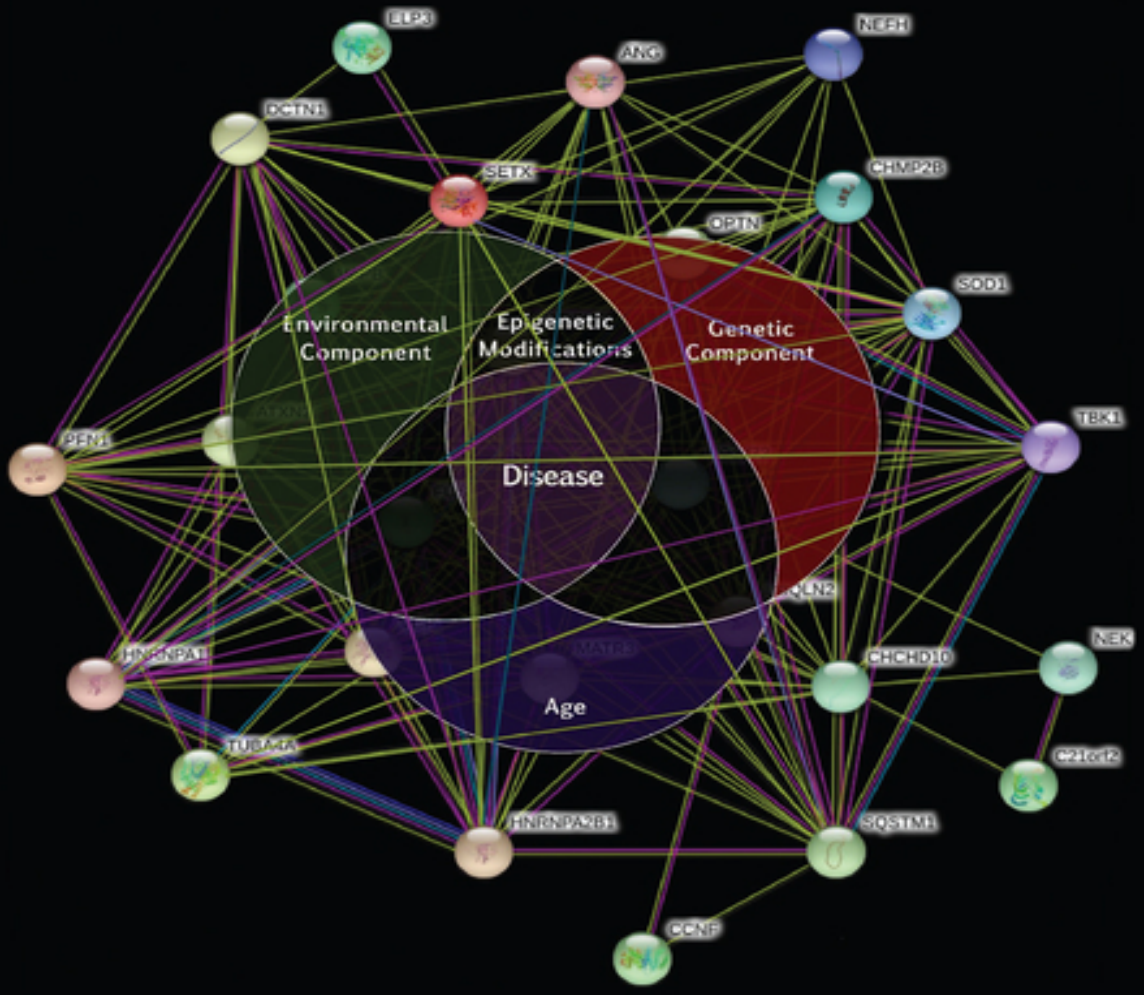
SPECTRUMS OF AMYOTROPHIC LATERAL SCLEROSIS

HETEROGENEITY, PATHOGENESIS AND THERAPEUTIC DIRECTIONS



EDITED BY
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This book is dedicated to people living with ALS, their families,
and those who care for them.

