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Review

Sporadic and hereditary amyotrophic lateral sclerosis (ALS)☆

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ABSTRACT

Genetic discoveries in ALS have a significant impact on deciphering molecular mechanisms of motor neuron degeneration. The identification of SOD1 as the first genetic cause of ALS led to the engineering of the SOD1 mouse, the backbone of ALS research, and set the stage for future genetic breakthroughs. In addition, careful analysis of ALS pathology added valuable pieces to the ALS puzzle. From this joint effort, major pathogenic pathways emerged. Whereas the study of TDP43, FUS and C9ORF72 pointed to the possible involvement of RNA biology in motor neuron survival, recent work on P62 and UBQLN2 refocused research on protein degradation pathways. Despite all these efforts, the etiology of most cases of sporadic ALS remains elusive. Newly acquired genomic tools now allow the identification of genetic and epigenetic factors that can either increase ALS risk or modulate disease phenotype. These developments will certainly allow for better disease modeling to identify novel therapeutic targets for ALS. This article is part of a Special Issue entitled: Neuromuscular Diseases: Pathology and Molecular Pathogenesis.

1. Introduction

Amyotrophic lateral sclerosis is an adult onset, fatal neurodegenerative disorder involving the large motor neurons of the brain and the spinal cord. It is clinically characterized by progressive paralysis and eventual death from respiratory failure within three to five years. ALS is in the main a sporadic disease but about 10% of ALS cases are familial. SOD1 was the first gene to be discovered about two decades ago, but in the last fifteen years, a number of new ALS causing genes have been discovered (Table 1). These genes can play an important role in our understanding of the pathogenesis of familial and sporadic ALS.

2. Clinical features and diagnosis

Progressive pure motor weakness starting focally is the most distinctive clinical feature of ALS. The disease starts with limb weakness in about two-thirds of patients, often preceded by cramps, and with bulbar weakness causing dysarthria and dysphagia in the remaining one-third. In rare instances, cognitive impairment, behavioral disturbances or early respiratory failure can be the initial manifestation of ALS. The characteristic combination of upper and lower motor neuron dysfunction is usually evident on neurological examination with the presence of weakness, atrophy and fasciculations together with hyper-reflexia and increased tone in the same motor segment and not infrequently an extensor response to plantar stimulation. Sensory findings are minimal or absent. Relentless, the disease contiguously spreads to other body parts and eventually to respiratory muscles leading to death from respiratory failure within 30 months on average [1]

The diagnosis of ALS is clinical, based on the history and physical examination showing progressive upper and lower motor neuron dysfunction. It is usually supported by electrophysiological studies and neuroimaging and laboratory tests to exclude mimickers. The El Escorial criteria have been developed to standardize the diagnosis for clinical research [2].

Familial ALS is more easily identified when there is a positive family history; however, familial ALS may present as sporadic disease on account of incomplete penetrance or incomplete family history. In the absence of family history, an early age of onset, atypical rapid or slow disease progression, pure lower motor neuron presentation or the presence of dementia may alert to a familial etiology.

3. Update in the pathogenesis of familial ALS

The initial paradigm shift in approaching ALS pathogenesis, although recently recognized [3], occurred about 30 years ago when the tools of molecular genetics were applied to ALS. This effort led to the identification of the first ALS gene: superoxide dismutase 1 (SOD1), which accounts for 20% of familial ALS cases [4–7], and turned the attention to hereditary ALS as a means to investigate motor neuron degeneration.

SOD1 catalyzes the dismutation of superoxide radicals and protects the cell against reactive oxygen species. More than 160 mutations in SOD1 have been reported. Almost all are dominant missense mutations

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Table 1
Causative ALS genes.

