Amyotrophic lateral sclerosis: new genes, new models, and new mechanisms
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Abstract
Research aimed at understanding amyotrophic lateral sclerosis (ALS) has seen exceptional growth in the past few years. New genes, new models, and new mechanisms have not only improved our understanding, but also contributed to the increasing complexity of ALS pathogenesis. The focus of this piece is to highlight some of the more notable developments in the field and to encourage a re-appreciation for the superoxide dismutase 1 (SOD1) mouse models.

Introduction
In 1869, the French neurologist Jean-Martin Charcot described a fatal and progressive paralysis known as amyotrophic lateral sclerosis (ALS). This devastating neurodegenerative disease, also known as Lou Gehrig’s disease (USA) or motor neuron disease (UK), has an incidence of 2–3 cases per 100,000 annually. In the United States, 25,000 patients are predicted to be affected at any one time, with only 50% of patients surviving 2–3 years after diagnosis. Patients experience muscle weakness and atrophy, leading to inevitable paralysis, due to the selective and progressive loss of motor neurons of the brain and spinal cord that is characteristic of the disease [1,2]. Most often, patients succumb to the disease because of denervation of muscles involved in respiration. Despite decades of research, we have yet to identify the “silver bullet” to cure, or even slow, the disease. There is only one therapeutic option for patients, riluzole, which offers a modest 3- to 6-month extension in survival [3,4]. The current lack of effective therapeutic options for patients is due, in part, to the general lack of understanding of how the disease initiates and progresses. However, in the past few years, there have been a significant number of advances that are expected to shed new light on this disease.

A total of 10% of all ALS cases are genetically inherited, while the remaining 90% have no known cause [1]. Of the familial cases, mutations in the gene encoding superoxide dismutase 1 (SOD1), one of the three superoxide dismutases responsible for destroying free superoxide radicals in the body, first described in 1993, are the most common [5]. So, it is not surprising that the bulk of research has focused on examining the consequences of pathogenic SOD1 mutations in a variety of models. Some researchers believe, maybe too cynically, that the inability to find an effective therapy is because the field relies too heavily on SOD1 mouse models; this cynicism stems from the fact that only a subset of ALS cases are due to mutations in this particular gene. Regardless, using SOD1 animal models, scientists have pushed the field forward by dissecting out a variety of molecular events in affected motor neurons [6].

New genes
In the past 3 years, two novel genes have emerged on the scene, bringing with them a new, previously little-considered mechanism of RNA metabolism and processing. The protein products of these new genes, TARDBP (encoding TAR DNA binding protein-43, or TDP-43) and FUS (also known as TLS, which encodes RNA-binding protein FUS), are known to participate in a multitude of
functions [7]. While these two genes appear (at first blush) to implicate very different mechanisms than those identified using the SOD1 models, much work remains to be done to define the pathogenic mechanism(s) affected by these mutations. In the meantime, there has been a veritable stampede in the development of animal models to investigate these new genes. How, and if, these different genetic causes converge on the selective killing of motor neurons in ALS, yielding a more or less clinically indistinguishable phenotype, is an important question in the field. Identifying the common link between them is a major research focus as this intersection would be expected to be an ideal therapeutic target. Mutations in a third gene, OPTN (which encodes the protein optineurin, reported to be involved in protein trafficking, autophagy, and gene expression) are known to be causative for primary open-angle glaucoma, and have also been recently implicated in ALS. While the role for OPTN in ALS is not yet clear, several mutations have been found in both Japanese and Italian ALS patients, the latter cohort including both familial and sporadic cases [8-11]. For TDP-43 and FUS, their discoveries have raised the very real possibility that RNA metabolism and processing is a central feature in neurodegeneration, including ALS. Of course, RNA metabolism and processing is a broad term that implies a wide range of cellular activities. To some extent, however, the field is expecting to see additional RNA-binding proteins surface in ALS, but the negative result of a recent mutational analysis for a FUS/TLS family member, TAF15, has dampened such expectations [12]. On the other hand, defects in the gene encoding the RNA-binding protein SMN1 (survival of motor neuron 1) have long been known in spinal muscular atrophy, a juvenile disease that is characterized by the loss of spinal cord motor neurons in young children [13].