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Foreword

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The past decade or so has seen a substantial increase in the extent of research directly or indirectly related to amyotrophic lateral sclerosis (*ALS*). Unfortunately, this research has had limited impact on the clinical course of patients with ALS, suggesting that in many fundamental ways we do not really understand this disease. Numerous observations still defy a clear explanation. For instance, how is it that mutations in various genes, seemingly without a clear interaction in a signaling cascade or pathophysiologic mechanism, all result in a disease with a superficially similar phenotype, a phenotype that is shared with patients where no known gene mutations are present? How is it that the rate of progression of ALS is so rapid and unresponsive to modulation in some patients, yet a lucky few will have the disease course slow substantially? Why are there specific patterns of nervous system involvement in ALS such as “classic” ALS (Charcot type), bulbar ALS (perhaps better described by the original name of “glosso-labio-pharyngeal paralysis”), progressive muscular atrophy, and primary lateral sclerosis? What is the relation between the loss of motoneurons and their axons and the progressive decline in corticospinal and other descending connections? What is the basis of fasciculations? How does

ALS “spread” so rapidly in the nervous system? These and other questions remain unanswered.

It is also interesting to look back at how our view of ALS research has changed over time. A clinician or scientist of 25 or 50 years ago would not have seen much investigation into ALS. To those of us who were involved with ALS at that time, the disease appeared neglected. Potentially, to a researcher investigating ALS 50 years ago, it also might have seemed that a treatment for this disease would be relatively straightforward, compared to the treatment of other neurological diseases like Alzheimer's disease or Parkinson's disease. ALS was characterized by the loss of neuronal populations that were well studied, even decades ago, and affected cells might be amenable to the delivery of intrathecal or intramuscular treatment to augment the health of dying neurons and so prolong patient survival.

How times have changed! Instead of being a neglected disorder, there has been considerable scientific and public interest in ALS, due not only to events like the Ice Bucket Challenge, but also to social media and increasing public awareness. Second, the initial hopes that the disease would turn out to be treatable and responsive to trophic molecules and other factors that would improve the “metabolism” of motoneurons have not yet borne fruit. In retrospect, it seems clear that given the complexity of motoneuron physiology, the difficulty of successful treatment may not have been fully appreciated. Furthermore, the scientific community generally has woken up to the challenge that ALS poses, and many labs around the world are investigating aspects of the disease: the genetics of ALS, the relation between viruses and ALS, RNA-binding proteins, risk genes and environmental toxins, as well as other topics that are reviewed in the present volume.

We can only hope that this new volume will be a stimulus for continued research on ALS and result in insights into this enigmatic, frustrating, and tragic illness.

Preface

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All humanity is on a train speeding through time. The name of the train is life. And like a train you might see in India, it's covered with people, inside and out. People inside are seated in different classes and are engaged in all manner of activities. The people on the roof would love to be inside. They are the sick. The wind buffets them, the rain drenches them, and the sun beats down on them. And each time the train jostles or turns, they have to quickly cling on to prevent them from sliding off and ending their journey.

The terminally ill cling precariously to the side of the train. They try to find perches on the thin window ledges or doorway openings. Some of them have ALS. They are exhausted from the relentless wind and weather, from

standing, and from the strain of grasping whatever they can to keep from falling. Often the exhaustion is so great that they feel it might be easier to just let go. But something miraculous happens. People inside the train have given up their seats, walked over to the windows, and put arms around those desperate people. They say, “Don't worry, I have you. Relax for a while, and I'll hold on to you.”

Who are these kind people? They are like those from the ALS Clinic or the ALS Society or its donors. By vocation, by volunteering, or by donating, they give help to people who urgently need it.

