittemer, Steinbuchel, Wasner, Laier-Groeneveld, & Borasio, 2003).

PERCEPTIONS OF QUALITY OF LIFE

It can be difficult to imagine the quality of life of another person, especially when the

other person's circumstances are out of the ordinary. One study of 89 patients with ALS and 188 healthy subjects (either caregivers of the patients with ALS or age-matched volunteers) assessed the situation of each other (a vignette about a typical patient with ALS was given to the age-match volunteers; Lule et al., 2013). Estimates of depression were similar between the patients with ALS and their caregivers, but patients and caregivers estimated less depression than volunteers. Global quality of life scores (Anamnestic Comparative Self-assessment) were similar between the patients and caregivers, but both patients and caregivers rated their quality of life lower than they estimated in the volunteers.

FACTORS AFFECTING QUALITY OF LIFE

Specific factors have been sought that might influence quality of life, and the large number of individual tests available with specific focuses allows for generalized conclusions. Two existential factors are existential elements and religiousness. Existential elements are those that concern a person's thoughts of how they relate to the world in general. They include how one orients oneself in the world and includes life's experiences and personal beliefs. Religiousness and spirituality are broadly considered and may or may not relate to traditional religious beliefs or practices.

Data from several studies support religion as a positive factor. Seventy-five caregivers were given the Idler Index of Religiousity and the McGill Quality of Life Questionnaire and it was found that religiousness, in particular private aspects, was correlated with quality of life (Calvo et al., 2011). Furthermore, from longitudinal surveys of patients with ALS and caregivers, religiousness may become more important over time, especially in the setting of a fatal disease (Walsh, Bremer, Felgoise, & Simmons, 2003; Pagnini et al., 2011).

Mood and depression might be expected in the setting of ALS, but low percentages of patients score in the depression range (<16%) based on individualized tests of depression. It has been noted that up to 38% of patients with ALS have a history of depression prior to the diagnosis of ALS, and half had been medically treated in the past (Rabkin et al., 2005). When patients are followed in the course of the disease to the terminal phases, rates of depression decline (Rabkin, Albert, Rowland, & Mitsumoto, 2009). Another life factor that can affect quality of life and self-esteem in the setting of ALS is marital intimacy before disease onset (Goldstein, Atkins, Landau, Brown, & Leigh, 2006). Thus, premorbid mood issues and coping mechanisms are important factors with MND.

Two frequently recommended interventions, noninvasive ventilation and gastric feeding tube placement, have been investigated for their affect on quality of life. One study of subjects followed in a negative drug trial used the McGill single-item quality of life visual analogue scale (Lou et al., 2010). Initiation of noninvasive ventilation during the study in 74 subjects revealed a nonsignificant trend to better quality of life, whereas placement of a gastric feeding tube in 52 subjects resulted in a significant slowing in the rate of decline of quality of life on the visual analogue scale. In a study of 27 patients with ALS, 22 of whom were receiving noninvasive ventilation and 5 requiring invasive ventilation, there were no significant differences between the two groups for the McGill open scale and the SF-36 health-related scale (Rousseau et al., 2011). Another study of 39 patients with ALS supported an increase in quality of life as measured by the McGill scale within 1 month of starting noninvasive ventilation (Mustfa et al., 2006).

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Chapter 29

Caregiver Issues

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Caregivers have key roles in providing emotional and physical support for patients with motor neuron disease (MND), which increases over the course of the disease. This imposes an emotional and physical burden on the caregiver that becomes more challenging to manage over time.

Many facets of providing care have been studied and a high degree of caregiver resiliency is noted. Most caregivers are spouses, and MND is a disorder of older adults, and premorbid issues and coping strategies have an affect on the caregiver burden. The emotional burden further increases when there are behavioral issues in the patient because of frontotemporal lobe syndrome or dementia. The overall burden is associated with level of care, and is greatest when the patient is using long-term mechanical ventilation. Spirituality is an important positive element for the caregiver and patient.

INTRODUCTION

The role of providing care is of paramount importance to the patient with MND for both physical assistance and emotional support. There is a burden for the caregiver in providing these functions that increases over time, as the patient requires more physical assistance. Additionally, other symptoms common in patients with amyotrophic lateral sclerosis (ALS) that further tax caregiver reserves include pseudobulbar affect and frontotemporal syndrome and dementia. In the setting of predictable and progressive issues, caregivers display a resiliency and derive a feeling of satisfaction from the efforts (Miller et al., 2000).

IMPACT OF MND ON CAREGIVERS

The impact of providing care affects a large number of areas, including general health, emotional status including anxiety and coping with strains, life satisfaction, socioeconomic situation, and relationships. Protective factors include social and structured support. These areas have been studied in a variety of settings and using different instruments, and the results have been summarized (Mockford, Jenkinson, & Fitzpatrick, 2006).

- General health: The number of hours required for patient care increases over time, particularly for patients on a ventilator, and most such patients are treated at home. Caregiver health status is somewhat lower than that of the general population.
- Emotional status: Depression is not common among caregivers but is slightly greater compared with their patients. Sadness over the progressive loss of patient function represents progressive grieving during the life of the patient. Anxiety is found to be variable and not correlated with disease progression. Coping with emotional strains is related to the extent and use of support systems, which include family and friends, and is more effectively used by female caregivers.
- Life satisfaction: Daily activities change with greater patient care needs, particularly when patients are placed on long-term ventilation, and the need to think about the patient before engaging in an activity represents "forced life changes."
- Socioeconomic situation: The progression of weakness affects patient and caregiver employment, but is offset somewhat by the higher average of disease onset that is in the retirement range. The costs of care vary among countries with respect to insurance coverage. However, home renovations represent a financial strain.
- Relationships: The relationship between the caregiver and patient depends on many factors, including premorbid interactions, and may show equally an improvement or reduction. Problematic issues related to sexuality are fears consequent to physical changes.
- Protective factors: Social support from frequent caregiver contact with family and friends is important. However, friends may retreat because of not knowing how to interact with the caregiver or patient. Formal support groups are helpful, perhaps less so very early or very late in the course of the disease. Support from religious groups is very helpful.

LONGITUDINAL QUALITY OF LIFE BETWEEN PATIENT AND CAREGIVER

Most caregivers are spouses, and MND is a disorder of older adults, and premorbid issues can affect quality of life for both. One study assessed quality of life, as measured by the McGill Quality of Life instrument, of both patient and caregiver at 6-month intervals (Roach, Averill, Segerstrom, & Kasarskis, 2009). Multilevel modeling was used to estimate variability within and between the pairs and over time. Both patients' and caregivers' quality of life varied, and analysis suggested that about half of the total variance was caused by stable differences between patients and caregivers, primarily with psychosocial aspects. Patient quality of life fluctuated over time but was not related to time since diagnosis, whereas caregiver quality of life also fluctuated but decreased over time.

LONGITUDINAL EFFECT OF CAREGIVER BURDEN

A range of instruments has been used to assess caregiver burden and its impact. Overall, there is a greater negative impact on quality of life for the caregiver than for the patient.

From a cross-sectional study comparing quality of life between patients with ALS and caregivers using the Schedule for the Evaluation of the Individual Quality of Life-Direct Weighting quality of life instrument, an open instrument where the responder defines elements making up their quality of life, index values were lower for the caregiver than the patient (Bromberg & Forshew, 2002). From a longitudinal study using the McGill quality of life instrument, an instrument that includes existential elements, there was a correlation in falling quality of life scores for the caregiver over time from diagnosis, but not for the patient (Roach et al., 2009).

Several studies show greater physiologic stress (anxiety and mood) that increases over time for spousal caregivers, but has only mild correlations with the physical burden of providing care (Goldstein, Atkins, Landau, Brown, & Leigh, 2006; Gauthier et al., 2007). Depression among caregivers, as measured by the Beck Depression Inventory, was not common (13%) and was mild in degree, and worsened little over the course of the patient's disease (Rabkin, Albert, Rowland, & Mitsumoto, 2009). Factors such as caregiver burden and fatigue also worsened over time, but to small degrees.

EFFECT OF PATIENT'S BEHAVIORAL DYSFUNCTION

Frontotemporal lobe dysfunction is experienced by up to 50% of patients with ALS, varying in severity from mild to sufficiently severe to fulfill diagnostic criteria for frontotemporal dementia (see Chapter 5 on frontotemporal lobe dementia). Symptoms of the syndrome have a negative impact on the caregiver.

From a set of instruments assessing behavior given to 70 patient-caregiver couples, 49% of caregivers ranked their patient as having frontotemporal behavioral symptoms, whereas only 13% of patients acknowledged such symptoms (Chio et al., 2010). Another study compared patients with ALS without frontotemporal syndrome with those with frontotemporal dementia, and a marked lack of insight was noted in the patients with dementia (Woolley, Moore, & Katz, 2010). The most common behavioral issues are apathy, executive dysfunction, and to a lesser extent disinhibition. Quality of life as measured by the McGill instrument and depression as measured by the Zung Depression Scale were negatively correlated with the magnitude of the behavioral symptoms and not with the patient's level of function. In another study, patient impulsivity and disinhibition were the more challenging issues for caregivers and negatively impacted their burden (Lillo, Mioshi, & Hodges, 2012).

Over time, patient symptoms tend to become more troublesome. From a study of 10 patients with ALS on long-term tracheal ventilation (median time, 48 months) aggressive irritability, greater interest in sex (despite being sexually inactive), and obsessiveness were viewed by the caregivers to have progressed over time (Marconi et al., 2012).

Pseudobulbar affect may be separate from frontotemporal lobe behavioral issues, and by itself affects the caregiver burden but only to a mild degree (Colamonico, Formella, & Bradley, 2012).

CAREGIVER COPING

Behavioral issues are major negative factors affecting the caregiver. A list of interventions has been compiled to aid the caregiver in managing cognitive and behavioral symptoms (including irritability, executive deficits, apathy, loss of insights, disinhibition, aggression, and rigidity; Merrilees, Klapper, Murphy, Lomen-Hoerth, & Miller, 2010). Behavioral interventions are emphasized over drugs, because pharmacologic intervention has not proved to be markedly effective. How these interventions can help the caregiver is not clear, and the final recommendation is working toward "acceptance" of the behavioral issues.

POSITIVE FACTORS FOR CAREGIVERS

Despite the overall challenges in providing care, positive factors have been identified. In a study of 75 caregivers assessed for their level of religiousness by the Idler Index of Religiosity there was a positive correlation between quality of life measured by the McGill instrument and religiousness (Calvo et al., 2011). Higher correlations were found for private religious activities (taking place in the home or generally in daily life) than for public (formal religious institutions) religious activities.

A study assessed the relationship between the patient's sense of well-being and the caregiver's sense and quality of life (Pagnini et al., 2011). Both the patients' and caregivers' sense of well being were based on several questions from the McGill quality of life scale that focus on existential issues; the patient was also asked two questions about the role of religion taken from the Amyotrophic Lateral Sclerosis Specific Quality of Life-Revised instrument; and the caregivers' quality of life was measured by the single question on quality of life over the past 2 days. There were correlations between the caregivers' sense of well-being and quality of life with positive patient responses to spirituality.

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Chapter 30

End-of-Life Care

Mark B. Bromberg

BACKGROUND AND NEW POINTS

All forms of motor neuron disease (MND) are progressive and are proximate causes of death. End-of-life care is important in the continuum of MND care and should include the patient, caregiver, and family members. One concept is that end-of-life care starts at the time of diagnosis and progresses in magnitude over the course of MND.

Patients are frequently concerned with the manner of death, and in particular the possibility of choking and pain. Some patients think about suicide or assisted death at some point in the course of MND. The issue of manner of death should be discussed frequently with the assurance that choking and pain are not common and can be managed through hospice care. Bereavement for the caregiver and family should be addressed, but a form of mourning by the patient for activities lost during MND progression should also be considered.

INTRODUCTION

End-of-life care focuses on amyotrophic lateral sclerosis (ALS) but also includes progressive muscular atrophy (PMA) due to similar survival statistics, and less commonly on primary lateral sclerosis due to its longer survival. Two terms commonly used toward the end of life are palliative care and hospice care. There are formal differences: palliative care focuses on relieving suffering in the setting of a serious illness, whereas hospice is palliative care at the final time period (6–12 months) of a life-shortening illness. In some countries the term palliative care includes hospice, and the term hospice can refer to a building or institution that specializes in palliative care. However, it is to be emphasized that end-of-life or hospice care for patients with ALS can represent a care approach, and does not depend on a physical care facility. The contemporary focus on palliative and hospice care dates to the 1960s. The palliative/hospice care movement includes ALS as a noncancer disorder (Oliver, Borasiio, & Walsh, 2000; Bede et al., 2011).

Box 30.1 Triggers for Initiating Discussions About End-of-Life Care

1. Inquiries by patient or family
2. Psychological, social, or spiritual distress or suffering
3. Marked degree of pain
4. Dysphagia associated with recommendation for gastric feeding tube
5. Dyspnea, forced vital capacity <50% or predicted, symptoms of hypoventilations
6. Loss of function in two body regions

Modified from Mitsumoto et al. (2005).

The state of end-of-life care in ALS was reviewed in 2005 (Mitsumoto et al., 2005). A list of clinical triggers for providers to initiate discussions about end-of-life care was recommended (Box 30.1). Areas where knowledge was deficient were identified, and included incorporation of an interdisciplinary approach to end-of-life care and guidelines for optimizing care.

TIME LINE OF MND CARE

Although hospice is considered in the context of end-of-life care, it is more appropriate to consider palliative care as "relief of suffering in the setting of a serious illness." Thus, palliative care can be considered in the broader context as supporting both a patient's psychosocial and physical well-being. The principles of end-of-life care as it relates to a fatal disease can be considered in a continuum, from the time of diagnosis with frank discussion, to hospice, and beyond for bereavement by the family (Fig. 30–1). Related to a time continuum of care is continuous surveillance of suffering. Among 100 patients with ALS surveyed, suffering was reported in 20%, and was related to pain, hopelessness, and greater sense of burden (Ganzini, Johnston, & Hoffman, 1999).

PATIENT DECISION-MAKING

At the time of diagnosis of ALS or PMA a statistical time course of disease progression is available (median survival 2–4 years from

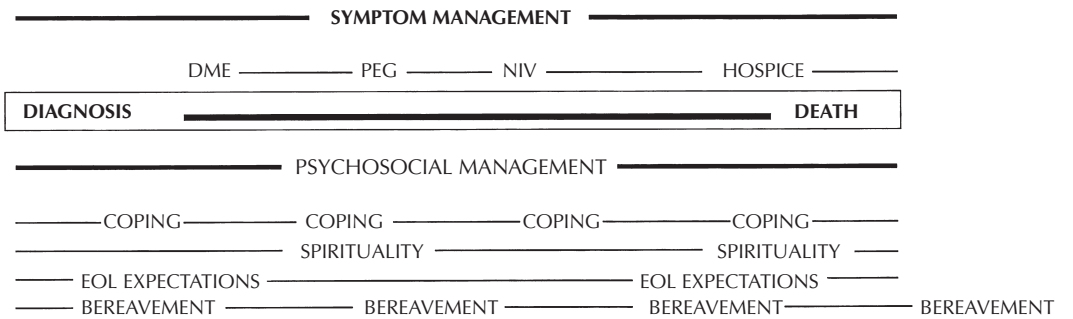

Figure 30–1. Concept of the principles of hospice across the full timeline of MND, and beyond for bereavement by the family. DME = durable medical equipment; EOL = end of life; NIV = noninvasive ventilation; PEG = percutaneous endoscopic gastroscopy.

symptom onset) and most patient either ask about the time course or read about it from outside sources. A comparison has been made between 32 patients with ALS and 59 with advanced cancer with a 2-year survival of less than 50% (Astrow et al., 2008). Patients with ALS more often than patients with cancer discussed options for end-of-life treatment, do-not-resuscitate choices, and hospice care.