Gene symbol	Locus	Function	Phenotype
SOD1	21q22	Superoxide metabolism	AD-ALS/ALS1
TARDBP	1p36	RNA metabolism	AD-ALS/ALS10, ALS-FTD
FUS	16p11	RNA metabolism	AD-ALS/ALS6, ALS-FTD
OPTN	10p13	Many functions including membrane and vesicular trafficking	AD & AR-ALS/ALS12
VCP	9p13	Ubiquitinated protein trafficking Autophagosme maturation	AD-ALS/ALS14, ALS-FTD, IBMPFD
SQSTM1	5q35	Protein degradation	AD-ALS, ALS-FTD, Paget's disease of bone
UBQLN2	Xp11	Protein degradation	X-linked adult and juvenile ALS/ALS15, ALS-Dementia
C9ORF72	9p21	Unknown	AD & sporadic-ALS/ALS-FTD
PFN1	17p13	Actin polymerization	AD-ALS

and account for 20% of familial ALS and for 2%–3% of apparently sporadic cases (www.alsod.org). The A4V mutation is the most common mutation in North America, followed by the I113T mutations [8]. Our current understanding of the pathogenic role of mutant SOD1 comes primarily from the study of transgenic rodents overexpressing mutant SOD1 and in particular from the G93A mouse model [9] as it recapitulates many features of the human SOD1 type disease. Although the exact toxic mechanism of SOD1 mutations is not completely elucidated, the mutant protein is misfolded and gains many toxic functions that can cause endoplasmic reticulum (ER) stress and include: overloading the unfolded protein response, mitochondrial dysfunction and disruption of axonal transport. Mutant SOD1 forms aggregates through an oxidation-mediated mechanism and recruit wild-type SOD1 by crosslinking of intermolecular disulifide bonds [10]. Inclusions containing these aggregates are found in the lower motor neuron and are a prominent pathological feature of human familial ALS due to SOD1 mutations and in the SOD1 mouse models [11–13]. Demetalled and unfolded apoform of SOD1 enters the intermembrane space of the mitochondria, where it is refolded by the chaperone of superoxide dismutase (CCS), copper and zinc ions are acquired and intermolecular disulphide bonds form between cysteine 57 and cysteine 146 [10,14]. In the SOD1 mouse models (G93A, L126X and A4V), the apoprotein forms intermolecular covalent bonds in the intermembrane space of the mitochondria, heralding the onset of symptoms. This is particularly notable because the A4V mice never becomes symptomatic until crossbred with mice overexpressing wild-type SOD1, and the onset of symptoms coincides with the formation of insoluble aggregates consisting of apo SOD1 with intermolecular covalent bond formation [10,14]. Not only mutant SOD1 but wild-type SOD1 has also been proposed to undergo post-translational modifications that can cause misfolding [15]. Using conformation-specific antibodies, misfolded wild-type SOD1 has been reported in the spinal cord of sporadic ALS patients and in non-SOD1 familial ALS but not in controls, implicating wild-type SOD1 aggregation in the pathogenesis of sporadic ALS [16,17]. Following the discovery of SOD1-familial ALS, several other genes causing ALS or ALS-like syndromes were unraveled (Table 2), starting with the gene for ALS2 coding the protein ALSIN that was discovered in 2001 [18]. A major step forward was the discovery of TAR DNA-binding protein (TDP-43/TADBP) in 2006 as a major component of ubiquitinated inclusions in ALS and subsequently in other neurodegenerative diseases [19]. ALS families were then screened and mutations in TARDBP were also found to cause familial ALS [20]. This important discovery provided a direct pathogenic link between TDP43 and sporadic ALS similar to the role of β-amyloid precursor protein in Alzheimer's disease and α-synuclein in Parkinson's disease [20]. TDP43 is a DNA/RNA binding protein involved in many cellular functions such as transcription and splicing regulation, mRNA stability and microRNA processing [21,22]. TDP43 is primarily a nuclear protein, but after acute neuronal injury, TDP43 translocates to the cytoplasm and forms stress granules that dissolve after recovery, suggesting that TDP43 shuttles between the nucleus and cytoplasm as a response to injury [23]. In the cortex of ALS and FTD patients, TDP43 is phosphorylated, ubiquitinated and cleaved, forming 20–25 kDa C-terminal insoluble fragments that aggregates in the cytoplasm with loss of the normal TDP43 nuclear staining in a sizable minority of neurons [19]. Over the years, several cell and transgenic animal models of TDP43 have been developed from yeast, zebra fish, drosophila, mice and rats. Although these animal models do not exactly replicate an ALS-like phenotype, most suggested that either the loss of nuclear or cytoplasmic TDP43 function or the gain of new toxic function through the sequestration of essential proteins in the aggregates plays an important role in neuronal degeneration. Recently, TDP43 was also found to play a role in the axonal transport of certain target mRNAs into distal neuronal compartments. ALS-causing mutations in TDP43 impaired axonal trafficking of these target mRNA in Drosophila, in mouse cortical neurons and in ALS patients' motor neurons derived from induced pluripotent stem cells, implicating the loss of this new cytoplasmic function in the pathogenesis of ALS [24].