ALS patients like me need much more than the love and support of their care givers and healthcare providers. We need hoists and slings to move us; specialized wheelchairs to help us to get around; and hospital beds for support, care, and comfort. As our needs grow more complex, the list gets longer and more expensive. But this equipment often makes the unbearable bearable. Some of it literally keeps us alive.

Please donate to the ALS Society of British Columbia. When you do, you are saying, “Hang on, fellow traveler: I see that you need help. Grab my arm.”

Typed on my eye gaze computer.
Ted Stehr

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CHAPTER 1
Clinical Heterogeneity of ALS – Implications for Models and Therapeutic Development

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) was first described in 1874 as a specific neurological disease by the French neurologist Jean-Martin Charcot, who chose this term to reflect both clinical observations and post-mortem pathological findings. *Amyotrophic* refers to clinical evidence of muscle atrophy as a consequence of the loss of lower motor neurons (LMNs). *Lateral sclerosis* refers to the pathological observation of hardness of the lateral columns of the spinal cord, following upper motor neuron (UMN) degeneration [1]. UMN degeneration is followed by the formation of a sort of scar. The disease leads to progressive paralysis, with death occurring due to respiratory failure within three to five years after symptom onset.

The classical form is characterized by the concomitant involvement of UMNs in the cerebral cortex and LMNs located in the brainstem and the spinal cord. Clinical manifestations of UMN damage are loss of dexterity of the hands and spastic gait associated with overactive tendon reflexes. These signs are frequently associated with pathological reflexes, including Chaddock and Babinski signs (extension of the big toe after rubbing the lateral malleolus and the sole of the foot, respectively) and Hoffmann sign (flexion and adduction of index finger and thumb when flicking the nail of the middle finger downward). Corticobulbar involvement leads to slurred speech and difficulty swallowing, often with pathological crying and laughing. The consequence of LMN degeneration is weakness, which may involve any muscle of the body including those of the tongue, pharynx, or larynx (innervated by bulbar motor neurons); those of upper and lower

limbs; and the respiratory muscles. Oculomotor and Onuf's motor neurons are usually spared. Muscular atrophy, reduced reflexes, and signs of hyperexcitability in motor neurons, such as fasciculation and cramps, are additional features of LMN degeneration.

The combination of the these symptoms and signs of UMN and LMN dysfunction results in a peculiar and stereotypical picture, which in most cases is easy for expert clinicians to identify. However, there is an evident clinical heterogeneity among ALS patients, which is determined by several independent elements. The age of onset and survival, two major phenotype features, show a marked variability among patients. Furthermore, the relative number of UMN and LMN signs may show substantial differences. An additional contributor to this heterogeneity is the evidence that the types of cells impaired in ALS may extend beyond UMNs and LMNs to include the frontal and temporal cortex, extrapyramidal system, peripheral nerves, and skeletal muscles, giving rise to variable and sometimes overlapping phenotypes.

Finally, genetic research has revealed that ALS is linked with several causative genes – a list that will probably increase in the coming years due to the rapid improvement of next-generation sequencing technologies. ALS-related genes are implicated in various cellular functions, including RNA metabolism, autophagy, and axonal transport, suggesting significant heterogeneity in disease mechanisms as well.

Thus, it appears that ALS is used as an umbrella term referring to a spectrum of disorders with diverse clinical manifestations, heterogeneous disease mechanisms, and (probably) different responses to therapies. On the other hand, all ALS patients, except carriers of superoxide dismutase 1 (*SOD1)* and fused in sarcoma (*FUS)* variants, appear to be unified by a single pathological signature: the presence of abnormal accumulation of the transactivation response DNA binding protein (TDP-43) in the cytoplasm of neuronal and glial cells [2].