LEGAL DOCUMENTS

Documents to direct one's health care and estate are important for everyone of adult age, but especially so in the setting of a progressive and fatal disease. However, surveys indicate that a minority of patients with ALS, and also a minority of neurologists, attorneys, and the general public, has prepared these documents. Among 21 patients with ALS, 7 patients with cancer, 14 healthcare providers, 12 estate planning attorneys, and 20 healthy control subjects in the United States queried for their knowledge about and completing of legal documents (Banks-Bromberg & Bromberg, unpublished data), estate planning attorneys completed the most documents and patients (ALS and cancer) more than healthcare providers and control subjects (Table 30–1). The most common reason was planning to complete at a later time. A sample of 15 patients with ALS and 15 neurologists from Germany analyzed attitudes of neurologist presenting the topic and patients receiving it (Burchardi, Rauprich, Hecht, Beck, & Vollmann, 2005). Both considered the topic appropriate close to the time of death. However, an argument can be made to consider these documents for everyone when they reach adult age, and in the setting of ALS at an early stage (Benditt, Smith, & Tonelli, 2001). If made late in the course of ALS, communication abilities may be compromised and unfinished business related to estate planning difficult to manage.

HOSPICE

Eligibility guidelines vary among countries (Bede et al., 2011). In the United States, there are guidelines for Medicare (Centers for Medicare and Medicaid Services) determination of terminal status (Box 30.2). There are no patient costs for such care (aside from possible small copayments for some drugs), but expenses are covered only when provided by Medicare-approved hospice agencies. Hospice care is nominally for 6 months, but can be renewed if progression is documented. It has been pointed out that only a small percentage of patients with ALS who could benefit from hospice care fulfill Medicare guidelines. In one study, only 5 out of 97 patients with ALS fulfilled Medicare guidelines; however, the remaining patients were accepted into hospice care based on local hospice guidelines (McCluskey & Houseman, 2004). For these patients, the average number of days in hospice was 84 (range, 1–534 days).

The range of hospice services varies among regions, and Box 30.3 lists services covered by

Table 30–1 **Percentages of Medical and Legal Documents Completed by Groups**

	Estate Planning Attorneys	Patients With ALS	Patients With Cancer	Healthcare Providers	Healthy Control Subjects
Living will	64%	43%	57%	29%	20%
Health care proxy	73%	38%	43%	21%	5%
Medical directive	36%	33%	14%	14%	5%
Emergency directive	0%	24%	0%	0%	5%
Will	91%	57%	57%	50%	65%
Trust	82%	33%	43%	29%	20%
General power of attorney	45%	33%	43%	21%	15%
Special power of attorney	45%	5%	0%	0%	0%
Joint tenancy	73%	48%	43%	93%	65%
Gifts	45%	14%	0%	21%	15%

Box 30.2 Medicare Eligibility Criteria for Admission to Hospice

All of the following within past 12 months:
Forced vital capacity <30% of predicted
Significant dyspnea at rest
Requirement for oxygen supplementation at rest
Artificial ventilation declined
Or demonstration of both:
Rapid progression, demonstrated by all of the following within past 12 months:
Independent ambulation to wheelchair or bed-bound status
Speech from barely intelligible to unintelligible
Diet from normal to pureed
Independence in most or all activities of daily living to need for assistance
Nutritional impairment by all of the following within past 12 months:
Oral intake of nutrients and fluids insufficient to sustain life
Continuing weight loss
Dehydration or hypovolumia
Artificial feeding methods declined
Or demonstration of both:
Rapid progression (above)
Life-threatening complication by one of the following with past 12 months:
Recurrent aspiration pneumonia
Upper urinary tract infections
Recurrent fever refractory to antibiotic therapy
Skin ulcers (multiple sites/episodes, stage 3 to 4)

Medicare. Because hospice is brought in at the terminal stage of MND hospice may not support the institution of life-prolonging interventions, such as gastric feeding tubes and assisted or long-term ventilation, although hospice does support the continued use of them. These interventions are usually introduced at an earlier stage and are considered by international guidelines to improve patient quality of life (Andersen et al., 2012). If not in place when hospice is being considered they should be reviewed and instituted before starting hospice.

TIME IN HOSPICE

The time period terminal patients (all diagnoses) spend in hospice care in the United States is short (median, 19.1 days; average, 69.1 days). Most receive care in their homes (66%). The diagnoses include cancer 37.7%, and less than 2% patients with ALS (National Hospice & Palliative Care Organization; Facts & Figures, 2013, http://www.nhpco.org/sites/default/files/public/Statistics_Research/2013_Facts_Figures.pdf).

END-OF-LIFE PATIENT CONCERNS ABOUT MANNER OF DEATH

Patients frequently articulate greater fear about the manner of death from ALS than the prospect of death. The magnitude of their internal thoughts about the manner of death can only be imagined, and it is important to enquire frequently after their thoughts and address them explicitly. One concern expressed is suffocating, but a study of 100 patients with ALS assessed by autopsy indicates that acute respiratory failure occurred in 9% and hypoxia in 3% (Corcia et al., 2008). Other concerns that

Box 30.3 Medicare Coverage for Underlying Diagnosis or Associated Injuries

Services
Physician
Nursing
Physical therapy
Occupational therapy
Speech-language
Dietitian
Social work
Hospice aide and homemaker services
Medical equipment
Wheelchairs, walkers
Medical supplies
Bandages, catheters
Drugs
Symptoms or pain relief (may require a copayment)
Counseling
Grief and loss
Short-term inpatient care
Pain and symptom management
Volunteer help

Modified from Centers for Medicare and Medicaid Services Product No. 02154 (revised August 2013).

are considered suffering are losing functional independence and being dependent on and a burden to the caregiver (discussed later).

PALLIATING PAIN

ALS and PMA are not intrinsically painful disorders, but pain is experienced to some degree by a large percentage of patients during the course of the disease (see Chapter 26 on symptom management; Chio et al., 2012; Rivera et al., 2013). The last days of any disease can include new-onset pain or a worsening of previous pain and with ALS can include discomfort from respiratory insufficiency. A study of palliative medications during the final 3 days for 62 patients from six hospices in the United Kingdom revealed the following (Oliver et al., 2010). Of note, 75% of patients died in a hospice, nursing home, or hospital. Most (6%) received morphine (mean equivalent oral doses, 50–80 mg; range, 10–520 mg), with only mild increases during the final 24 hours. Midazolam was given to 78%. Other medications included management of oral secretions. Medications were administered by a variety of routes but morphine was frequently administered by mouth.

SUICIDE IN PATIENTS WITH ALS

Patient suicide represents self-management of the end of life, and is a concern. There are many factors involved and personal ones can only be assessed remotely. Some countries and states in the United States allow or do not regulate physician-supported suicide. Suicide is taking one's own life, and euthanasia is defined as another person directly assisting in the death of a patient at the patient's explicit request. It is challenging to assess numbers of patients falling into each category.

A study of patient attitudes toward suicide was conducted in Washington and Oregon in the United States (Ganzini, Johnston, McFarland, Tolle, & Lee, 1998). Fifty-six percent of patients with ALS agreed with the statement: "Under some circumstances I would consider taking a prescription for a medicine whose sole purpose was to end my life." Hopelessness, but not depression, was more common. A similar study was conducted among patients in Switzerland and Germany and showed that 42% had thought about suicide and 21% percent thought about euthanasia (Stutzki et al., 2013).

A population-based cohort study in Sweden, where neither suicide nor physician-suicide is regulated, supported a 5.8-fold risk for suicide (identified from International Classification of Diseases codes) among patients with ALS (Fang et al., 2008). Factors were younger age and within the first year after diagnosis. A directed survey of dying patients with ALS was conducted in the Netherlands where suicide and physician-assisted suicide is illegal but not punished if performed under strict conditions (Veldink, Wokke, van der Wal, Vianney de Jong, & van den Berg, 2002). Among 203 patients, 17% chose euthanasia and 3% were directly assisted by a physician.

Queries sent to 53 caregivers from the Northeast region of the United States about their patients' thoughts and actions at the end of life revealed the following (Albert et al., 2005). Although 43% expressed thoughts about ending their life, only three (6%) did so with the assistance of hospice. Greater hopelessness and less comfort in religion were factors.

Comparisons of euthanasia rates and underlying factors between patients with ALS and those with other fatal medical diagnoses has been performed in the Netherlands (Maessen et al., 2010). Twenty percent of patients with ALS died due to euthanasia or physician-assisted suicide compared with 5% of patients with cancer and 0.5% of patients with heart failure. Questionnaires indicated that among patients with ALS, fear of suffocation was a factor in 45% and being dependent in 37%, compared with patients with cancer, where pain was a factor in 46% and fatigue in 38%.

MANAGING THE PROCESS OF DYING

A challenge for any physician is managing the circumstances of a patient's death, from any cause. There are issues that can be identified and that deserve individual patient consideration and attention, and should be addressed openly with the family, especially if the physician (neurologist or hospice/palliative care physician) is not planning to be present at the time of death (Twaddle, 2001).

1. Disease reality and end of life: Preparing a patient and family that MND will be the proximate cause of death is important for all.
2. Prognostication: Estimating the time of death is difficult with ALS, and it is not wise to set a time because patients may miss the time on either side; if death is earlier families feel not prepared, if it is later emotions may have already peaked.
3. Nutrition and fluids: Patients at the end of life do not require nutrition and fluids.
4. Pain: It is important to assess for pain and offer relief.
5. Delirium and agitation: Patients may drift in and out of consciousness and family should be aware of this. If agitated, medications should be given to treat symptoms.
6. Spontaneous movements: Myoclonic jerks and agonal movements may occur and the family should be informed to expect them; occasionally a seizure occurs.

PLACE OF DEATH

The place of death is influenced by several factors, including medical condition at time of death, local availability of nursing facilities, and patient wishes (Oliver & Turner, 2010).

A study from a North American patient registry of 1,014 patients with ALS who had died showed that 64% died at home, 21% died in a hospital, 8% in a nursing home, and 8% in a hospice (Mandler et al., 2001). Another study of UK and German patients noted that 52% and 52%, respectively, died at home (Neudert, Oliver, Wasner, & Borasio, 2001).

BEREAVEMENT

The definition of bereavement focuses on the reaction of an individual to the loss of someone close to them, but it can also be expanded to include the patient who frequently feels the impending loss for what they expected to be able to do physically or be a part of in the future. Such issues may surface for the patient at different time periods. Similarly, there can be bereavement by the caregiver and family members while the patient is living as they realize that the patient's participation and presence will be limited. Recognition of this form of contemporaneous grieving can be helpful for the patient and family, and is considered in the time line presented in Figure 30–1.

Issues related to bereavement by the caregiver and family have not been well studied in ALS. The number of United States ALS clinics (certified by two major US organizations) offering bereavement support in 2005 was 39% (Hebert, Lacomis, Easter, Frick, & Shear, 2005). The primary method of active bereavement support was referral to outside resources, including grief support groups, social workers, and mental health specialists, and only 39% had the availability of professional counseling within the clinic.

Another survey included 27 families conducted over an unspecified number of years after the death of the patients (Martin & Turnbull, 2001). From the survey, 22% believed they were coping well; 33% coping with moderate ongoing difficulties; but 37% were coping poorly, with no relationship of improvement in coping with the passage of time. There was no formal bereavement program in effect for these family members. Negative feelings about the disease included sadness, fear, frustration, hatred and anger, depression, and a lack of hope. Despite the fact that there was no family history of ALS, there remained concern about passage to the offspring. It is interesting that only 37% of families participated in fundraising for ALS.

Factors contributing to caregivers' feelings included 82% experienced burn-out and 63% did not use respite care (may or may not have been available). Most families incurred considerable expense, 22% severe and 26% moderate, related in part to 48% of patients having to stop working and 52% of homes requiring renovations. Providing care resulted in 67% feeling that it pulled the family together, but 15% feeling that it was detrimental to family relationships.

ORGAN DONATION

Requests to donate organs are frequently enquired about by patients with ALS and their families. Criteria for donation after cardiac death excludes donors with high-risk diseases, but a report from a consensus meeting allowed donors with "end-stage musculoskeletal diseases" (but diseases were not specified; Bernat et al., 2006). Several patients with ALS have donated organs (Toossi et al., 2012; Smith et al., 2012). However, a note of caution has been raised as to the safety of organ tissue donation based on concerns for a prion-like propagation of misfolded protein from the donated organ to the unaffected host (Holmes & Diamond, 2012). Furthermore, patients with ALS without a family history of ALS or dementia may have unsuspected mutations associated with hereditable ALS and dementia (Majounie et al., 2012).

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Chapter 31

Costs of Amyotrophic Lateral Sclerosis Care

Mark B. Bromberg

BACKGROUND AND NEW POINTS

Amyotrophic lateral sclerosis (ALS) is a disorder with progressive needs over time for equipment and interventions. Prior to the approval of riluzole in the early 1990s the costs of ALS care were relatively low and based on durable medical equipment, but since approval the drug became a major cost factor.

The costs of ALS care are largely driven by medications (riluzole and Nuedexta), durable medical equipment (power wheelchairs), interventions (noninvasive ventilation and gastric feeding tube placement and nutritional supplies, and diaphragm pacing [NeuroRx DPS]), and alternative communication devices. Complementary alternative medicines and treatments are commonly used, but cost data are not available. Costs of home care related to the caregiver, and lost wages of the patient and caregiver, are difficult to estimate. Hospice care costs are low because of the short time a patient with ALS is under hospice care. Overall, ALS has higher associated costs among chronic disorders characterized by progressive muscle weakness. Funding in the United States comes from private insurance, Medicare (most patients with ALS are older, and those who have paid into the Federal Insurance Contributions Act are eligible based on ALS as a disability), and Veterans Association for military veterans (ALS is considered a full service connected disability).

INTRODUCTION

Data on the cost of health care for patients with ALS are important for patients and funding agencies. Direct costs incurred for care increase over time and include those for medications, durable medical equipment, interventions,

hospital care, provider expenses, and end of life care (hospice). Indirect costs are lost wages for both the patient and caregiver. There is also an issue of cost-effectiveness for therapies and interventions in a disease with progressive disability where current disease-modifying therapies have a modest effect on prolongation of life and where interventions cannot markedly improve function (Ginsberg & Lowe, 2002). Costs of care studies are for ALS and there are no studies for other forms of motor neuron disease, but costs for progressive muscular atrophy are likely similar to those for ALS. Costs for primary lateral sclerosis are likely less, at least on an annual basis, but life expectancy is longer.

DRUGS TO TREAT ALS

Two drugs are approved for ALS in the United States, riluzole as a disease-modifying drug and Nuedexta for treatment of pseudobulbar affect. However, many patients seek alternative treatments in hopes of modifying the course of ALS, but the frequency of use and costs cannot be readily calculated.

Riluzole

Riluzole is approved for treatment of ALS in the United States and other countries. Riluzole became generic in 2013 and the cost dropped (Table 31–1). The costs and gains attributable to riluzole are based on an extension of life by 2–6 months, and have been evaluated by a variety of models. Comparisons between use of riluzole and best supportive care are based on "quality-adjusted life years," which is a measure of disease burden factoring in the quality and quantity of life that would be added by the intervention, with scaling values for each year from perfect health (1.0) to death (0.0). Analysis yields a wide range of cost-benefit values per quality-adjusted life years, from $US30,000 to more than $US60,000 (based on the year 2000). Conclusions vary with respect to cost effectiveness: support for cost effectiveness is largely based on prolonging survival at an early stage of ALS when a patient has good function (Messori, Trippoli, Becagli, & Zaccara, 1999; Arnold et al., 2005; Tavakoli & Malek, 2001; Tavakoli, 2002).

Nuedexta

Nuedexta is a combination of dextromethorphan, 20 mg, the active drug, and quinidine sulfate, 10 mg, a drug that reduces the metabolism of dextromethorphan. It was approved in the United States in 2010 for pseudobulbar affect. When drug trial results for efficiency were published the drug could be compounded at low cost, but after Food and Drug Administration approval the cost increased (Table 31–1).

COMPLEMENTARY ALTERNATIVE MEDICATIONS AND PROCEDURES

A significant percentage of patients with ALS (~50%) seek alternative treatment options with the hope of slowing the progression of weakness, improving strength and function, or curing the disease (Vardeny & Bromberg, 2005; Wasner, Klier, & Borasio, 2001). Treatments include vitamins and supplements; acupuncture; and more complex treatments, which include stem cell therapies. Given the wide spectrum of alternative medications, costs are difficult to assess, but one estimate from a German ALS population in 2001 reported a median of $4,700 and a high value of $40,000 (Wasner et al., 2001). Stem cell treatment has captured the interest of patients as an alternative to riluzole. With only formal Phase I/II safety trial data available in the United States, an unknown number of patients with ALS seek unproven and unregulated stem cell therapies abroad. Such treatments have substantial costs, including transportation to a foreign country; accommodations; and the treatment itself, which can reach $10,000 to $40,000. Most complementary alternative medications and procedures are not covered by insurance and represent out of pocket expenses.