Table 2
Other genes causing ALS or ALS-like syndromes.

Gene	Locus	Function	Phenotype
ALSIN	2q33	Vesicle trafficking	AR-Juvenile ALS/ALS2/PLS, infantile onset spastic paraplegia
SETX	9q34	RNA/DNA helicase	AD-juvenile ALS/ALS4 AR-ataxia with oculomotor apraxia
VAPB	20q13	Vesicle trafficking	AD-ALS/ALS8, AD-distal SMA
DCTN1	2p13	Axonal transport	AD-ALS, PMA
ANG	14q11	Hypoxia responsive ribonuclease	AD-ALS/ALS9
CHMP2B	3p11	Vesicle trafficking	AD-ALS, ALS-FTD
FIG4	6q21	Vesicle trafficking	AD-ALS/ALS11, PLS, CMT4J
DAO	12q24.11	Unknown	AD-ALS
ATXN2	12q24.12	Unknown	AD-ALS/ALS13, SCA2
hnRNPA2B1	7p15	RNA metabolism	AD-ALS, multisystem proteinopathy
hnRNPA2A1	12q13	RNA metabolism	AD-ALS, multisystem proteinopathy

Shortly after the discovery of TDP43 mutations causing familial ALS, mutations in fused in sarcoma/translocated in liposarcoma (FUS/TLS), another RNA binding protein, were identified as causing about 4% of familial ALS and rare sporadic ALS cases [25,26]. Like TDP43, FUS/TLS is a nuclear protein with many RNA regulation and processing functions. FUS immunoreactive inclusions are also found in sporadic ALS, in non-SOD1 familial ALS and in ALS/dementia tissue [27].

It is presently not clear whether the role that TDP43 and FUS play in motor neuron degeneration is through common or divergent pathways. Prior genome-wide deletion and overexpression screens in yeast failed to show significant overlap in genetic modifiers of TDP43 and FUS toxicity [28]. However, FUS was recently found to bind thousands of human and mouse brain mRNAs, some shared with TDP43 [29]. The depletion of FUS/TLS and TDP43 in human neurons, differentiated from pluripotent stem cells, resulted in the down-regulation of long intron containing TDP43 or FUS targets, some with important neuronal functions, suggesting a common pathway for FUS and TDP43 in motor neuron death [29]. In addition, using computational algorithms to

identify prion-like domains, both FUS and TDP43, ranked highly (1st and 10th, respectively) among RNA-binding proteins harboring a canonical RNA recognition motif and a putative prion domain [30]. This prion-like domain has been recently implicated in TDP43 and FUS toxic misfolding and aggregation *in vitro* [31].

This newly discovered role of RNA metabolism and processing in the pathogenesis of ALS was given a boost with the identification of mutations in C9ORF72. Expanded GGGGCC hexanucleotide repeats in the first intron of C9ORF72 were found to be the most common cause of ALS and FTD (Fig. 1), responsible for about 30% of the familial and 5% of the sporadic ALS cases [32,33]. This gene encodes a protein of unknown function and has provided additional evidence that impaired RNA function is crucial for ALS. Intranuclear RNA foci, containing expanded RNA transcripts, have been described in ALS and FTD tissue. Similar to other repeat expansion diseases, it was recently demonstrated that the toxicity of these RNA foci are length-dependent and sequester specific RNA binding proteins such as hnRNP-H and multiple RNA transcripts leading to significant dysregulation of RNA processing [34]. Furthermore, this G4C2 expansion mutation can also be expressed in both directions through repeat-associated non-ATG (RAN) translation, producing sense and antisense C9-RAN proteins that are detected in the P62-positive aggregate in C9ORF72-positive brains, linking RAN translation to ALS pathology [35,36].