In the case of TDP-43 mutations, a recent major advance is the identification of the target transcripts bound by TDP-43 [14-17]. However, despite the plethora of potential TDP-43 regulatable transcripts that these approaches have yielded, the field is a long way from understanding the biology. And surprisingly, despite widespread prediction, as yet there has been no convincing demonstration that either TDP-43 or FUS mutants alter the splicing pattern of any known transcripts. However, both have been localized to, and implicated in, the formation and regulation of stress granules, which is a normal cellular response to exogenous stress stimuli [18-24], and there is significant speculation that this pathway, not previously implicated in ALS, may be a factor in the disease. Another piece of evidence for this comes from studies showing that recognized markers of stress granules colocalize with TDP-43 and FUS inclusions in postmortem patient material [20,25].

Likely one of the most exciting recent developments in ALS research is the identification of genes that can modify aspects of the disease. While disease gene modifiers, alleles/genes that either are associated with a higher than normal risk of developing the disease in the population or modify the onset and/or duration of the disease, have been reported in other disease contexts, few such examples were known to exist for ALS. Some years back, an association was reported between a marker influencing KIFAP3 expression and patient survival; KIFAP3 (kinesin-associated protein 3) participates with the motor protein kinesin in the movement of organelles and molecules towards the neuromuscular junction [26]. However, an independent follow-up study did not confirm this association [27]. Conversely and most recently, ATXN2 (encoding ataxin-2), known to cause spinocerebellar ataxia type 2, was linked to ALS in several different studies [28-32]. Specifically, intermediate length expansions of ataxin-2’s polyglutamine repeats (27-33 glutamines) are found in ALS patients. Intriguingly, the possibility that this locus could be implicated in ALS was presented 3 years ago using a very different genetic approach [33]. What is exciting here is that the recent emergence of ATXN2 is akin to the identification of APOE (encoding apolipoprotein E) allele e4, which confers an increased risk in Alzheimer’s disease [34]. And as we have seen for APOE in Alzheimer’s disease, the biology behind the ATXN2 association in ALS is certain to be an area of intense investigation.

The implication of ATXN2 in ALS, as well as in spinocerebellar ataxia type 2, highlights the recent general acknowledgement that there is genetic overlap between neurodegenerative diseases. Most recently, mutations in the gene encoding valosin-containing protein (VCP), previously identified as causative for both frontotemporal dementia [35] and the rare syndrome IBMPFD (inclusion body myopathy with early-onset Paget disease of the bone and frontotemporal dementia) [36], were described in a cohort of ALS patients using exome sequencing [37]. The idea that ALS and frontotemporal dementia might share some common pathways was raised in 2006, when TDP-43 was identified as a major component of neuronal aggregates that figure significantly in the pathology of patients with ALS or frontotemporal dementia [38] and, clinically, the association between ALS and frontotemporal dementia has been recognized for more than a decade [39]. This overlap was further reinforced by the identification of TDP-43 mutations in frontotemporal dementia patients without ALS features [40,41]. Thus, it is now widely
believed that ALS and frontotemporal dementia are part of a disease spectrum. It is evident that genes such as ALS2, SETX, and VAPB, which are thought to cause familial forms of ALS, may be allelic for, or implicated in, other neurological diseases. As our genetic understanding of ALS advances, what has become very apparent is that SOD1 mutations have never been reported in any other disease. So is the SOD1 mouse model really so bad? Wouldn’t a purely ALS-causing gene be the ideal choice with which to dissect mechanism(s) and develop therapeutics?