There are formal efforts to investigate alternative medications and procedures that are listed on the Internet. ALSUntangled (ALSUntangled.com) represents the investigatory efforts of a group of ALS neurologists to determine the science behind complimentary offerings.

Table 31–1 **Costs for Pharmacy (Prescription Drugs), Durable Medical Equipment, and Interventions Used for Patients With ALS**

Drugs to treat ALS	
• Rilutek/riluzole	
- Rilutek	$2,500/month
- Riluzole	$525/month
• Nuedexta	$670/month
Drugs to treat symptoms	
• Amitriptyline	$4/month
• Glycopyrrolate	$80/month
• Scopolamine patch	$160/month
• Botulinum toxin	$500/injection
• Baclofen	$4/month
• Mood-stabilizing drugs (selective serotonin reuptake inhibitors)	$4–$12/month
• Pain	
- Meloxicam	$4/month
- Trazodone	$4/month
Durable medical equipment	
• Walkers	$130
• Manual wheelchairs	$3,000
• Power wheelchairs	$20,000
• Alternative communication	
- Eye-gaze tracking system	$20,00
Interventions	
• Noninvasive ventilation	$500–$1,000/month
• Diaphragm pacing	
- NeuroRx DPS, device only	$21,250
- Avery Biomedical Devices, device only	$58,000
• Gastric feeding tube	
- Placement	Highly variable
- Feeding formula	$400/month

Pharmacy costs based on single academic center's pharmacy; durable medical equipment and interventions are estimates; costs vary because of insurance contracts.

DRUGS TO MANAGE SYMPTOMS

A spectrum of drugs is used off-label to treat symptoms associated with ALS. Symptoms include sialorrhea, spasticity, mood, sleep, constipation, urinary urgency, and pain, and their use is discussed in Chapter 26. Costs are relatively modest (Table 31–1).

INTERVENTIONS

Two common interventions for ALS are noninvasive ventilation and gastric feeding tubes, and recently diaphragm pacing.

Noninvasive Ventilation

Noninvasive ventilation requires a ventilator and hardware and is usually managed in an outpatient clinic, most commonly as part of a multidisciplinary clinic or in a pulmonology clinic, but occasionally is set up during a formal sleep study or overnight in a hospital. Ventilators and expendable supplies are expensive but are usually borne by insurance or other health agency, with little expense to the patient.

Invasive Ventilation

Full time tracheal ventilation has higher costs associated with it, including the ventilator (and a back-up ventilator is commonly recommended in case of primary ventilator failure),

and more expendable supplies. Furthermore, there is the issue of 24-hour care, and costs for care vary widely because care can be assumed by some combination of family support (after training) or professional care.

Gastric Feeding Tubes

Gastric feeding tubes are placed by gastroenterologists in an endoscopy suite, radiologists in an interventional radiology suite, or by surgeons in an operative suite. The procedure may require coordinated care by an anesthesiologist. Most are placed in an outpatient setting or 1-day surgery. Overall costs are difficult to determine because of regional variability in pricing and contracted discounts.

Diaphragm Pacing

Diaphragm pacing is proposed to augment noninvasive ventilation. There are two types of devices: one uses electrodes implanted around the phrenic nerves in the neck (Avery Biomedical Devices) and the other is by electrodes implanted in the diaphragm muscle as the phrenic nerves enter the diaphragm (NeuroRx DPS). They were developed for cervical spinal cord injuries, but in 2011 the Food and Drug Administration, under a Humanitarian Device Exemption, approved NeuroRx DPS for ALS. Both procedures require surgical intervention and are performed in an operative suite, and also require an anesthesiologist. The cost of the respective devices (Table 31–1) does not include surgical and operating suite or anesthesia costs.

DURABLE MEDICAL EQUIPMENT

Equipment needs for patients with ALS commonly includes walking aids (cane-walking stick, braces, walker), wheelchairs (manual and power), ventilators (most commonly noninvasive types, less commonly positive pressure types), and gastric feeding tubes (which have surgical intervention costs). One cost analysis assessed equipment and intervention needs during the course of ALS (Bromberg, Brownell, Forshew, & Swenson, 2010). It was determined that by the time a patient reaches 50% of their disease course they begin to require ambulatory aids and wheelchairs, noninvasive ventilators, and gastric feeding tubes (Fig. 31–1). Furthermore, the time-to-need for these items was largely independent of site of symptom onset (bulbar, arm, leg). In terms of cumulative costs, patients with leg onset reach $33,000 and those with bulbar or arm onset $20,000, the difference attributed to the universal need for wheelchairs among patients with leg onset (Fig. 31–2).

Alternative communication devices, especially eye tracking systems, can be expensive, and the cost may or may not be borne by insurance companies (Fig. 31–1).

A cost-effectiveness analysis has been made for noninvasive ventilation for patients with ALS (Gruis, Chernew, & Brown, 2005). The model is based on determine quality-adjusted life years for patients started at time of diagnosis compared with when force vital capacity reaches 50% of predicted. The results indicate that if noninvasive ventilation were started early it would be cost effective at $3,132 per year (2004 dollars) if it resulted in an improvement in the EuroQol ED-5D visual analogue scale ("health today": 0–100) of 13.5%.

CLINICAL CARE COSTS

Overall, ALS patient costs have been estimated in the Netherlands, comparing 35 patients cared for by a general provider with 63 patients cared for in a multidisciplinary ALS clinic (van der Steen, van den Berg, Buskens, Lindeman, & van den Berg, 2009). Costs, calculated monthly but expanded to annually included direct and indirect costs (€ converted to US$ in 2003) were similar for general provider care (€15,252/$17,040) and for multidisciplinary care (€16,032/$17,911). Costs were 22% higher for bulbar-onset patients than for spinal onset, attributed to costs of gastric feeding tube placement and supplies. Costs also rose with greater functional disability (determined by the ALS Functional Rating Scale), with a two-fold increase from mildly disabled (score 30–40) to severely disabled (score 0–20).

A similar study in Spain of 63 patients with ALS showed average annual costs (both

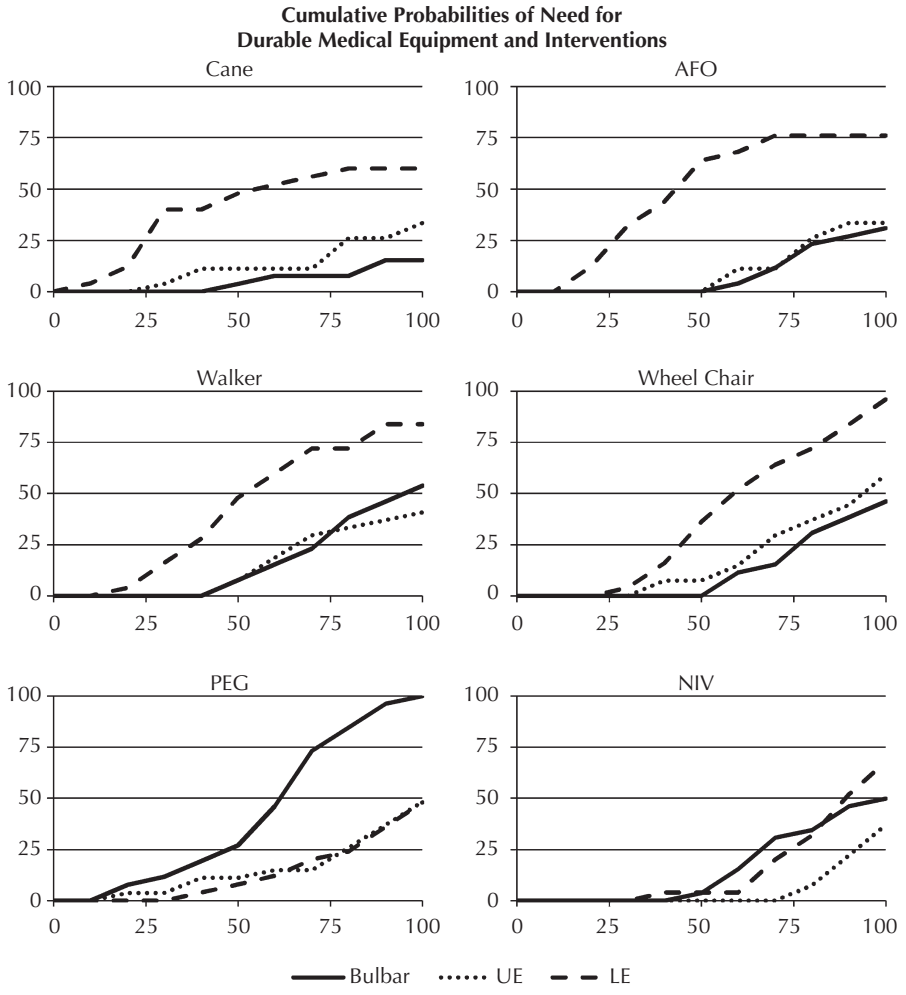

Figure 31–1. Time line of cumulative needs for durable equipment. X axis: percent of disease (ALS) duration. Y axis: percentage of patients needing durable item. AFO = ankle-foot orthosis; LE = lower extremity; NIV = noninvasive ventilation; PEG = percutaneous endoscopic gastrostomy; UE = upper extremity. (From Bromberg et al., 2010, with permission.)

dire ct and indirect costs; € converted to US$ in 2008) of €36,194/$50,310, with a range of €17,000/$23,630 for low severity and €42,728/$59,392 for high severity patients (Lopez-Bastida, Perestelo-Perez, Monton-Alvarez, Serrano-Aguilar, & Alfonso-Sanchez, 2009). The greatest difference between the two patient groups was the estimated costs of nonprofessional home care (Lopez-Bastida et al., 2009). This is greater than the costs in the Netherlands, but many factors may differ between the studies. A comparison of costs for other diseases estimated by the same group of investigators ranked annual ALS costs higher than for HIV/AIDS (€13,823/$19,214), stroke (€13,826/$19,218), and Alzheimer disease (€28,198/$39,195).

Efforts have been made to place patients with ALS into stages based on their degree of disability, and this has allowed estimates of the costs per stage (Chio, Hammond, Mora, Bonito, & Filippini, 2014). Yearly costs based on 1 year of data indicate the following: stage 0 (weak but no loss of independence), €280; stage 2 (loss of independence in one domain), €560; stage 3 (loss in another domain), €625; stage 4 (loss in another domain), €825; and stage 5 (loss in another domain), €825.

Multidisciplinary clinics are common coordinating centers of care, and a study from the

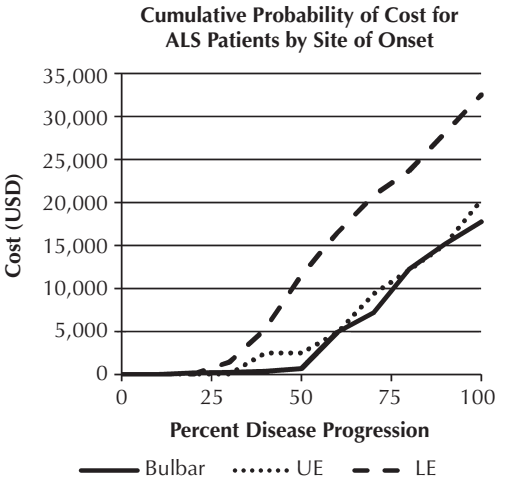

Figure 31–2. Time line of cumulative costs for durable equipment. X axis: percent of disease (ALS) duration. Y axis: cumulative costs over disease duration. LE = lower extremity; UE = upper extremity. (From Bromberg et al., 2010, with permission.)

Netherlands compared 63 patients with ALS who received care in a multidisciplinary clinic with 35 who received general care (van der Steen et al., 2009). Costs (both direct and indirect and indexed to 2003) were tracked over 3 months, and costs for the multidisciplinary group were €1,336/$1,493 compared with €1,271/$1,420, with higher costs for weaker patients as measured by lower ALS Functional Rating Scale scores.

HOSPITALIZATION COSTS

The rate and pattern of hospitalization has been found to vary among patients with ALS cared for by general neurologists compared with care by a multidisciplinary ALS clinic (Chio, Bottacchi, Buffa, Mutani, & Mora, 2006). In a study from a registry in Italy of 124 patients compared by the former and 97 by the latter, there was an average of 3.3 hospital admissions per patient followed by general neurologists compared with 1.2 admissions for patients followed by ALS clinics. Among admissions, more were for acute events among those cared for by general neurologists and more were for procedures (feeding tube or tracheal tube placement) among those cared for by ALS clinics with resultant shorter hospital stays. Survival was also enhanced.

In another study in the United States using 1996 Nationwide Inpatient Sample, which included data from 20% of US hospitals, 1,600 hospital admissions for patients with ALS were compared with 5,364,728 admissions for non-patients with ALS (Lechtzin, Wiener, Clawson, Chaudhry, & Diette, 2001). Compared with non-ALS admissions those for patients with ALS were significantly more likely to be through the emergency department, have more life-threatening issues with more hospital deaths, longer hospital days, and required more institutional after hospital care.

A recent cost analysis of ALS in the United States included direct medical and nonmedical costs and indirect loss of income (Larkindale et al., 2014). Medical costs were calculated based on commercial insurance database and Medicare claims data; nonmedical and indirect costs were determined from a survey sent to families registered with the Muscular Dystrophy Association. Most patients with ALS (83%) were covered by private insurance or by Medicare. Total annual per-capita costs (2010) was $63,693; broken down as $31,121 annual per-patient medical costs, $17,889 nonmedical costs (weighted by the need to change or modify residences because of infirmities of ALS), and $14,682 indirect costs.

HOSPICE CARE COSTS

Hospice care for ALS is difficult to calculate because the number of eligible patients for hospice care is not known, nor is the length of stay for those in hospice care. A comparison of hospice costs from one hospice program in the United States from 2001 to 2003 between 25 patients with ALS and 159 with lung cancer indicated for patients with ALS a significantly longer length of stay (87 vs. 35 days), and greater average daily expense ($65.00 vs. $76.00) attributed to greater need for durable medical equipment and staff services (Elman, Stanley, Gibbons, & McCluskey, 2006).

ALS COSTS COMPARED WITH OTHER DISEASES

Determining total care costs of rare diseases is difficult because comprehensive data are not

readily available. A cost comparison of ALS with two chronic disorders with loss of strength and function, Duchenne and myotonic dystrophies, has been made (Larkindale et al., 2014). Data were collected from insurance databases and Medicare claims and nonmedical and indirect costs were determined from family surveys; costs are from 2010 US$. Annual costs of ALS were $63,693, for Duchenne dystrophy were $32,236, and for myotonic dystrophy were $32,236. A breakdown of the total costs for ALS includes medical cost, $31,121 (medical and pharmacy claims); nonmedical cost, $17,889 (home costs, travel costs, nutritional supplements, paid aids); and indirect cost, $14,682 (family income loss).

VETERANS ADMINISTRATION AND ALS

Several epidemiologic studies show a higher incidence of ALS among US military veterans (Weisskopf et al., 2005). This led to a review by the Institute of Medicine in 2006 with the conclusion that "there is limited and suggestive evidence of an association between military service and later development of ALS." This, in turn, led the Secretary of Veterans Affairs to declare in 2008 ALS as "presumptive and compensable disease" and 100% disability benefits for veterans, who can receive their care in Veterans Administration Medical Centers and durable medical equipment and structural alterations to their home.

MEDICARE AND ALS

Patients with ALS in the United States may receive Medicare health coverage if they have contributed to the Federal Insurance Contributions Act a certain number of quarters. Those eligible receive full disability status 5 months after the diagnosis of ALS and Medicare becomes the primary insurance carrier for health care and durable equipment.