The second paradigm shift in understanding ALS pathogenesis occurred in 2011 [3] with the identification of mutations in Ubiquilin 2 (UBQLN2) and Sequestosome 1 (SQSTM1 or P62) in patients with familial ALS and ALS/dementia. Although UBQLN2 and SQSTM1 (P62) contribute to a small subgroup of ALS cases, they are an important component of the cytoplasmic inclusions not only in familial ALS tissues but also in sporadic ALS, in FTD and in other degenerative diseases including Alzheimer's (P62), Parkinson's (P62), Huntington's (UBQLN2) and in muscle disease resembling inclusion body myopathy (P62) [37–39].

UBQLN2 is a member of the ubiquilin family of proteins characterized by the presence of an N-terminal ubiquitin-like domain (UBL) and a C-terminal ubiquitin-association domain (UBA). This structure is characteristic of the proteins that deliver ubiquitinated proteins to the proteasome for degradation [40]. In addition, UBQLN2 also plays an important role in autophagy. The UBL domain of ubiquilins mediates both its association with autophagosomes and its protective effect against starvation-induced cell death. Ubiquilins co-localize with autophagosomes and bind LC3. Depletion of ubiquilins delays the delivery of autophagosomes to lysosomes, inhibiting autophagosome-lysosomal degradation [41]. ALS-causing mutations in UBQLN2 lead to ubiquitin-mediated impairment of proteasomal degradation [42], underscoring the role of UPS in the removal of misfolded and damaged proteins.

Similar to UBQLN2, SQSTM1 (P62) also has a UBA C-terminal domain and its N-terminal domains share considerable homology with the UBL domain. P62, a multifunctional protein, also interacts with ubiquitinated proteins and acts as a cargo receptor for the degradation of ubiquitinated proteins through both autophagic and proteosomal pathways [43]. P62 mutations were identified in familial and sporadic ALS [44,45], in FTD and in FTD-ALS [45,46]. Although the exact role played by UBQLN2 and P62 in motor neuron degeneration has yet to be elucidated, the presence of ubiquitin-P62-UBQLN2 positive inclusions in brain and spinal cord tissues of all types of ALS [42] and other degenerative disorders, directly implicates impaired protein degradation pathways in the pathogenesis of ALS and highlights the importance of these proteins in the final common pathway of neuronal degeneration.

Furthermore, mutations in P62 also cause about 25%–50% of familial Paget's disease of bone, a disorder characterized by rapid bone turnover [47]. Two additional proteins: optineurin (OPTN) and valosin-containing protein (VCP) are also implicated in ALS/FTD and in Paget's disease of bone. OPTN is involved in a variety of functions including regulation of endocytic trafficking, immune response, mitosis, NF-κB signaling transduction and autophagy [48]. VCP belongs to the AAA + protein family of ATPases, which have diverse biological functions; among them, the possible delivery of degradation-destined ubiquitinated protein to the 26S proteasome, autophagosome maturation, placing it at the crossroad of protein degradation through both