CLINICAL HETEROGENEITY OF ALS

Familial and Sporadic ALS

The disease occurs sporadically in the majority of cases (sporadic amyotrophic lateral sclerosis [sALS]), and nearly 10% of patients have a positive family history (familial amyotrophic lateral sclerosis [fALS]) [3]. However, the dichotomy between fALS and sALS is less clear than previously assumed, since several clinical, pathological, and genetic observations support the view that they are linked with each other over a continuum. From a clinical point of view, patients with sALS are indistinguishable from those with fALS. Both conditions show similar pathological patterns – the presence of ubiquitinated TDP-43 positive

inclusions in neuronal cells – with the only exception being patients with *SOD1* and *FUS* mutations in which the SOD1 and FUS proteins are detected, respectively [4]. Importantly, fewer than 50% of fALS patients show a clear Mendelian inheritance, usually autosomal-dominant (definite fALS). In the remaining fALS cases, the genetic architecture is less clear as familiarity is assumed by the presence of a single relative with ALS beyond the propositus. These cases are defined as probable fALS when the affected subject is a first- or second-degree relative and possible fALS when the subject is more distant than second-degree. Finally, the most consistent link between sALS and fALS is the observation that all genes involved in fALS are invariably found to be mutated in patients with apparently sporadic disease [3]. Genetic variants in major ALS genes have been detected in about 15% of sporadic forms [5, 6].

Age of Onset

ALS affects people of all ages, with a peak between ages 60 and 79. Recent population-based studies reported a prevalence of ALS between 4.1 and 8.4 per 100 000 [7]. Patients with onset in the first two decades are extremely rare; such cases are termed *juvenile ALS*. This appears to be a different condition than classic ALS as it is familial in most cases, generally has autosomal recessive inheritance, and shows a very prolonged course. Patients with onset between 20 and 40 years are said to have *young-adult ALS*; this is otherwise classic ALS, although it has peculiar clinical features including predominant UMN signs, male prevalence, and more prolonged survival (usually greater than five years). It remains unclear whether distinctive clinical features of young-adult ALS are related to a different disease mechanism. Finally, very rare patients with onset before 20 years show an otherwise classic ALS with sporadic occurrence and an aggressive course. Most of the reported cases harbor a *de novo* mutation in the *FUS* gene.

Survival

The median survival of ALS is approximately three years from the onset, and about 70% of patients die within five years from onset. However, the duration of the disease differs widely in individual patients, ranging from a few months to over 10 years. Such remarkable variability is a major factor in favor of the hypothesis of ALS as a syndrome rather than a single disease. Median survival is worse in patients with bulbar onset ALS than with the spinal onset. Patients with disease onset before the age of 40 and patients with predominant UMN signs show a better prognosis. In most ALS patients, the cause of death is respiratory failure due to the degeneration of motor neurons controlling thoracic and diaphragmatic muscles. Of note, both the temporal and spatial patterns of the disease spread are important determinants of survival. Regarding the temporal

pattern, the spreading rate of the degenerative process may vary among patients, with some patients showing a very rapid, aggressive course and others a slow progression. The spatial pattern is also important, since the sequence in which various body regions are involved is extremely variable and the survival changes if respiratory muscles are among the first or last to be affected.