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Chapter 32

Clinical Trial Designs in Amyotrophic Lateral Sclerosis

Dan H. Moore and Mark B. Bromberg

BACKGROUND AND NEW POINTS

Clinical trials for motor neuron disease (MND) focus on amyotrophic lateral sclerosis (ALS), but progressive muscular atrophy and primary lateral sclerosis have largely been included. Drugs or interventions that treat the underlying pathology in MND are unlikely to result in improvement in strength, and a slowing in the rate of progression is more realistic. However, natural rates and patterns of progression vary markedly, challenging statistical trial design. Furthermore, with only one successful trial there is little practical experience with endpoint measures.

A goal of trial design is short duration and low number of subjects together with sensitive endpoint measures. New trial designs have been tested and several endpoint measures have been used and others proposed, but experience with achieving the goals has been hampered by ineffective drugs.

INTRODUCTION

There are several challenges in demonstrating an effective drug for MND. One is that the underlying pathophysiology is not known and mechanisms targeted by the drugs are hypothetical. A related issue is that more than 5% of ALS has a genetic basis but is indistinguishable clinically from sporadic ALS, and mechanisms for sporadic or hereditary forms may or may not overlap. A second issue is that a drug cannot restore degenerated neurons, and realistic expectations are a slowing in the rate of progress. Among the forms of MND, ALS is the most rapidly progressive and drug trials focus on ALS and not primary lateral sclerosis because of its intrinsic slow rate of progression and its rarity. Progressive muscular atrophy is usually excluded from drug trials because the El Escorial Criteria and its revisions (Brooks, 1994) were intended for clinical trials and do not have a category for progressive muscular

atrophy. A third issue is that within ALS there is a spectrum of sites of onset and rates of progression, which can influence the sensitivity and choice of primary endpoint measures. A fourth issue is that there are interventions that can prolong survival, such as noninvasive ventilation and nutritional support by gastric feeding tubes. A fifth and practical issue is the number of subjects needed to be enrolled, and several trials have required over 1,000 subjects. These challenges have not dampened clinical trial efforts, and a review of therapeutic agents for ALS in 2008 listed 43 clinical trials of 32 different compounds involving a total enrollment of more than 10,500 patients during the period 1993–2007 (Lanka & Cudkowicz, 2008), and since the review another two compounds involving another 1,000 patients were negative (Box 32.1). Despite these efforts, only one agent, riluzole, has been shown to be effective in two formal (pivotal) trials and also in trials of other drugs that were negative for the study drug but showed a positive effect for study subjects taking riluzole. Of note, the therapeutic mechanism of riluzole is not known. Thus, independent of a better knowledge of the pathophysiology or steps involved in ALS there is a need for a more efficient ways to test drugs.

CLINICAL TRIAL PHASES

Clinical trials usually proceed in a three-step sequence. Phase I trials are for drug tolerability and toxicity, and investigating pharmacokinetics of the agent. There may be a dose escalation scheme, where small groups of patients are given the same dose and, if there is no evidence of toxicity, the next group is given a higher dose. This is continued until there is evidence of toxicity and various rules are used to determine the maximum tolerated dose. In ALS, dose escalation to find the maximum tolerated dose is not often used in phase I trials, and testing different doses is deferred to a phase II trial.

Phase II trials provide the basis for deciding whether to proceed to a phase III trial, and if so, provide critical data to help design the phase III trial with respect to dose, endpoint measures, and statistical design. Unfortunately, earlier ALS phase II trials often neglected examination of effect size, biologic impact, and dose ranging. Recently, the design of phase II trials has been the focus of statistical design innovations in ALS trials and provides an opportunity to improve efficiency of drug screening by reducing the numbers of patients required for phase III trials and reducing the cost of phase II trials (Fig. 32–1).

Phase III trials are primarily designed to establish efficacy, often in hopes that it will be a pivotal trial of the agent and will receive Food and Drug Administration (FDA) approval for the disease. However, in ALS, phase III trials are often performed at a time when only animal or laboratory data provide support for proceeding to the large and expensive trial. Recent experience shows that this practice is associated with a low chance of efficacy and high costs. For a comprehensive review of clinical trials and definitions go to the website http://clinicaltrials.gov/.

CLINICAL TRIAL DESIGN

There are several trial designs and endpoint measures to choose from for ALS trials. Efforts to explore designs and endpoint measures have the goals of shortening the duration and lowering the number of subjects to provide statistically robust answers to candidate drugs with minimal time and expense. Four features of trial design are reviewed:

1. The comparison group (concurrent vs. historical controls or hybrid design including both)
2. The endpoint (mortality, rates of decline over time)
3. The method of analysis (log-rank for mortality or number of days to drop X points, linear mixed effects for declines over time, simple *t* test for number of events at a selected time)
4. The type of trial (parallel 2-arm or multiarm, cross-over, sequential, lead-in, two-stage)

COMPARISON GROUPS

Most clinical trials for ALS use a concurrent comparison group (control). If the trial is

Box 32.1 Drug Efficacy Trials for ALS, Most were Phase III

Riluzole (two trials)
Gabapentin (two trials)
Topiramate
Lamotrigine
Dextromethorphan
Talampanel
Ciliary neurotrophic factor (rhCNTF; two trials)
Insulin-like growth factor-1 (three trials)
Brain-derived neurotrophic factor
Thyrotropin
Releasing hormone
Xaliproden (two trials)
Vitamin E (two trials)
N-acetyl-L-cysteine
Selegeline
Coenzyme Q10
Creatine (two trials)
Branched chain amino acids (two trials)
Nimodipine
Verapamil
TCH346
Pentoxifylline
Minocycline (two trials)
Sodium phenylbutyrate
Cyclophosphamide
Bovine gangliosides (two trials)
Interferon-beta (IF1a)
Celecoxib
Glutathione
Oxandrolone
Tamoxifen
Lithium (three trials)
Ceftriaxone
Dexpramipexole

Modified from Lanka & Cudkowicz (2008).

blinded, the comparison group receives a placebo but subjects do not know whether they are getting the drug that is being tested or a placebo. The requirement for adequate controls in phase III trials is generally accepted. However, for phase II trials there has been discussion about the utility of using historical controls. Use of historical controls, in place of concurrent controls, is highly attractive to subjects (guaranteed of receiving active drug), and also for efficiency of design. Knowing the behavior of the comparison group instead of having to measure it in a trial results in a four-fold savings in sample size, provided that the historical control group is quite large. Half the savings comes from eliminating a new set of controls; the other half comes from not having to estimate parameters for these controls.

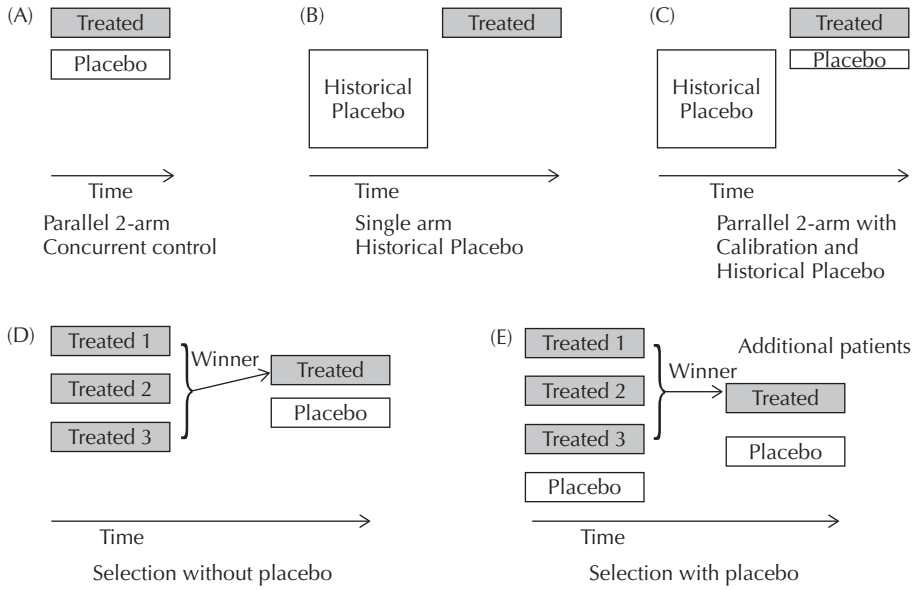

Figure 32–1. Diagrams for phase II designs. For each diagram time goes from left to right. Box lengths indicate length of trial. Box heights represent sample size. (A) Standard parallel 2-arm design where patients are randomly assigned to treatment or placebo. Results are compared at the end of the trial. (B) All enrolled patients are treated. At the end of the trial they are compared with matched historical control subjects who were placebo patients in earlier trials, indicated by the offset in time. (C) A small number of patients are randomized to placebo and compared with historical control subjects. If the control groups do not differ, they are pooled for comparison with treated. (D) Several treatments are compared and a winner is selected. Patients are rerandomized to the winning treatment or placebo. Final comparison for efficacy at the end of the second stage. (E) Several treatments are compared and a winner is selected (stage 1). New patients are recruited and randomized to the winner or placebo (stage 2). At the end of stage 2, the combined data are tested for futility. If utility is not rejected, the agent is eligible for phase III.

There is debate in the ALS drug trialist community: a 2004 consensus statement concluded that "It is not appropriate to use historical controls for comparisons, due to a large degree of variability in disease course and evolving standards of patient care" (Leigh et al., 2004). However, more recent publications support the use of historical controls, provided that they are matched with respect to symptom duration and initial clinical features that affect the outcome under study (Czaplinski et al., 2006; Gordon, 2009). Of note, the FDA accepts the use of historical controls in clinical trials under certain conditions: "Although. . . the regulations consider a historical controlled trial as a potential adequate and well-controlled trial design, this design is only acceptable when the natural history of the untreated condition (which would serve as the historical control with which to compare the treated group) is known with great precision" (Katz, 2004, p. 10).

An important point is that it is not clear that the natural history of ALS is known with sufficient precision, and there is a major concern for the potential for drift of the endpoint over time (e.g., survival), which could also bias comparisons. A study of the natural history of ALS found that survival has improved over time, but other metrics have not: the rates of decline for functionality (ALS Functional Rating Scale [ALSFRS] or ALS Functional Rating Scale-Revised [ALSFRS-R]; Cedarbaum & Stambler, 1997; Cedarbaum et al., 1999), muscle strength, and forced vital capacity (%FVC) did not change during 1990–2008 in placebo groups enrolled in different clinical trials (Qureshi, et al., 2009).

Historical controls have been used in two recent trials. Thalidomide was found to be ineffective for ALS in a small (N = 23) open label (i.e., no concurrent control) trial. The ALSFRS-R slopes for subjects in the trial were compared with the slopes of placebo controls from a previous trial of topiramate (Stommel

et al., 2009). To ensure comparability, initial clinical characteristics of subjects enrolled in the thalidomide trial were compared and matched with those of the historical topiramate placebo controls. There were no differences in slopes.

Lithium was found to be ineffective in an open label trial (N = 107) when the slopes of the ALSFRS-R of subjects on lithium were matched to placebo controls from six previous ALS clinical trials (Miller et al., 2011). Supplementary data analysis showed that the matched controls from the six studies conducted between 2000 and 2009 were similar and thus met the FDA's requirement that the historic controls are "known with great precision." No change was noted in the primary endpoint (slope of ALSFRS-R) over calendar time from 2000 to 2009. An additional comparison available for this lithium study was with another contemporaneous placebo controlled trial of lithium (Aggarwal et al., 2010), which showed that there was no difference between the randomized placebo group and the matched historical placebo database.

Historical controls that are carefully matched to subjects in a trial are less likely to suffer from the deficiencies of many historically controlled clinical trials where there has not been sufficient attention paid to matching, nor to selection of an endpoint that has been shown to remain stable over time.

There has also been discussion of a hybrid design that uses a mixture of concurrent and historical controls. This design uses only a small concurrent placebo group (called a calibration placebo group) so that most subjects are randomized to the treatment group. Two comparisons are made to determine whether the treatment shows signs of efficacy. The first comparison is a futility test of treated subjects compared with historical controls. If the treated group does not exceed the threshold for futility, then the (concurrent) calibration placebo group is compared with the historical placebo control group. If the calibration placebo group falls outside the confidence bounds for the historical placebo group, Bayesian methods are used to update progression rates for the historical placebo group and the treated group is tested against the updated information. Significance levels for the two-stage testing are adjusted so that the overall significance level is equal to the desired level (Cudkowicz et al., 2010; NINDS NET-PD Investigators, 2006). This design is currently being used for testing drugs for Parkinson disease.

Another issue to consider with comparison groups in phase II trials is whether or not subjects were taking riluzole. There is no consensus on this point, and some trials require all enrollees to be taking riluzole, whereas others stratify by riluzole usage. With stratification, it is possible to determine whether the effect of an agent is modified by riluzole usage. This may be important, because a new agent could be synergistic or antagonistic with riluzole.

ONLINE SELF-REPORTED PATIENT DATA

A unique approach to assessing drug or intervention efficacy is made available by the website PatientsLikeMe (PatientsLikeMe.com), an online patient community where patients with ALS can enter data and engage in observational studies that has been used to track medication and supplement use (Wicks et al., 2010). It was used to track changes in the ALSFRS-R score before and after taking lithium (prescribed by personal physicians), which was occasioned by the publication of an abstract showing promising results of lithium based on a small number of patients (Forani et al., 2008). The PatientsLikeMe study included 149 treated patients compared with a selection of 447 matched control patients (selected using a matching algorithm), and data at 12 months showed no treatment response (Fig. 32–2; Wicks et al., 2011). The results were available before the first of three formal studies began recruiting subjects and were confirmed by the formal trials (Aggarwal et al., 2010; Miller et al., 2011; UKMND-LiCALS Study Group, 2013). This study design has advantages of short study time without the need for multicenter and institutional review board approval, access to a control population, and cost; limitations are use of self-reported data, although the ALSFRS-R has good self-report reliability (Montes et al., 2006), lack of blinding, possible patient optimism leading to a marked placebo effect, and inability to control for certain variables. Further limitations of active subject participation is to influence the outcome of formal trials either by publishing online their functional status during the trial and making

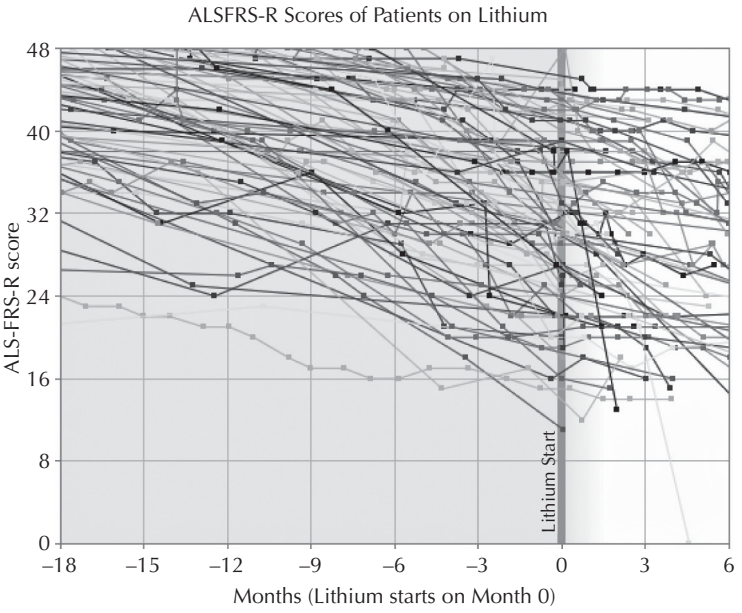

Figure 32–2. Aggregate view of ALSFRS-R scores for 348 patients with ALS who self-rated their function before and after starting lithium for ALS. (From Wicks et al., 2011, with permission.)

efforts to break the blind drug/placebo allocation. Recognition and involvement of patients in the design of studies is important to minimize patient-led "disobedience" to trial design (Wicks, Vaughn, & Heywood, 2014).

ENDPOINT MEASURES

A variety of primary endpoint measures have been used in ALS trials. Survival, or the time to need for invasive ventilation, is considered the "gold standard" because it captures the endpoint of the disease, and was the endpoint for each of the trials used in the only FDA-approved drug, riluzole. However, trials using survival require long durations and large sample sizes because the death rate per year is relatively low. Furthermore, respiratory insufficiency may be managed with noninvasive ventilation leading to prolonged survival, and nutritional supplementation by way of a gastric feeding tube may also prolong survival. As a result, more practical endpoint measures have been used in recent clinical trials. These endpoints measure loss or decline of subject's function, and trial length can be shortened because losses can be measured monthly and the slope of a line fitting functional measures versus time is significantly different from zero within 4–6 months for most patients with ALS.