**New models and mechanisms**

This brings us back to the utility of the SOD1 mouse models. As alluded to earlier, these models have uncovered several biological mechanisms thought to actively participate in the selective motor neuron degeneration characteristic of ALS (please see [6] for a recent comprehensive review). Of these possibilities, there has been a considerable focus on mitochondria, specialized organelles that are primarily responsible for meeting the energy demands of the power-hungry motor neurons, and how SOD1 might affect their normal function. This focus on mitochondria is due in large part to the observation that SOD1 preferentially associates with mitochondria from affected spinal cords but less so, if at all, with mitochondria from other tissues [42-44]. This work has developed in parallel with a series of groups developing tools designed to specifically recognize a disease-specific conformation of SOD1 [45-47]. Using these tools, it is now recognized that a misfolded form of SOD1 does exist and it is found to be bound to the mitochondrial surface [44,48]. Thus, it fits well that further investigation has found that mitochondrial functions at the surface, such as protein import [49] and metabolite conductance [48], are disrupted by the presence of misfolded SOD1. Other aspects of mitochondrial function may also be affected, as significant changes in axonal mitochondrial morphology have recently been reported in two different mutant SOD1 models [50]. What remains to be clarified is the sequence of these events in disease development. Regardless, it is significant (and exciting) that a misfolded form of SOD1 can be detected in the motor neurons of some sporadic ALS patients who lack a genetic mutation in SOD1 [47,51]. One possible explanation for this may come from recent studies showing that the wild type SOD1 protein can become mutant-like in vitro through oxidation [52,53]. Together, these findings firmly link SOD1 to sporadic disease and reinforce the utility of the SOD1 animal models.

However, it is surely true that other genes might exist that modify the disease in humans. Using the power of mouse genetics, a recent report using the well-characterized ALS model of mice expressing SOD1 with a glycine to alanine mutation (G93A) bred into multiple different genetic backgrounds yielded several disease modifying loci [54]. The timing of disease onset and the progression/duration of the disease was variable in the different backgrounds. While much additional work needs to be done, an exciting list of candidate genes will undoubtedly emerge from these studies.

The ultimate goal of every ALS research team is to define how the disease starts and progresses so that we can craft a therapeutic to halt the disease. To do this, we need to have confidence in our animal models. While the TARDBP and FUS/TLS mice are ways away from demonstrating their true utility in this regard, this is not true for the SOD1 models. In fact, of all the neurodegenerative disease models, the mutant SOD1 mice are some of the most faithful mimics of human disease. So why have the dozens (if not hundreds) of clinical trials in ALS not yet yielded our “silver bullet”? Is it poor preclinical trial design? Unrealistic expectations/outcome measures for patients in clinical trials? Both of these have recently been addressed by the field in the revamped way SOD1 mouse models are to be used in the preclinical evaluation of potential therapeutics. This is summarized nicely in the recently developed ALS mouse handbook [55]. The implementation of these consensus guidelines should hopefully avoid erroneous interpretations and thus rationalize the choice of therapeutics pushed forward into Phase I/II human trials. At the patient level, there has been a significant paradigm shift with regards to trial design. Improvements to patient quality of life are now considered to be more important and/or indicative of success than time of survival. Such new designs are also more attractive to potential patients enrolling in the trial [56].

**Conclusion**

In a relatively short period of time, the ALS field has acquired a remarkable amount of information about the potential cellular mechanisms underlying the disease. The challenge lying ahead is how to integrate and assimilate the recent and emerging data to identify common mechanisms as these will likely represent the best potential targets for future therapies. However, let’s suppose for a moment that we don’t get that. What if we find a handful of targets without finding the unifier? Perhaps we should start the discussion now about personalized patient care? Given the stochastic nature of ALS, the variability in age and site of onset, the inherited and noninherited forms, perhaps it is naïve to think that a “one size fits all” approach will benefit every ALS patient. Perhaps customized, combination therapies...
should be our goal? Maybe we should be thinking in terms of finding the “silver bullets”, not “bullet”.

Abbreviations
ALS, amyotrophic lateral sclerosis; ALS2, alsin; APOE, apolipoprotein E; ATXN2, ataxin-2; FUS/TLS, fused in sarcoma/translocated in liposarcoma; KIFAP3, kinesin-associated protein 3; OPTN, optineurin; SETX, senataxin; SOD1, superoxide dismutase 1; TARDBP and TDP-43, TAR DNA binding protein-43; VAPB, vesicle-associated membrane protein-associated protein B.

Competing interests
The authors declare that they have no competing interests.

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