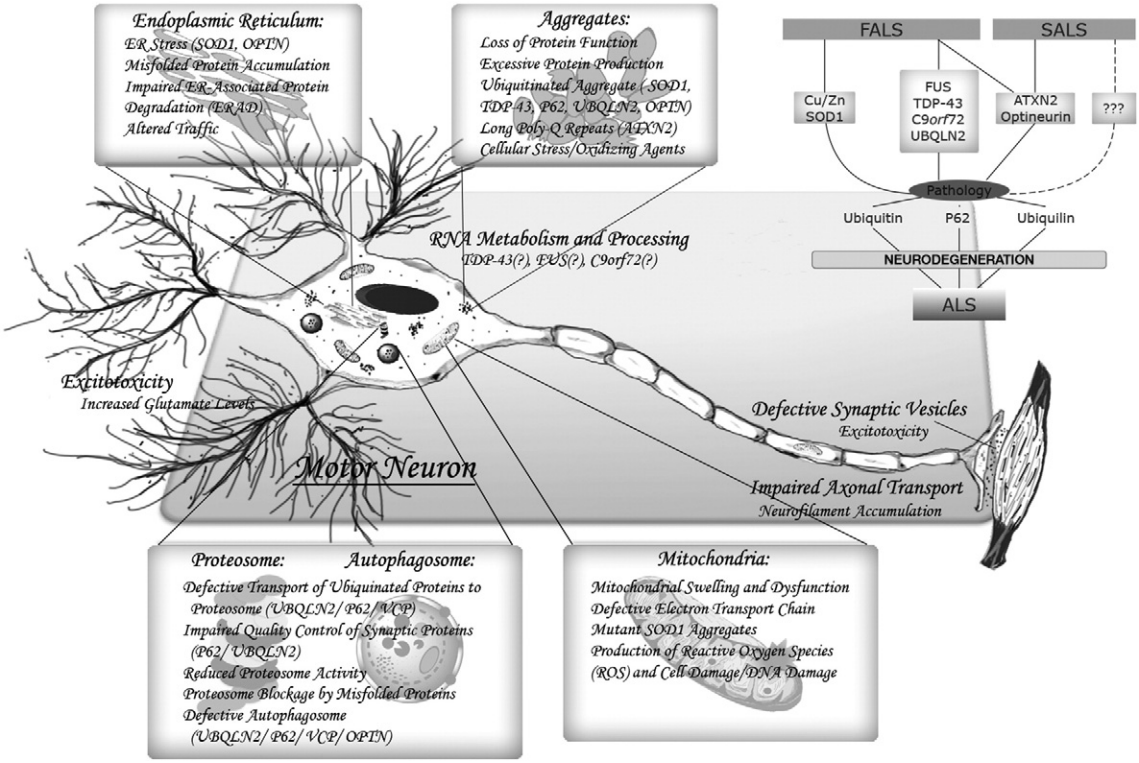

Fig. 1. Pathogenic mechanisms in motor neuron degeneration.

the ubiquitin-proteasome system (UPS) and autophagy [49]. Alteration in P62, OPTN and VCP in ALS and Paget's disease of bone also suggests an overlap between bone metabolism and motor neuron degeneration that needs further investigation.

4. Update in the pathogenesis of sporadic ALS

Despite this enormous progress, the etiology of sporadic ALS remains largely unknown. Going from the hypothesis that sporadic disease may arise from complex interactions between genetic susceptibility and environment, the scientific community turned to exploring the genetic landscape of sporadic ALS to identify novel disease mechanisms.

Several genome-wide association studies (GWAS) have been performed in sporadic ALS identifying a variety of risk loci. Many of them were not replicated in large cohorts. The most robust GWAS result was the locus on chromosome 9p that contributed to the identification of C9ORF72. However, that study had an admixture of familial and sporadic cases. UNC13A was also identified through GWAS to confer susceptibility to ALS and modulate survival [50,51]. UNC13A is a presynaptic protein that regulates neurotransmitter release [50]. In mice, this family of proteins is essential for synaptic vesicle priming. Mice lacking UNC13A have disrupted glutamate signaling and morphological abnormalities in spinal cord neurons and the neuromuscular junction [52]. ELP3, a component of RNA polymerase II involved in RNA processing, was found to be associated with ALS in three different populations. Mutagenesis experiments in Drosophila and knock-down experiments in zebra fish showed that ELP3 plays an important role in neuronal biology [53]. Ataxin 2 was identified through a genetic screen in yeast to modulate TDP43 toxicity. Intermediate repeat lengths, between 27 and 32 in Ataxin 2, were shown to predispose to ALS [54] with the CAG repeat size between 29 and 33, conferring a higher risk (Santana and Siddique, unpublished).

Besides identifying susceptibility loci, genome-wide association studies were also used to discover genetic variants that can influence ALS phenotype. A recent meta-analysis of the International Consortium on Amyotrophic Lateral Sclerosis Genetics genome-wide association samples identified a locus on chromosome 1p34 that modulates age of disease onset. Other loci were also implicated in either modifying survival (KIFAP3, UNC13A) or age of onset (VEGF, APOE…); however, some of these studies have not been successfully replicated. Finally, the paraoxonase gene cluster has been extensively examined in the past years and has emerged as a potential risk factor; unfortunately, the outcome of the GWAS examining its association with sporadic ALS provided conflicting results [55,56].