Classic ALS, LMN Form, and UMN Form

By definition, ALS is characterized by a combination of LMN and UMN clinical and electrophysiological signs. However, the relative mix of UMN and LMN impairment is highly variable among patients, and clinical manifestations of ALS exist on a continuum whose extremes are represented by cases showing pure LMN dysfunction on one side and cases with pure UMN signs on the other side. Classic ALS (Charcot type) is the most frequent form, accounting for about 70–90% of cases, and is characterized by predominant LMN signs combined with slight to moderate pyramidal signs. Patients with pure LMN signs without any accompanying clinical or electrophysiological UMN signs are labeled as having progressive muscular atrophy (PMA) and represent about 5–10% of cases. However, the demonstration that UMN pathology is present at autopsy in 50% of PMA patients indicates that, in at least some cases, pyramidal signs are simply masked by LMN dysfunction on both clinical and electrophysiological grounds. For this reason, the presence of preserved but not hyperactive reflexes in atrophic limbs should be interpreted as UMN impairment. PMA and ALS are not distinct entities, as they show significant phenotypic and genetic overlap. About 2–5% of patients with motor neuron disease show a pure pyramidal form with predominant spino-bulbar spasticity, known as primary lateral sclerosis (PLS). The onset of PLS is generally after 40 years, and the disease duration is significantly longer than in classic ALS. A small proportion of PLS patients develop a clear ALS phenotype, usually within three to four years from the onset, while others show only minimal LMN impairment; most cases remain PLS for decades. ALS patients with predominant pyramidal signs consisting mainly of severe spino-bulbar spasticity are said to have upper motor neuron-dominant amyotrophic lateral sclerosis (UMN-D ALS). These signs are associated with slight LMN signs, usually in the hands. This phenotype is frequent in the young-adult group and males, and it has a better prognosis than classic ALS [8–10].

Site of Onset

ALS begins focally at a seemingly random location and progresses to involve other body regions through anatomically connected pathways and/or neighboring regions. Approximately one-fourth of patients show initial manifestations in the muscles innervated by motor neurons residing

in the medulla (bulbar onset), one-third in the upper limb muscles, and one-third in the lower limb muscles whose motor neurons lie in the spinal cord (spinal onset). A small proportion of patients (2–5%) show respiratory symptoms at presentation. These cases are often difficult to diagnose because the absence of additional neurological signs can be misleading. The clinical phenotype at the onset, when temporal–spatial summation hasn't yet occurred, together with additional characteristics, may be important tools to delineate peculiar phenotypes, including spinal, bulbar, pseudopolyneuritic, emiparetic, and flail-arm forms. It remains to be clarified if these clinical pictures correspond to distinct nosological entities or are the simple consequence of stochastic phenomena.

Bulbar ALS usually presents with dysarthria and dysphagia due to a variable combination of impairment of LMNs located in the IX, X, and XII nuclei and of the corticobulbar fibers. Bulbar symptoms and signs may be the only manifestation for several months before limb symptoms occur and when only corticobulbar signs are present, the diagnosis of ALS is frequently overlooked. Bulbar onset is more frequent in females and has a worse prognosis than the spinal onset form. In pseudopolyneuritic ALS (Patrikios' disease), weakness and atrophy start in distal limb muscles with frequent absence of tendon reflexes, thus mimicking a neuropathy [11]. The flail-arm form (Vulpian-Bernhart syndrome) is characterized by symmetric, predominantly proximal, wasting and weakness of both arms with relative sparing of lower limbs in the initial phases. This ALS form is prevalent in males, starts after the age of 40, and shows a slightly slower disease progression than classic ALS [12, 13].

Diagnosis of ALS

To date, there are no reliable diagnostic tests for ALS, and clinicians rely on the clinical evidence of a combination of UMNs and LMNs in the same body region, electromyographic confirmation of ongoing LMN degeneration, and the exclusion of mimicking conditions. Motor multifocal neuropathy, Kennedy disease, inclusion body myopathy, Sandoff disease, Morvan syndrome, paraneoplastic encephalomyelitis, inflammatory multineuropathies, and compressive myelopathies are conditions that may be confused with ALS and should be accurately evaluated. Criteria for the diagnosis of ALS have been established and are known as the El Escorial criteria, but they are more useful in the research field than in the clinical setting [14, 15].