Currently, the rate of decline (slope) of the ALSFRS-R is the most commonly used endpoint in several trials (Stommel et al., 2009; Miller et al., 2007, 2011; Gordon et al., 2007; Kaufmann et al., 2009; Gordon, Cheung, & Levin, 2008). The ALSFRS-R consists of the sum of ratings (scored 0, no function to 4, normal function) for 12 common activities (e.g., handwriting, walking, and breathing). Decline of ALSFRS-R has been shown to be linear with time, at least for periods up to 9 months following enrollment into a clinical trial (Traynor et al., 2004). Over longer periods of follow-up, the rate of decline is curvilinear with gradual slowing of the rate as the ALSFRS-R decreases in value (Fig. 32–3; Gordon et al., 2010). The ALSFRS-R creates other efficiencies because it does not require the subject to visit the treatment center, reducing loss of data from weak subjects. Several studies have established the reliability of the scale when assessed by telephone interviews (Kasarskis et al., 2005; Kaufmann et al., 2007; Mannino et al., 2007).

Manual muscle testing is another endpoint measure that declines as ALS progresses. It has replaced the older, time-consuming method of

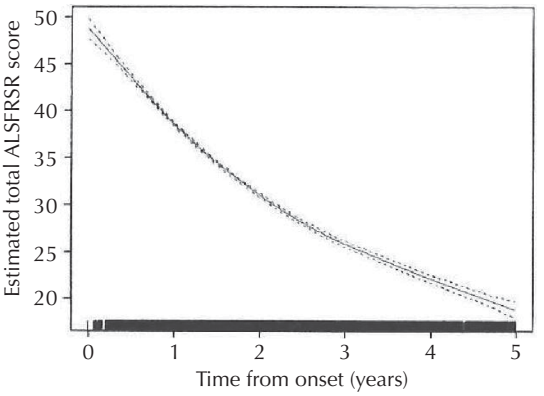

Figure 32–3. Slope of ALSFRS-R score from symptom onset through 5 years, based on linear mixed effects additive model. (From Gordon et al., 2010, with permission.)

quantitative muscle testing using fixed apparatus (exemplified by the Tufts Quantitative Neurologic Examination). Both methods require a trained evaluator to rate the subject's muscle strength for several different muscle groups because ALS characteristically begins focally. Manual muscle testing has been used as a primary endpoint in some trials (Sorenson et al., 2008) but has several disadvantages. A major disadvantage is the requirement for the patient to be measured at a center where a trained evaluator can perform the test, or for the evaluator to visit the patient. Also, there are missing data when the muscle is incapacitated (e.g., from an injury). It is more expensive to administer than the ALSFRS-R, is fatiguing to the patient, and it does not closely correlate with survival.

Other endpoints used in recent clinical trials include FVC, measured as percent of normal for healthy persons because this measure shows a decline over time. However, it requires evaluator training and quality control (Sanjak et al., 2010). Estimates of the number of motor units innervating a group of muscles (motor unit number estimation), before the muscle has lost all strength is attractive (Shefner et al., 2011). However, it can be applied only to a limited number of muscles and requires evaluator training for quality control (Shefner et al., 2011), and there are methodological issues that have resulted in modifications (Shefner, 2009). Another approach is a neurophysiologic index that combines several neurophysiologic metrics (de Carvalho et al., 2010; Cheah et al., 2011). Among these, FVC has greater within- and between-patient variation than ALSFRS or manual muscle testing, and all have been designated in most trials as a secondary endpoint measures.

A review of different endpoints (survival, ALSFRS-R, manual muscle testing, and FVC) was studied in a small (N = 30 patients) clinical trial of glatiramer acetate (Cudkowicz et al., 2006). The comparison found that the ALSFRS-R endpoint was the most reliable, easiest to administer, and correlated well with survival.

METHODS OF ANALYSIS

The method of statistical analysis depends on the endpoint, the comparison group, and the study design. Kaplan-Meier curves are used to estimate survival over time for the treatment and control groups in clinical trials with survival as the primary endpoint. A log-rank test can be used to compare survival in the two groups. An alternative is to fit a Cox proportional hazards model to the data and perform a test of the hazard ratio for treated versus control subjects using a z statistic (equal to the hazard ratio divided by its standard error) or a similar test statistic. Covariates (e.g., symptom duration, bulbar or limb onset, and so forth) can be added to the model to adjust for possible differences in the randomly assigned treatment arms (Cudkowicz et al., 2006; Gordon et al., 2007).

A linear mixed effects model has proven to be useful for analysis of continuous measures that decline with disease progression (e.g., ALSFRS-R, FVC%; Gordon et al., 2007; Miller et al., 2007). The model specifies

a mixture of fixed and random effects. Fixed effects are those that affect all patients in the study. Random effects reflect individual patient variations from the fixed effects. The simplest model includes fixed effects for intercept (initial value), slope and treatment effect on slope plus random effects for intercept and slope. Under this model the treatment effect is assumed to be the same for each treated patient, and is usually expressed as a reduction in slope. The analysis weights each patient inversely proportional to the variance of that patient's estimated slope. The equation for the simple model is

$$Y(ij) = (B0 + b0i) + (B1 + B2 * D(i) + b1i) * T(ij) + e(ij),$$

where Y(ij) is the measured score (e.g., ALSFRS-R) for the ith patient at time j; B0, B1, and B2 are fixed effects for the intercept (B0), "natural" slope (B1) and change in slope (B2) due to treatment. D(i) is the dose assigned to the ith patient and b0i, b1i and e(ij) are random effects. T(ij) is the time that measurement is made on the ith patient at the jth test, and is measured from the date of the first measurement. For trials longer than 9 months, it is useful to add an additional fixed term to allow the slope to change over time.

A key concern with this model is that patients who drop out of studies tend to have faster rates of progression, which would introduce bias, especially if drop-out rates are higher in one of the study arms. A suggestion for dealing with this is to assign a score of 0, or a score equal to the minimum of those remaining in the trial, to those who die or have tracheostomy during the trial. Assigning 0 scores to drop-out increases the variance of the slope and makes the linearity assumption for the rate of decline unlikely to be true. This method has been used in some recent clinical trials as an index of sensitivity (Miller et al., 2007; Kaufmann et al., 2009).

An alternative method for dealing with drop-outs is a rank-based procedure that allows combining deaths with other outcomes (Finkelstein & Schoenfeld, 1999). This method, identified in an ALS setting as Combined Assessment of Function and Survival, was used in a large phase III trial of dexpramipexole (Cudkowicz et al., 2011; Berry et al., 2013). Combined Assessment of Function and Survival ranks all deaths in order of their survival time, and then survivors are ranked by their slopes, starting with the fastest progressor ranking immediately after the last death. The sum of the ranks in the two treatment groups (treatment vs. placebo) are then compared using standard rank tests. Another advantage of this method is the ability to determine if a drug has a differential effect on function or survival

TYPES OF TRIALS

Randomized Trials

The most common design of ALS trials is a randomized, two parallel arm trial where one arm receives the agent being tested and the other a placebo. Traditionally, this design tests the null hypothesis of no treatment effect against the alternative that the treatment is effective. A modification, suggested for phase II trials, interchanges the two hypotheses. This is a futility design and predicts a level of improvement under the null hypothesis and the agent is advanced to further testing if the null hypothesis is not rejected. This design can increase the likelihood that agents with moderate effects are considered for phase III testing by using a one-sided test and a more liberal choice of type I and type II errors. This design was proposed for phase II trials in ALS (Palesch & Tilley, 2004) and subsequently used in some recent ALS trials (Aggarwal et al., 2010; Kaufmann et al., 2007).

Multiarm Trials

In clinical trials efficiency is defined as the number of patients required to achieve a specific power to detect a prespecified effect. Efficiency can be increased by comparing several treatments against placebo in a single trial, rather than in separate trials. Efficiency can be increased by conducting the trial in two stages. In the first stage, the treatments are compared against each other and the winner is compared with placebo or historical control to determine whether to proceed to a phase III trial. In the second stage, a futility design is used to determine whether the winning treatment in the first

stage should be tested further. This design was described in 2006 (Cheung, Gordon, & Levin, 2006) and used in a trial comparing minocycline combined with creatine versus celecoxib combined with creatine (Gordon et al., 2008), and in another trial to compare two doses of coenzyme Q10 (Kaufmann et al., 2009). The coenzyme Q10 trial was stopped after the second stage because the winner (high-dose coenzyme Q10) was declared to be futile. In the creatine trial (Gordon et al., 2008), the winning combination passed futility, when compared with historical controls, but to date no phase III trial has occurred.

In theory a selection design with up to 10 agents tested against each other (with no concurrent control) in the first stage and the winner compared against concurrent control in the second stage would reduce the total number of patients required by more than 50% compared with doing 10 individual trials (Schoenfeld & Cudkowicz, 2008). An additional benefit of a multiarm selection trial is that a large percentage of patients receive an active agent. This facilitates recruitment and retention, and the results provide a ranking of the agents. Disadvantages of a selection trial are that information about potential efficacy is limited, if no placebo group is used, and that agents that are not selected for further testing may still be effective (Cudkowicz et al., 2010).

Lead-in Trials

Another type of trial uses a lead-in design where patients are enrolled but not treated for several months, then randomized to treatment with drug or placebo for an additional period of observation (Miller & Moore, 2004; Moore & Miller, 2004). This design compares slope changes, after versus before treatment, and was used in two large trials (Gordon et al., 2007; Miller et al., 2007). Theoretically, the design is more efficient because a baseline (untreated) rate of decline is estimated for each patient and this information is incorporated into the analysis. However, results from these trials revealed that the rate of decline of ALSFRS-R (the primary endpoint for both studies) gradually increased over time for most patients. This required the addition of a fixed effect coefficient to model the increased rate of decline after randomization. Also, the variability of change in slope within patients was almost as large as between-patient variability in slopes, so that there was little gain in efficiency with this design.

The lead-in design has also been proposed for selecting patients with ALS whose rates of decline may make them more sensitive to an effective agent (de Carvahlo & Swash, 2006). Rates of decline vary widely among patients and it is difficult to show a drug effect in patients that are slowly progressing. This study showed that rates of progression for three measures of decline (ALSFRS, estimated motor unit number, and neurophysiologic index) at 1 month were significantly predictive of changes at 3 months. This suggests that a 1-month lead-in might be sufficient to identify those patients that would require shorter follow-up to see the effect of an agent. This design has not yet been tested in a clinical trial, but results from a recent trial of dexpramipexole support the utility of selecting patients based on their disease severity (this trial is discussed later in Subject Selection.)

Cross-over Trials

A cross-over design, where the treatment and placebo are switched (after a wash-out period) for patients enrolled in a 2-arm, parallel trial is recognized to suffer from several disadvantages that make it unsuitable for trials in ALS. First, because ALS is progressive with rate of decline increasing over time, patients receiving drug first may have different responses than those who receive drug later (i.e., a drug may be more, or less, effective if given early in the course of the disease). In addition, there is the added possibility of a carryover effect that is common to all cross-over designs. Nevertheless, this design has been used recently to study the effects of treatment to manage ALS symptoms (Weber, Goldman, & Truniger, 2010).

Sequential Trials

Sequential designs, where results are monitored continuously or at preplanned intervals, have not been widely used in ALS trials, although they may be worth considering. A theoretical comparison of the continuously monitored sequential design with results

from a 2-arm, parallel trial for efficacy with survival at 18 months as an endpoint revealed that the trial could have been stopped earlier for futility (Groeneveld et al., 2007). However, this trial required lengthy follow-up so that all patients had already been enrolled before the trial could be stopped. As a result there was no savings in numbers of patients, but many could have been spared lengthy treatment with a futile agent.

A recent group sequential trial of lithium was conducted by the NEALS consortium. This trial used ALSFRS-R as the primary endpoint measure, and there were three preplanned interim analyses. This trial had several innovative features. The primary endpoint event was a 6-point or greater drop in the ALSFRS-R or death. The trial was double blind so that neither patients nor those evaluating the primary endpoint knew whether the patient was receiving lithium or placebo. Patients assigned to placebo were switched to lithium, without being informed of the switch, once they registered a 6-point drop, allowing a greater percentage of patients to be treated. The trial was designed to have 80% power to detect a 40% decrease in the rate of decline of ALSFRS-R. A futility stopping boundary was defined by a one-sided p value for the log-rank test based on a comparison of the numbers of events in lithium versus placebo arms of the trial. The trial used a stopping rule for futility at the first interim analysis if the p value for the log-rank test was greater than 0.68. This trial was stopped for futility at the first interim analysis with 84 of the planned 250 patients enrolled (Aggarwal et al., 2010). It is possible that longer treatment could have proven to be effective, a disadvantage of this type of design. This is an important design consideration to take into account in sequential trials. The advantage of being able to stop a trial early when the agent seems to be ineffective must be traded off against the possibility that longer treatment is required to show an effect.

Bayesian Trials

There has been increased interest in Bayesian designs for clinical trials. In fact, a Bayesian approach has become the standard in designing clinical trials conducted at the M. D. Anderson Cancer Center (Biswas et al., 2009). This method uses three components in determining if an agent is effective: (1) an opinion about the plausibility of different values for the treatment effect (known as the prior); (2) the results from the actual clinical trial (expressed as a likelihood); and (3) an updated calculation about the treatment effect based on the first two components (known as the posterior distribution; Spiegelhalter, Abrams, & Myles, 2004). Advantages of the Bayesian approach include (1) continuous updating of information as data accumulate; (2) use of information from sources outside the trial, such as the likelihood that any agent will work, in general; (3) hierarchical modeling to incorporate information from different therapies; (4) using currently available data to calculate predictive probabilities of future outcomes; and (5) use of posterior probabilities to make inferences regarding efficacy rather than calculated values. (Biswas et al., 2009) The inferences about efficacy are generally expressed as a probability distribution that is valuable in the determining the risks and benefits of going forward with a larger study. Many articles and books have been written about Bayesian methods and the interested reader is encouraged to learn more about them. To date, we are unaware of any Bayesian trials in ALS.

SUBJECT SELECTION

Results from a recent phase III clinical trial of dexpramipexole suggest that it may be advantageous to study subgroups of patients with ALS. This trial did not show an effect in the treated population considered as a single group, in contrast to promising results from an earlier phase II trial. A comparison of the patient populations in the two trials revealed that patients enrolled in the earlier trial tended to have more advanced disease than those enrolled in the phase III trial. In particular, phase III enrollees had longer symptom duration, were less likely to be taking riluzole, and fewer of them were in the "definite" El Escorial category. When the analysis of phase III results was restricted to those in the definite category with symptom duration less than 15.3 months and who were taking riluzole, it was found that the treatment

was effective. This suggests it may be useful to test new treatments in more advanced patients first and then, if the treatment is shown to be effective, extend the trial to less advanced patients.

CLINICAL TRIAL OPERATIONS

Clinical trials in ALS require the participation of multiple clinical centers to enroll a sufficient number of subjects over a reasonable amount of time. Challenges with a trial are the start-up time for each center to enroll and subject retention. Within centers, start-up time includes establishing a financial contract and institutional review board approval. Data from five large clinical trials shows a median contract approval time of 105 days and institutional review board approval time of 125 days and a total mean start-up time of 252 days (range, 70–596 days; Atassi et al., 2013). Retention factors (cause of withdrawal from the study) are consent withdrawal, death of the subject, and adverse events, resulting in a mean attrition rate of 33%. Adequate subject retention to trial completion is important, and factors favoring retention are higher ALSFRS-R scores (supporting earlier disease burden) and longer time from diagnosis to enrollment (supporting slower disease progression). Suggestions for improved retention are shorter trials, simpler testing burden, remote assessments (telephone and telemedicine assessments), and self-administered assessments.

A further issue is whether subjects that enter drug trials are representative of all patients with ALS. A survey of 813 patients found that 66% met entry criteria (El Escorial Criteria definite, probable, probable laboratory-supported) but only 30% actually entered clinical trials (Chio et al., 2011). Those who entered trials tended to be younger, had longer diagnostic delay, were more likely to have limb onset, were more likely to be male, and ultimately had longer tracheostomy-free survival than those in an epidemiologic cohort. Inclusion/exclusion criteria for trials often include requirements for FVC values to be above a threshold and symptom duration no longer than a specified number of years. Recently, these requirements have been adjusted with FVC greater than 70–75% of predicted and disease duration of 2 years to select subjects early in the disease process to minimize drop-outs and maximize treatment effect (under the assumption that treatment is more effective early than late in the course). Minimizing drop-outs is important, especially when trials are of long duration. However, any restrictions to trial enrollment have the negative effect of selecting trial patients who are not representative of all patients with ALS.

Subject enrollment has been found to vary among participating clinical centers (Bedlack et al., 2008). Trial enrollment is low, about two per month per center, but the range was wide (0.1–7.5 per month). A number of factors likely influence enrollment rates: a high percentage of patients chose alternative therapies over formal trials, centers may vary in enthusiasm for and encouragement to enter trials,

DISCUSSION

During the past 5 years, the ALS field has seen an increasing interest in designing and implementing more efficient phase II trials, resulting in several specific changes. The use of historical controls has begun to gain acceptance, particularly in the WALS group, which has assembled a database of placebo controls from previous clinical trials. The NEALS and the WALS consortia are sharing deidentified individual patient data to determine whether outcome measures are stable over time. The shared databases have been used to measure within- and among-patient variability to select measures that are the most efficient and to determine the shape of response over time (linear vs. nonlinear). Efforts to identify factors that influence trial outcomes, such as El Escorial Criteria status, symptom duration, and vital capacity at enrollment are also important, so that studies with differing entry criteria can be compared. The knowledge gained from these studies will facilitate the use of historical controls in future clinical trials, to reduce the number of patients enrolled in trials of futile therapies, and to decrease the financial risk associated with large phase III trials.

There has also been a shift toward endpoints that can be assessed with shorter duration trials (e.g., slope of ALSFRS-R in place of survival). Several studies have shown that patients with slower rates of decline tend to survive longer than those with faster rates of decline. There is also concern that patient choice can affect survival (e.g., whether or not or when to accept artificial life support). Thus, it is reasonable to use rates of decline in place of survival. However, the optimal length of a trial using ALSFRS-R or other functional measures as a primary endpoint is not known. It may turn out that longer trials are required to give an agent time to alter the course of the disease through a beneficial effect on the motor neurons.

New endpoints, such as the neurophysiologic index (de Carvalho et al., 2003), enhanced measurement of motor unit numbers (Shefner et al., 2011; Nandedkar, Barkhaus, & Stålberg, 2010), and electrical impedance (Rutkove et al., 2007), are being identified and compared with more established ones. These new endpoints are appealing because they estimate the number of motor neurons directly rather than indirectly through loss of motor function or changes in muscle due to loss of motor units. However, to date they have been used only in studies that estimate rates of change and reliability.

Other methods to increase efficiency include a search for biomarkers that determine whether an agent is "hitting the target." This will help direct future research by resolving whether failure to show an effect is due to missing the target or the target simply not affecting disease progression. Several trials currently underway are measuring biomarkers for this purpose (Neuraltus Pharmaceuticals, Inc NP001; ClinicalTrials.gov, 2011).

Methods for analysis of data from clinical trials are becoming more sophisticated. These include increased use of the linear mixed effects model for measuring rates of decline and ranking different outcome measures into a single statistic (Aggarwal et al., 2010; Finkelstein & Schoenfeld, 1999). Bayesian methods are starting to be considered for use in ALS trials (Cudkowicz et al., 2010).

Innovative trial designs are becoming widespread. Sequential designs allow for trials to be stopped early for futility or for efficacy. ALS trialists, through their consortia, with appropriate support from the pharmaceutical industry, may also be able to test more than one drug at a time using selection designs. With increased interest in genetic and other markers, it may be possible to divide ALS into stages so that clinical trials can be more focused as they are in cancer.

Methods for increasing patient participation in clinical trials have also been considered. Some trials feature open label access to all patients upon trial completion. However, to date there has not been a comparison of recruitment in trials with versus without this feature.

Several of the designs discussed previously are diagrammatically illustrated in Figure 32–1. In conclusion, this review suggests an increasing desire to increase efficiency in testing new agents for ALS. All aspects of the design of clinical trials are being studied to achieve greater efficiency. It is hoped that this will speed the identification of useful therapies in ALS.

DISCLAIMER

The text of this chapter is an adapted and expanded version of a previous publication by the first author (Moore, Katz, & Miller, 2011).

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Chapter 33

Stem Cell Therapy for Motor Neuron Disease

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BACKGROUND AND NEW POINTS

There is no markedly effective therapy to slow the progression of amyotrophic lateral sclerosis (ALS), despite many drug trials. The mechanism of motor neuron (MN) degeneration and death is not known, but growth factors can protect against MN degeneration in cell culture studies. This has led to clinical trials of neuroprotective growth factors in ALS, but none have succeeded in slowing degeneration.

Stem cells have attractive properties as a potential treatment modality for ALS. While it is unlikely that stem cells can replace MNs that have degenerated and restore strength, both nerve and glial stem cells produce neuroprotective growth factors, metabolic factors, and immunologic factors that may be protective. Certain formidable aspects include the source of stem cells and the route of delivery; however, formal trials are underway.

INTRODUCTION

ALS involves the progressive degeneration of both upper and lower MNs. Patients present with either bulbar signs or limb-onset muscle atrophy and weakness, and symptoms progress throughout the disease course to ultimately culminate in complete loss of muscle function. The average survival following diagnosis is only 2–5 years, with death typically caused by respiratory failure or pneumonia resulting from the loss of MNs innervating the diaphragm. Riluzole, the only available treatment for ALS, acts by attenuating glutamate toxicity but only improves survival for approximately 3 months (Borasio & Miller, 2001). The ability to identify novel efficacious treatments for this devastating disease is hampered by the yet undetermined pathogenic mechanisms of ALS onset and progression. Although only 10–15% of ALS cases are familial and attributed to known genetic mutations in genes, such as Cu^{2+}/Zn^{2+} superoxide dismutase (SOD1),

TAR DNA binding protein 43 (TDP43), or hexanucleotide repeat expansions in the 5' noncoding region of the C9orf72 gene, most ALS is sporadic with no known cause (Ilieva, Polymenidou, & Cleveland, 2009; Kabashi et al., 2008; Rothstein, 2009; Sreedharan et al., 2008; van Blitterswijk, DeJesus-Hernandez, & Rademakers, 2012; Gordon, 2013). Research indicates that multiple cell types in the spinal cord, including astrocytes and glia, play a role in ALS pathogenesis (Ilieva et al., 2009; Lee et al., 2012). Several disease mechanisms, including glutamate toxicity, loss of trophic support, RNA dysregulation, and inflammation, are also probable (Rothstein, 2009; Bruijn, Miller, & Cleveland, 2004; Polymenidou et al., 2012; Evans et al., 2013), and evidence supports the contention that differing insults influence disease progression at the level of the MN cell body, distal axon, and at the neuromuscular junction (Fischer et al., 2004; Fischer & Glass, 2007; Gould, et al., 2006). This complexity makes the development of effective targeted therapies challenging; therefore, comprehensive therapies that impact multiple aspects of disease offer the best potential for improved outcomes.

Stem cells have gained tremendous popularity in the 21st century as a cellular therapy for ALS and numerous other conditions (Lunn et al., 2011a, 2011b; Miller, 2006). The term "stem cell" denotes a population of cells that maintains the ability to self-renew and differentiate along a diverse set of cellular lineages. Several stem cell classifications exist that differ in their source of origin and their differentiation capacity, including embryonic stem cells (ESCs), progenitor cells, mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs; Lunn et al., 2011a, 2011b). The cells' inherent properties, along with cell availability and supply, constitute both advantages and limitations for each class depending on the desired application. In this chapter we present the rationale behind and current progress toward the development and application of stem cell therapies for ALS. We begin by introducing the various types of stem cells, with a specific emphasis on the characteristics of the different stem cell types that support their application for mechanistic discovery and the treatment of ALS. We also introduce important considerations for cellular delivery approaches that are critical for development of cellular therapies, and present preclinical data supporting the clinical translation of stem cell therapies to patients with ALS. Finally, we assess completed and ongoing clinical trials examining various stem cell therapy approaches in patients with ALS.

CONSIDERATIONS FOR STEM CELL THERAPY DEVELOPMENT

Stem Cell Classifications and Properties

ESCs are derived from the inner cell mass of a developing blastocyst and they are pluripotent, possessing an unrestricted ability to differentiate into cells from all three germ layers (Vazin & Freed, 2010). Studies using known developmental cues have demonstrated that ESCs can differentiate into neural lineages, including MNs and other cell types that reside in the spinal cord microenvironment (Gaspard & Vanderhaeghen, 2010; Wichterle et al., 2002). The number of ESC lines available for scientific use, however, is limited due to strict federal requirements and restrictions that have hindered the development and translation of ESC-based therapies to patients. Preclinical advances in cellular transplantation research have been made using murine ESC lines, which are more accessible and easier to work with. Although these studies have shed light on the potential applications of stem cell–based therapies for ALS, the direct translational application to human patients is limited. Furthermore, it is essential to ensure there is no major risk for tumorigenicity, which may accompany the use of undifferentiated cells.

The use of progenitor cells and MSCs for disease modeling and therapeutic application is more advanced, however, and the beneficial properties of these stem cell classes for cellular therapy development are multifaceted. Progenitor cells are derived from more developed sources, either embryonic or adult, and maintain the ability to self-renew. However, the cells' potential range of differentiation is a result of their relative developmental age and source of derivation. Neural progenitor cells (NPCs) are harvested from the nervous system

of fetal or adult tissue and give rise purely to neural lineages (Kim, 2004; Xu et al., 2006). There are also populations of neural stem cells that naturally reside in the adult body, although they are typically rare and in limited supply. Within the brain, the two prominent niches of stem cells are found in the subgranular zone in the hippocampus and in the subventricular zone of the lateral ventricles (Garzon-Muvdi & Quinones-Hinojosa, 2009). These cells give rise to new neurons predominantly associated with memory and learning or olfactory function, respectively. Studies in transgenic G93A-SOD1 mice have shown that endogenous neural stem cell populations respond to MN degeneration by increasing proliferation, migration, and neurogenesis (Chi et al., 2006). Although this suggests that the body may be attempting to combat MN degeneration and restore functional neuronal circuitry, it is likely that the limited number of adult neural stem cells is not sufficient to completely halt disease progression, and with time can become overwhelmed or depleted. Similarly, alterations in the properties of these endogenous stem and progenitor cell niches in the brain of ALS mice have been reported (Liu & Martin, 2006). These data indicate, however, that NPC transplantation may simulate the natural response to MN degeneration in ALS. In vitro and in vivo data using NPCs demonstrate that in addition to differentiating into neural cells, NPCs are capable of the production and secretion of neuroprotective factors, providing support to cells within the spinal cord, and improving the toxic spinal cord microenvironment in ALS (Boulis et al., 2011). These findings are discussed in more detail later in the chapter and support the continued development and application of NPCs as cellular therapies for ALS.

Alternatively, MSCs are a more abundant and readily accessible source of multipotent, self-renewing cells that offer an option for the development of autologous cellular therapies. Derived from bone marrow, they naturally differentiate into osteoblasts, chondrocytes, and adipocytes, but recent evidence has demonstrated approaches to differentiate MSCs across lineages to establish neural cell populations (Caplan, 2007; Mezey, Mayer, & Nemeth, 2010; Satija, et al., 2009). Because they can be derived and expanded from a patient's own bone marrow, MSCs may circumvent the issue of immunosuppression when developed for patient-specific administration. Similarly, umbilical cord blood stem cells (UBCs) offer an alternate source of multipotent cells that may be harvested for cellular therapy development (Silani et al., 2004). Yet, caveats to the use of autologous MSCs and cord blood stem cells include lineage restrictions and any underlying genetic predisposition those cells carry. This feature, however, supports the use of MSCs as potential diagnostic and prognostic markers for ALS. In one study, MSCs harvested from ALS patient bone marrow exhibited specific alterations in pluripotency and growth factor secretion profiles, which may be correlated with disease progression rates and be predictive of prognosis (Koh et al., 2012). Similarly, other studies identified genes in MSCs from small cohorts of patients with ALS that may serve as novel disease biomarkers (Gupta et al., 2012, Nachmany et al., 2012). These results suggest that in addition to providing an autologous source for cellular therapies, MSCs may represent an accessible cellular option for the identification of diagnostic and prognostic biomarkers for ALS.

Finally, the recent development of and advances in iPSC technology has introduced an enticing alternative option to obtain pluripotent, self-renewing cells from nonembryonic tissue (Gunaseeli et al., 2010, Inoue, 2010; Kiskinis & Eggan, 2010). iPSCs are generated from fibroblasts obtained following a skin punch biopsy by introducing a selection of transcription factors that reprogram the cells to a more ESC-like state. Although the original approach involved the expression of *OCT 3/4, KLF, SOX2*, and *C-MYC*, various alternative combinations of factors delivered by vector, virus, protein, or RNA-mediated vehicles have now been used by several groups to successfully drive iPSC reprogramming (Cho et al., 2010; Hanley, Rastegarlari, & Nathwani, 2010; Judson et al., 2009; Yakubov et al., 2010; Yamanaka, 2008; O'Doherty, Greiser, & Wang, 2013). These cells can then be differentiated using standard neural developmental cues to establish neural populations for disease modeling and therapeutic discovery. For example, iPSCs from patients with sporadic ALS offer a means to study disease mechanisms and test therapies that could be efficacious in this more common form of ALS. Dimos et al. (2008) described the first iPSCs derived from an 82-year-old patient with ALS who exhibited a slowly progressing

disease with clear clinical symptoms, and iPSCs derived from a 54-year-old patient with ALS have also been reported (Luo et al., 2012). Both studies demonstrated that the iPS lines could be differentiated into MNs, and although long-term characterization of the cells is still in progress, these studies indicate the feasibility of developing iPS from fibroblasts from patients of various ages with neurodegenerative disease. iPS lines have also been developed from a cohort of patients with sporadic ALS and control subjects, and from a patient with familial ALS with a mutation in TDP43, and used for drug screening assays, further demonstrating the potential of iPS lines for therapeutic discovery (Egawa et al., 2012; Burkhardt et al., 2013). Despite the tremendous advantages iPSCs offers for mechanistic discovery and therapeutic screening, however, the potential for iPSC grafting and cellular therapy development is much less evolved. On one hand, autologous patient-specific cells might circumvent the need for immunosuppression with grafting and may be generated following relatively noninvasive skin punches. On the other hand, establishment of sufficient numbers of iPSCs can be slow and further characterization of cellular properties must be completed before translation of such a new technology is possible. Additional considerations include the need to understand the long-term consequences of transcription factor–based reprogramming, issues such as genetic stability and immunogenicity of the iPSCs, and the permanence of transcription factor silencing following line development (Lunn et al., 2011b; Okita, Nagata, & Yamanaka, 2011). Furthermore, the use of any ALS patient-based cellular therapy includes the risk of perpetuating any inherent predisposition to disease or global epigenetic modifications. With further advancement of the field, however, iPSC technology has the potential to provide tremendous insight into disease mechanisms that are relevant to both sporadic and familial ALS, and further development of iPS technology for ALS is warranted.