ALS and Its Relationship with Frontotemporal Dementia and Myopathies

ALS has long been considered a paradigm of pure motor neuron disorder. However, genetic discoveries have shown that other cell types may be involved, linking ALS to other diseases. The most common and well-

established condition connected with ALS is frontotemporal dementia (FTD). Frontotemporal lobar degeneration (FTLD) consists of the degeneration of the frontal and temporal lobes of the brain, leading to atrophy, and occurs with an incidence of 3.5–4.1/100 000 per year in individuals under 65 [16, 17]. Clinically, this is the second most common cause of early-onset dementia, referred to as FTD, and is familial in 20–30% of cases. Variants of FTLD have been described based on clinical signs. Behavioral variant frontotemporal dementia (bvFTD) is the most frequent form and is characterized by behavioral problems – apathy and disinhibition – and a decline in executive functions. Progressive nonfluent aphasia (PNFA) is characterized by language problems including nonfluent speech, dysarthria, poor articulation, and agrammatism with preserved comprehension. The third variant is semantic dementia (SD), also called progressive fluent aphasia (PFA), characterized by the loss of semantic and conceptual knowledge. All these FTLD variants have been described in patients with ALS.

Insoluble proteins aggregate in the neurons of patients with FTLD, leading to three different pathological variants: FTLD-Tau, characterized by the accumulation of the microtubule-associated protein and often by mutations in the gene encoding for the same protein (*MAPT*) (~30–40% of cases) [18]; FTLD-FUS, containing the FUS sarcoma protein (~10% of cases) [19]; and the most frequent, FTLD-TDP, with TDP-43 aggregates (~50–60% of cases) [18–21].

From a clinical point of view, FTD and ALS overlap since 15–18% of ALS patients have FTD and 15% of FTD patients show motor dysfunctions [22, 23]. ALS and FTD also share genetic and neuropathological features, thus leading to the definition of the ALS/FTD spectrum where ALS and FTD are the extremes of a continuum. From a genetic point of view, this idea has been consolidated by the identification of the gene *C9orf72* [24, 25], whose pathogenic expansion has been described in 30–50% of fALS, 25% of familial FTD, 5–7% of sALS, and 6% of sporadic FTD cases in different populations [26, 27]. Furthermore, other genes have been associated with the ALS/FTD spectrum: *TBK1, TARDBP, FUS,* and *SQSTM1* [28]. Finally, with regard to neuropathology, TDP-43 inclusions in neuronal cells are a hallmark of ALS as well as of a proportion of FTD.

Recent genetic evidence, along with clinical and pathological observations, indicate that ALS may be linked to primary muscle disorders as well. Mutations in valosin-containing protein (VCP), previously identified in a proportion of patients with hereditary inclusion-body myopathy (IBM), were later detected in a subset of sALS and fALS cases [29]. Additional genes, including *MATR3, hnRNPA1, hnRNPA2B1,* and *SQSTM1,* have been identified, which are responsible for an ALS/myopathy spectrum with overlapping phenotypes [30–32]. Interestingly, most myopathies associated with ALS are distal myopathies with evidence of rimmed vacuoles at muscle

biopsy. These structures represent the accumulation of autophagic vacuoles due to lysosomal dysfunction or protein accumulation.

Paget's disease of the bone, extrapyramidal syndromes, psychiatric disorders, and peripheral neuropathies are additional conditions that are mechanistically linked to ALS. The spectrum of clinical phenotypes associated with major ALS-associated genes is listed in Table 1.1 [24, 25, 29, 30,32–66].

PLEIOTROPY OF ALS GENES

SOD1 is the only ALS-associated gene that has been associated exclusively with an isolated motor phenotype. A common phenomenon for all other ALS genes is pleiotropy, which means a genetic variant can be associated with multiple phenotypic traits. The same genetic variant can cause not only different ALS subtypes in families, in terms of age of onset and disease course, but also different diseases. Examples of pleiotropic genes are *C9orf72* and *VCP*. In the same family, *C9orf72* carriers can have only ALS, only FTD, or overlapping ALS/FTD phenotypes. Furthermore, the same pathogenic variant in *VCP* has been detected in patients with ALS, FTD, IBM, and Charcot-Marie-Tooth type 2 (CMT2) [67]. The opposite is also true: different pathogenic variants in the same ALS-associated gene can cause an identical phenotype.