CELLULAR THERAPY EXPECTATIONS FOR ALS

When considering a cellular therapy approach using stem cells for ALS, understanding what the stem cells are expected to do is essential to determine the most appropriate stem cell type for therapeutic development. To improve outcomes in patients with ALS, one advantage to using stem cell–based therapies is the ability to replace lost or damaged cellular populations. However, it is unlikely that direct MN replacement is practical or warranted for ALS (Lunn et al., 2011b; Boulis et al., 2011; Thonhoff, Ojeda, & Wu, 2009). Transplanted MNs must project axons over long distances through inhibitory white matter in a diseased environment lacking the developmental cues that supported the original differentiation and axonal outgrowth of MNs. Although studies using mouse ESCs and MNs derived from mouse ESCs and NPCs have demonstrated that these cells are capable of extending axons peripherally and forming functional neuromuscular contacts in embryonic, injury, and disease models (Wichterle et al., 2002; Deshpande et al., 2006; Harper et al., 2004; Wichterle & Peljto, 2008; Wichterle, Peljto, & Nedelec, 2009; Yohn et al., 2008), intraspinal transplantation in G93A-SOD1 rats indicated improved motor function but no evidence of axonal outgrowth and neuromuscular junction formation, and graft survival was an issue (Lopez-Gonzalez, Kunckles, & Velasco, 2009). This is not surprising given the susceptibility of MNs to cell death under toxic in vitro conditions that mimic the ALS spinal cord microenvironment (Lopez-Gonzalez et al., 2009; Boillee, Vande Velde, & Cleveland, 2006; Clement et al., 2003; Di Giorgio et al., 2007), and supports the idea that other protective mechanisms are likely responsible for the beneficial effects of NPC transplantation on motor function following transplantation. Instead, replacement of other neuronal and nonneuronal cellular populations in the spinal cord has shown promising results in preclinical studies. As mentioned previously, multiple cell types are likely involved in ALS pathogenesis, including astrocytes and microglia, through effects on metabolic support for MNs and release of toxic metabolites (Ilieva et al., 2009; Lee et al., 2012); therefore, cellular therapies replacing glial populations may ease the demand on endogenous populations to improve the spinal cord milieu and support remaining MNs. In vivo, wild-type astrocyte transplantation is capable of attenuating the death and degeneration of MNs expressing G93A-SOD1 (Clement

et al., 2003, Boucherie et al., 2009), and transplantation of glial-restricted precursors that differentiate into astrocytes improves survival in mutant SOD1 rats, likely via restoration of glutamate transporter levels (Lepore et al., 2008). Overall, these studies indicate that stem cell transplantation and differentiation into supporting cell types may alleviate MN degeneration by preserving a less toxic spinal cord microenvironment.

Defects at the neuromuscular junction and in MN axons are a prominent early feature of ALS, occurring well before symptom onset and MN loss (Fischer et al., 2004; Fischer & Glass, 2007). These defects are associated with a loss of neurotrophic support, which indicates that neurotrophic factor production by stem cells may provide benefit in ALS. Stem cells naturally express an array of growth factors, and also respond favorably to growth factor production or treatment. A study that evaluated the effect of intrathecal infusion of epidermal growth factor and fibroblast growth factor 2 on endogenous NPCs demonstrated that growth factor treatment enhanced neuronal differentiation and migration to the ventral horn in transgenic G93A-SOD1 mice (Ohta et al., 2006). Similarly, human NPCs exhibit increased neurite outgrowth and decreased proliferation in response to increased levels of the neurotrophic factor insulin-like growth factor-I (IGF-I), as well as increased survival in the presence of glutamate (Lunn et al., 2010). Protection by IGF-I is well-established in ALS (Sakowski, Schuyler, & Feldman, 2009) and has supported clinical trials examining the efficacy of subcutaneous IGF-I treatment in patients with ALS (Borasio et al., 1998; Lai et al., 1997; Sorenson et al., 2008). Although IGF-I administration did not yield the expected outcome in these trials, it is likely that IGF-I failed to reach MNs in the spinal cord following a subcutaneous administration paradigm, a hurdle that could be circumvented by intraspinal transplantation of stem cells expressing IGF-I and other neurotrophic factors. Numerous stem cell lines have been developed that overexpress protective growth factors. Cortical NPCs engineered to express glial cell–derived neurotrophic factor (GDNF) are protective against MN death in G93A-SOD1 rats and also differentiate into astrocytes and glia, forming a multifaceted attack on the ALS disease course (Suzuki et al., 2007). Similarly, intraspinal transplantation of NPCs expressing increased levels of IGF-I or GDNF confers neuroprotection (Park et al., 2009) and intramuscular transplantation of GDNF-producing MSCs attenuate MN death and increase survival in G93A-SOD1 rats (Suzuki et al., 2008). Together, these studies indicate that restoration of neurotrophic factor levels can have a significant impact on MN health and ALS disease progression.

Stem cells also afford an array of additional benefits that may improve ALS disease outcomes. One of the many proposed mechanisms for ALS involves a role for inflammation in progressive MN degeneration (Papadimitriou et al., 2010; Pollari et al., 2011; Rizzo et al., 2014). As demonstrated in a recent study reported by Canzi et al. (2012) where intracerebroventricular injection of skeletal muscle–derived stem cells that differentiate to neural lineages improved motor function through neuromuscular junction protection and anti-inflammatory cytokine upregulation, selecting stem cell lineages that are capable of regulating inflammation may improve outcomes in ALS. Within the spinal cord, loss of functional motor circuitry is also a consequence of MN degeneration and the toxic spinal cord milieu. Studies characterizing human spinal stem cell (HSSC) transplantation in G93A-SOD1 rodents indicate that HSSCs predominantly differentiate into neuronal cells expressing GABAergic and glutamatergic markers, in addition to astrocytes (Yan et al., 2007). These neuronal cells expressed synaptophysin and integrated into the MN circuitry on transplantation (Xu et al., 2009), indicating that HSSC transplantation may represent a direct mechanisms to maintain functional connections and support vulnerable MNs in a toxic environment.

Overall, each class of stem cell exhibits an array of attributes that support their applications for cellular therapy development, and for mechanistic and therapeutic discovery through disease modeling. The advantages and limitations for each class of stem cell for applications in ALS are summarized in Figure 33–1. Stem cell therapies that are capable of alleviating toxicity, attenuating inflammation, and enhancing neurotrophic support for remaining MNs in the diseased spinal cord microenvironment may offer the best options for cellular therapy development to combat the multitude of insults MNs face during ALS.

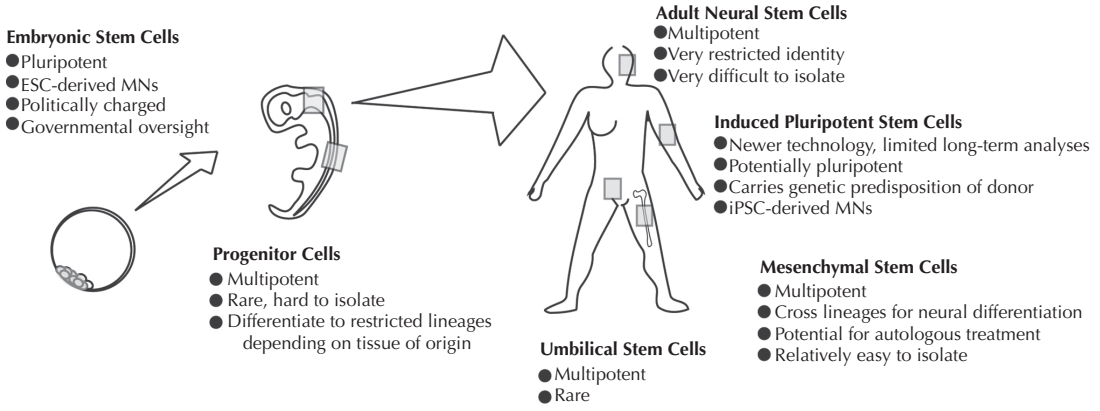

Figure 33–1. Comparison of stem cell properties. Various classes of stem cells exist, including ESCs, UBCs, MSCs, adult neural stem cells, and NPCs, which exhibit differing characteristics. Here, we highlight the beneficial features that distinguish the different stem cell classes and the suitability of each type for mechanistic and therapeutic discovery and/or as cellular therapies for ALS.

DELIVERY APPROACHES FOR CELLULAR THERAPY

Although stem cells exhibit an array of characteristics that warrant their development as a therapeutic approach for ALS, the ability to safely and accurately deliver stem cells to the precise regions affected by ALS is a delicate and important matter. Numerous approaches have been tested, including systemic stem cell administration, ventricular and intrathecal injections, and intraspinal transplantation using freehand or table-mounted injection methods (Chen et al., 2007; Deda et al., 2009; Mazzini et al., 2010). For all these approaches, however, controls and long-term data regarding the efficacy, accuracy, and safety of these procedures in patients are required. Our experience examining cellular therapy administration approaches in a large animal, the Gottingen minipig, indicates that freehand injection paradigms fail to produce sufficient accuracy and reproducibility. Furthermore, occurrences of sheering injuries, pressure injuries, and graft reflux following injection of stem cells into the spinal cord using freehand or table-mounted devices are also possible because of patient respiration, spinal cord pulsation, and other minor unpredicted movements during surgery. Therefore, a spinal cord stabilization and injection device for intraspinal therapeutic delivery that anchors to the patient vertebrae has recently been developed to offer a novel approach for controlled precise targeting of intraspinal injections (Boulis et al., 2011; Raore et al., 2011; Riley et al., 2011). This device underwent iterative changes and extensive optimization using minipigs to validate its safety for lumbar intraspinal injections, and was used in the Phase I clinical trial investigating the feasibility and safety of intraspinal HSSC transplantation in patients with ALS (Feldman et al., 2014; Glass et al., 2012; Riley et al., 2012; Riley et al., 2014; discussed later in the chapter). Depicted in Figure 33–2, this innovative device allows for minimized procedural complexity while still maintaining safety and accuracy (Boulis et al., 2011; Raore et al., 2011; Riley et al., 2011). Consisting of a platform and gondola that are mounted directly to the patient's spine, precise positioning of the injection cannula is possible while compensating for any slight movement of the platform application. A mechanical z-drive is used to precisely position the needle, which places a floating cannula into exact locations in the spinal cord. Once the rigid outer sleeve is retracted to expose the flexible catheter attached to the floating cannula, the stem cell preparation may be slowly injected into the spinal cord ventral horn at regular intervals. A pump is used to administer injections to ensure controlled and reproducible delivery rates. Measurements for injection positioning are predetermined using magnetic resonance imaging to ensure accurate anatomic targeting. Moreover, a modification of the device has been developed to accommodate cervical intraspinal injections; this device was also used

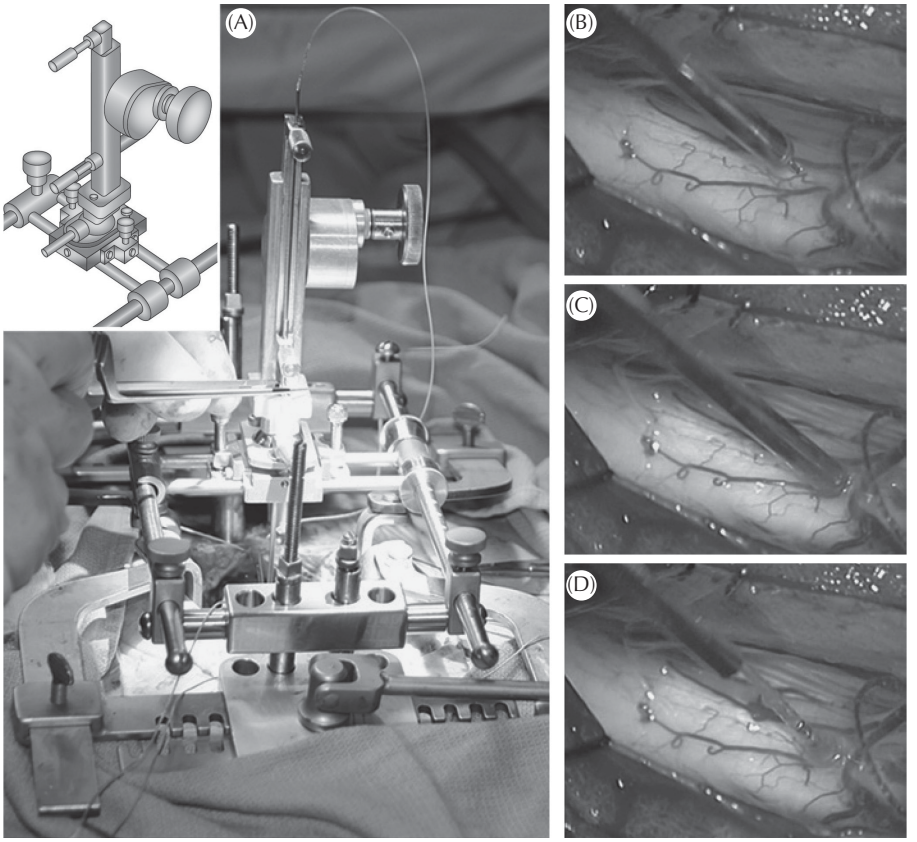

Figure 33–2. Spinal cord stabilization and injection systems for intraspinal stem cell transplantation. (A) Platform anchored to patient's spine consists of two bridge rails (blue), one of which is scored at 2-mm intervals to aid regular positioning of injections. Gondola (green) compensates for slight movements in the platform application. Mechanical z-drive (orange) allows precise raising and lowering of floating cannula. (B) Cannula tip is positioned 1-mm medial to dorsal root entry zone. (C) Needle penetrates into spinal cord ~4 mm from pial surface. (D) Once needle tip is positioned at the target, metal outer sleeve is pulled up, leaving flexible tubing exposed. (From Boulis et al., 2011.)

in the Phase I trial and is now being used in the ongoing Phase II trial (Raore et al., 2011; Riley et al., 2014). Overall, the device has applications for the delivery of a vast array of biologics to the spinal cord.

SUPPORTING DATA FOR ALS STEM CELL THERAPIES

In Vitro Mechanistic Rationale

In vitro studies using stem cells have provided important insight into the possible applications and therapeutic benefits of stem cells. The establishment of in vitro differentiation techniques to generate functional MNs from mouse ESCs, MSCs, and iPSCs has aided in the ability to generate ALS models (Thonhoff et al., 2009; Park et al., 2012; Wu et al., 2012; Hu & Zhang, 2009, 2010; Guo, et al., 2010; Gonzalez-Garza et al., 2013). MNs generated from iPSCs of a patient with familial ALS exhibit multiple pathologic features of ALS (Bilican et al., 2012), suggesting that similar studies in iPSCs from patients with sporadic ALS will enable a previously unattainable means to model this more prevalent form of ALS for mechanistic investigation and screening. In fact, drug screening in ALS patient–derived iPSCs has already demonstrated that it is possible to identify compounds that effectively rescue abnormal MN phenotypes

(Egawa et al., 2012; Burkhardt et al., 2013). On the therapeutic development front, studies characterizing MSCs from patients with ALS demonstrate that early passage MSCs are stable and express anti-inflammatory chemokines and neuroprotective growth factors, such as vascular endothelial growth factor (VEGF), supporting the use of early passage MSCs as a cellular therapy (Choi et al., 2010). Similarly, NSCs grown adjacent to spinal cord sections are capable of inducing axonal outgrowth, and addition of conditioned media from NSCs to spinal cord sections indicates that GDNF and nerve growth factor production and secretion underlie these effects (Llado et al., 2004). Cocultures of NSCs with spinal cord slices also conferred neuroprotection in the presence of excitotoxic stress, further validating that the NSCs have potential therapeutic benefit for ALS (Llado et al., 2004). A positive effect of enhanced glutamate transporter expression has also been identified in in vitro studies using primary and immortalized glial precursor cells (Maragakis et al., 2005). Together, these studies indicate that in addition to the possibility for disease modeling and therapeutic screening, stem cells exhibit a multitude of beneficial properties that may mitigate many of the proposed pathologic insults in ALS, and warrant their continued therapeutic development.

In Vivo Support for Clinical Translation

Perhaps the most convincing preclinical rationale for the use of stem cell therapies to combat neurodegeneration in ALS comes from the extensive amount of in vivo evidence supporting stem cell efficacy. These studies, which have investigated the therapeutic ability of various stem cell types, including ESCs, UBCs, MSCs, and NPCs, are summarized in Table 33–1. As mentioned previously, ESCs have been used to derive MNs for cellular replacement therapies (Thonhoff et al., 2009; Lopez-Gonzalez et al., 2009). Although motor function was transiently improved in these studies, the lack of peripheral axonal projections, a paucity of neuromuscular junctions, and issues with graft survival support the contention that mechanisms other than direct MN replacement contribute to the benefits of cellular therapies for ALS.

Studies using UBCs have provided insight into the indirect protection that stem cell therapies afford to MNs in the spinal cord. In one study, intracerebroventricular injection of UBCs demonstrated significant improvements in disease progression in transgenic ALS mice that was attributed to decreased inflammation through the production of cytokines and chemokines, despite a lack of UBCs localized in the spinal cord ventral horn (Bigini et al., 2011). Further validation of systemic effects from UBC-based therapies is seen with intravenous infusion of UBCs in ALS mice, which resulted in modulation of the immune system inflammatory response, improvements in disease progression, MN protection, and increased survival (Garbuzova-Davis et al., 2003, 2008). Direct intraspinal transplantation of UBCs also improved motor function, increased survival, and attenuated MN loss and astrogliosis in transgenic G93A-SOD1 mice (Knippenberg et al., 2012b). Furthermore, genetic modification of UBCs to express VEGF and fibroblast growth factor 2 resulted in enhanced growth factor profiles and differentiation of the UBCs to an astrocyte phenotype in the spinal cord following retro-orbital injection in an ALS mouse model, demonstrating that stem cells can serve as a vehicle to increase local growth factor production and support adjacent cellular populations (Rizvanov et al., 2011).

MSCs confer similar beneficial effects in ALS animal models. In G93A-SOD1 mice, intravenous administration of MSCs attenuated aggregate accumulation and astrocyte and microglial activation (Uccelli et al., 2012), and astrogliosis and microglial activation were also attenuated following intraspinal MSC transplantation (Vercelli et al., 2008); significantly improved motor symptoms were observed in both studies. Similarly, MSC intrathecal delivery in ALS mice resulted in increased MN numbers, slowed motor function decline, and prolonged lifespan (Kim et al., 2010). Intrathecal delivery in ALS rats promoted differentiation into astrocytes along with attenuated MN loss, lower inflammation, and improved survival (Boucherie et al., 2009). Systemic administration of MSCs that are induced with neurogenin-1 also enhanced MSC migration to the central nervous system and significantly increased motor performance in ALS mice (Chan-Il et al., 2013). Further evidence of MSC neuroprotective

Table 33–1 **Application of Stem Cell–based Therapies in ALS Models**

Cell Type	Themes	Effective*	References
ESCs	Motor neuron replacement	No	**Lopez-Gonzalez et al. (2009)**
UBCs	Reduced astrogliosis; impact on inflammation	Yes	**Garbuzova-Davis et al. (2003, 2008); Knippenberg et al. (2012b)**
	Vehicle for delivery of growth factors	Yes	**Rizvanov et al. (2011)**
	Other unknown mechanism	Yes	**Bigini et al. (2011)**
MSCs	Reduced astrogliosis; microglia activation	Yes	**Boucherie et al. (2009); Uccelli et al. (2012); Vercelli et al. (2008); Kim et al. (2010)**
	Synergistic grafting	Yes	**Forostyak et al. (2011)**
	Neural induction	Yes	**Chan-Il et al. (2013)**
	Vehicle for delivery of neuroprotective factors	Yes	**Suzuki et al. (2008); Pastor et al. (2012); Knippenberg et al. (2012a)**
NPCs	Cell integration into the spinal cord	Yes	**Xu et al. (2006, 2009, 2011); Yan et al. (2007); Lepore et al. (2011); Corti et al. (2007); Hefferan et al. (2012); Mitrecic et al. (2010)**
	Growth factor production	Yes/No	**Suzuki et al. (2007); Park et al. (2009); Klein et al. (2005); Hwang et al. (2009)**
	Addressing issues for clinical translation: cellular dosing, immunosuppression and cell graft tracking	—	**Raore et al. (2011); Hefferan et al. (2011); Yan et al. (2006); Usvald et al. (2010); Lamanna et al. (2012)**

ESC, embryonic stem cell; MSCs, mesenchymal stem cells; NPCs, neural progenitor cells; UBCs, umbilical cord blood stem cells.
* Study considered effective if modulation of at least one of the following aspects of disease was observed: onset, motor neuron loss, survival.

effects is observed following GDNF upregulation accompanying intraspinal transplantation in ALS mice (Pastor et al., 2012) and through GDNF delivery using engineered MSCs to ALS rat muscle (Suzuki et al., 2008). MSCs expressing GLP-1, which exhibits antioxidant effects and protects against excitotoxicity, also improve outcomes in ALS mice following intracerebroventricular administration (Knippenberg et al., 2012a). Likewise, synergistic effects of MSC therapy via intravenous and intraspinal administration are also observed (Forostyak et al., 2011), further supporting the efficacy of MSC-based therapies and delivery strategies for the treatment of ALS.

NPCs also harbor a diverse range of features that denote a strong ability to contest the neurodegenerative processes of ALS. One study examining glial-restricted progenitor cell replacement in ALS mice revealed that progenitor cells survive, differentiate into astrocytes, migrate within the spinal cord, and do not form tumors in an ALS spinal cord microenvironment, supporting the safety and potential capacity of cellular therapies (Lepore et al., 2011). The potential to use stem cells to deliver neuroprotective growth factors has similarly been investigated. Transplantation of NPCs engineered to express either IGF-I, VEGF, neurotrophin-3, or GDNF into ALS mice and rats via intraventricular, intrathecal, or intraspinal injection approaches indicates that engineered cells engraft into the brain and spinal cord and are capable of differentiating into neurons, oligodendrocytes, and astrocytes to protect MNs (Suzuki et al., 2007; Park et al., 2009; Klein et al., 2005) and delay disease onset, prevent apoptosis, and prolong survival (Hwang et al., 2009). Therapeutic mechanisms and efficacy have also been assessed in both ALS mice and rats. NPC grafting in the G93A-SOD1 mouse delayed disease onset and progression, resulting in a significant improvement

in survival (Corti et al., 2007), as did intraspinal NPC transplantation in G93A-SOD1 rats (Xu et al., 2006, 2009, 2011; Yan et al., 2007; Hefferan et al., 2012). Detailed characterization of cellular grafts in these rats indicated that the cells differentiated into neurons, formed axons, and synapsed with the existing host neurons to reestablish neuronal circuitry (Xu et al., 2006, 2009, 2011; Yan et al., 2007; Hefferan et al., 2012). Furthermore, transplantation into multiple spinal cord segments to target both cervical and lumbar regions enhances the therapeutic efficacy of NPC transplantation in ALS rats (Xu et al., 2011). Finally, in vivo studies using NPCs have shed light on critical issues required for clinical translation. Studies in ALS rodents demonstrated that combined immunosuppression regimens consisting of FK506 and mycophenolate or rapamycin promoted enhanced HSSC or NPC graft survival following intraspinal transplantation, respectively (Hefferan et al., 2011; Yan et al., 2006). Although clinical application for ALS uses human cells in patients, these analyses in rodents receiving human stem cell transplants provide important insight into the response to grafting. Detailed characterization of graft survival, as well as comparisons of cellular dosing, has also been assessed in a large animal, Gottingen-Minnesota minipigs. These studies in immunosuppressed minipigs characterized cell survival, density, distribution, and maturation to establish feasible and safe dosing ranges for direct intraspinal injections of HSSCs for clinical translation (Raore et al., 2011; Usvald et al., 2010). Studies in rodents and minipigs are also underway to assess the ability to track cellular grafts in vivo using magnetic resonance imaging (Lamanna et al., 2012). Together, these studies address the neuroprotective mechanisms and efficacy, as well as critical considerations for human application, which strongly support the clinical translation of cellular therapies to patients with ALS.

CLINICAL APPLICATION AND PROGRESS

MSC Trials

The clinical translation of cellular therapies for ALS is still a relatively new concept; however, a small number of trials are paving the way for the continued development and optimization of stem cells as a therapeutic entity. Although MSCs do not naturally adapt a neural fate, MSCs are readily accessible, offer a means for the development of autologous cellular therapies, and protection is evidenced in multiple in vivo studies (Table 33–1). At this point, most cellular therapeutic approaches to reach clinical testing are MSC-based trials, which are centered in various countries across the globe. A study in Israel on 19 patients with ALS and a pilot study in India on 10 patients with ALS demonstrated that intrathecal MSC delivery is safe and well-tolerated, and the study in Israel further showed that combination therapy with intravenous MSC administration is feasible (Karussis et al., 2010; Prabhakar et al., 2012). Characterization of the effects of MSC administration on the immune response in this trial ascertained that MSCs confer immediate immunomodualtory effects for patients with ALS, and demonstrated that prelabeling of MSCs with ferumoxides may provide an avenue for future cell graft tracking studies (Karussis et al., 2010). In South Korea, the safety of intraventricular MSC administration using an Ommaya reservoir was also validated, suggesting that future studies examining repetitive MSC administration would be possible (Baek et al., 2012). Injection of MSCs into the frontal motor cortex has been examined in Mexico, where a preliminary study involving 10 patients with ALS revealed that the application of autologous peripheral blood mononuclear cells is safe, well-tolerated, and efficacious, because patients receiving MSCs exhibited a statistically significant increase in survival compared with a control ALS patient cohort who did not receive the therapy (Martinez et al., 2009). The safety results were further confirmed in a larger follow-up study involving 67 patients with ALS, which exhibited a 90% survival after 1 year and a mean long-term survival rate of over 40 months (Martinez et al., 2012). Finally, intraspinal injection of MSCs has been examined in four reported trials. Two consecutive Italian trials on 19 patients with ALS demonstrated that direct injection of MSCs into the thoracic spinal cord using a table-mounted fixed-arm injection device was safe (Mazzini et al., 2010, 2012). Although postmortem analysis data was not reported for the first trial to determine survival of the cellular grafts

(Mazzini et al., 2010), and no major clinical improvement was observed in the second analysis, long-term postoperative monitoring for up to 9 years supports the safety of the approach and the need for larger future trials (Mazzini et al., 2012). Intraspinal grafting in 11 patients with ALS in Spain using a table-mounted vertical injection approach, however, supports the safety of MSC therapeutic applications in ALS and demonstrated increased MN numbers with attenuated pathologic features and no acceleration of disease progression rates, suggesting neurotrophic effects of MSC grafting (Blanquer et al., 2010, 2012). Lastly, a trial in Turkey injected MSCs into the high cervical spinal cord of 13 patients with ALS and demonstrated safety with positive effects on electroneuromyography measures in most patients at 1 year (Deda et al., 2009). Overall, much larger controlled trials are necessary to accurately determine the best approach for MSC delivery and evaluate the efficacy of MSCs for the treatment of ALS; however, the safety evidenced in these initial steps translating MSC-based cellular therapies to patients with ALS indicates that continued development of MSC therapies is warranted.

Neural Stem Cell Trials

As described, the therapeutic efficacy of NPCs is well-supported by in vitro and in vivo studies (Table 33–1), and NPCs are capable of mitigating a variety of the proposed insults complicating ALS pathogenesis. Specifically, the NSI-566RSC HSSC line differentiates into neural cell types, produces neurotrophic factors, forms synapses with host neurons and integrates into the neural circuitry, attenuates MN loss, and improves disease symptoms and survival following intraspinal grafting in ALS rodents (Xu et al., 2006, 2009, 2011; Yan et al., 2007; Hefferan et al., 2012). Based on the amount of preclinical support for HSSCs, along with the design, optimization, and validation of an innovative device to safely administer cellular therapies directly to the spinal cord (Fig. 33–2), in 2009, the Food and Drug Administration approved the first Phase I trial investigating the safety and feasibility of intraspinal HSSC transplantation in patients with ALS in the United States. The trial was designed to follow a risk-escalation paradigm, where the relative risk of surgery increased across cohorts, as measured by patient disease severity, the targeted spinal cord region, and the number of injections (Lunn et al., 2011a, 2011b; Boulis et al., 2011). Twelve individuals with ALS, beginning with six nonambulatory patients followed by six ambulatory patients, received either 5 unilateral or 10 total bilateral injections of 100,000 cells per injection into the lumbar spinal cord (Lunn et al., 2011a, 2011b; Boulis et al., 2011). Results indicate that the approach and introduction of stem cells into the spinal cord is safe and well-tolerated (Glass et al., 2012; Riley et al., 2012). Although this was a safety trial, patient symptom monitoring revealed no acceleration of disease progression in any patient and one patient exhibited improvement of clinical symptoms; however, larger studies are required to accurately assess efficacy of the intervention (Glass et al., 2012). Because there was no previous data on the immunogenicity of HSSCs in patients with ALS available before the trial, results from the first 12 patients indicted that no HLA antibodies against the cells were detected in any patient, including those who discontinued or reduced dosing of the immunosuppressive drugs because of immunosuppression-related toxicity; however, postmortem analyses are required to fully assess cellular graft survival in these patients (Glass et al., 2012).

Based on the positive safety results from the first 12 patients receiving intraspinal lumbar HSSC transplants, Food and Drug Administration approval for six additional patients was also been granted to examine the safety and feasibility of targeting HSSC transplants to the cervical spinal cord. Cervical intraspinal HSSC transplantation has the potential to support critical MNs controlling the diaphragm, because most ALS patient deaths result from consequences of diaphragmatic innervation loss. Design iterations to the spinal cord stabilization and injection device were made, optimized, and validated in minipigs to support the safety and accuracy of cervical intraspinal injections (Raore et al., 2011; Usvald et al., 2010). These final six surgeries have been completed, and all six patients received five unilateral HSSC injections of 100,000 cells per injection in the cervical spinal cord, with the final cohort including three individuals that previously received lumbar stem cell transplants. Data analysis

and detailed trial outcomes demonstrate that no major adverse effects of the surgery have been observed, suggesting that the approach is safe, feasible, and well-tolerated (Feldman et al., 2014; Riley et al., 2014). Based on the encouraging data from Phase I, Phase II of the trial commenced in 2013 and is currently underway. With continued progression and completion of this and future clinical trial phases, it is hoped that this trial will provide important and groundbreaking insight into the development of novel therapies for the treatment of ALS.

LOOKING INTO THE FUTURE

Although remarkable progress has been made in recent years concerning the characterization and translation of stem cell–based therapies for ALS, there are still many aspects of cellular therapy development that are left to be determined. Techniques to reliably track and monitor stem cell grafts in vivo and in patients with ALS are already being developed (Usvald et al., 2010; Lamanna et al., 2012), but continued optimization of these novel approaches will provide tremendous insight into the migratory potential, distribution, and survival of cellular grafts. Similarly, continued investigation into the neuroprotective benefits conferred by stem cells will improve the understanding of not only ALS pathogenesis, but also how to enhance therapeutic approaches to achieve maximal benefit. Along these lines, stem cell therapies targeted at multiple spinal cord segments have exhibited increased protection in animal models (Xu et al., 2011), and the ability to safely target both lumbar and cervical spinal cord segments in patients with ALS has been demonstrated in the Phase I trial with HSSCs described above (Glass et al., 2012; Riley et al., 2012). Combination therapies using stem cells to deliver neurotrophic support in addition to cellular support are also supported by in vivo evidence and may be a viable possibility in the future, because trials examining growth factor therapies in ALS and stem cell therapies in ALS have already been completed or initiated (Borasio et al., 1998; Lai et al., 1997; Sorenson et al., 2008; Glass et al., 2012; Riley et al., 2012). Targeting therapies to both MN cell bodies in the spinal cord and to neuromuscular junctions may also be warranted based on in vivo data, because protection of neuromuscular junctions has established efficacy in ALS (Suzuki et al., 2008; Krakora, Macrander, & Suzuki, 2012) and attenuation of MN loss in the spinal cord is not always sufficient to impact motor function or survival (Fischer et al., 2004, 2007; Gould et al., 2006). Understanding the requirements for immunosuppression is also essential to establish future stem cell–based therapy approaches for patients with ALS. In rodents, immunosuppression improves survival of human cell grafts (Hefferan et al., 2011; Yan et al., 2006); however, many current trials are assessing and characterizing the effect of autologous patient-specific MSCs or HSSCs where cross-species incompatibility is not an issue. Continued analyses, including determination of graft survival and characterization of local immune responses in postmortem tissues from ongoing and future trials, will provide important insight into requirements for immunosuppression in future clinical applications. Finally, further development and characterization of alternative stem cell types, such as iPSCs, may provide another more accessible resource for cellular therapy development. Overall, although these are just some of the issues at hand, continued progress and advances in technology are driving the development of clinically relevant stem cell therapies for ALS. Incredible strides have been made toward this goal in the past decade, and with vigilant attention to the issues at hand, meaningful advances toward much-needed treatment options for patients with ALS can be made.

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