5. Update in the pathology of familial and sporadic ALS

The pathology of ALS is characterized by the loss of pyramidal Betz cells in the motor cortex as well as loss and degeneration of the large anterior horn cells of the spinal cord and lower cranial motor nuclei of the brainstem [57]. The degenerating motor neurons display intracellular aggregates that form distinct ubiquitinated inclusions, the pathological hallmark of the disease. Unveiling the molecular makeup of these inclusions proved crucial in our understanding of the ALS pathomechanism.

Subsequent to the identification of TDP43 as the major component of the ubiquitinated inclusions in sporadic ALS [19] and in non-SOD1-linked familial ALS, we showed that FUS-immunoreactive inclusions were also present in spinal anterior horn neurons in all sporadic ALS and in non-SOD1-familial ALS cases. The FUS-containing inclusions were also immunoreactive with antibodies to TDP43, P62 and ubiquitin [27]. OPTN immunoreactivity was also found in skein-like inclusions of anterior horn neurons and their neurites in spinal cords of sporadic ALS and in non-SOD1 familial ALS cases [58]. Finally, UBQLN2-positive inclusions were identified in spinal cord sections of patients with X-ALS and found to co-localize with ubiquitin, P62, TDP43, FUS and OPTN but not SOD1. UBQLN2 immunoreactivity was also observed in spinal cord sections of sporadic ALS, ALS with dementia and in non-SOD1 familial ALS [42], suggesting that SOD1 mutations may perform their deleterious effects through distinct pathways. Widespread UBQLN2-positive inclusions were also observed in the hippocampus of ALS patients with dementia, including patients with expanded hexanucleotide repeats in C9ORF72 [42].

The pathology of C9ORF72 is characterized by the presence of TDP43 inclusions in the shape of compact or granular neuronal cytoplasmic inclusions in a minority of neurons containing ubiquitinated inclusions of diffuse neuronal cytoplasmic staining, dystrophic neuritis, glial cytoplasmic inclusions and variable numbers of neuronal intranuclear inclusions. These inclusions are mainly found in pyramidal, frontal and temporal cortex and hippocampus neurons [59]. In addition to TDP43 staining, a unique and characteristic feature of C9ORF72 is the presence of cytoplasmic inclusions in the cerebellar granule cell layer, hippocampal pyramidal neurons and neocortex that stain positively for UBQLN2, P62 and ubiquitin but are negative for TDP43 [60].

Although the direct link between the presence of these inclusions and motor neuron degeneration is not fully understood, the mechanistic dysfunction of UBQLN2 and P62 offers (currently) the most attractive molecular mechanism of disease linked to etiology and pathology.

6. Novel therapeutic approaches to ALS

Despite tremendous progress in the field of genomics, ALS remains an incurable disease. Phenotypic heterogeneity, divergent pathogenic pathways and clinical trial methodologies may account for the negative results obtained thus far and riluzole remains the only available therapy with only a modest effect on disease progression. Nevertheless, many compounds are either under development or in clinical trials.

Gene silencing through selective manipulation of RNA processing to reduce toxic protein level is an exciting novel therapeutic opportunity for familial ALS. Antisense oligonucleotide effectively decreased mutant SOD1 expression and prolonged survival in the transgenic rats [61]. A pilot study using intrathecal administration of antisense oligonucleotides in patients with SOD1 familial ALS proved to be safe and well tolerated [62], setting the stage for future gene silencing studies through antisense approach, RNA interference or small molecules in all genetic forms of ALS. Regenerative therapy using fetal human stem cells delivered through intraspinal injections to 12 ALS patients reached its safety endpoint [63] and a phase two clinical trial is underway. Finally, the effect of autologous bone marrow-derived mesenchymal stem cells (NurOwn) or human hepatocyte growth factor delivered through intramuscular injections will soon be, or is currently being investigated.

7. Conclusion

Significant genetic progress has been accomplished in recent decades with the discovery of several familial ALS genes. These genes offered a unique opportunity to decipher the molecular mechanisms of neuronal degeneration. Technological innovation in recent years allowed for improved disease modeling, including the generation of induced pluripotent stem cells and hastened therapy testing. The complex genetic landscape of sporadic ALS and the presence of low-penetrance loci that mimic sporadic ALS underscore the role that genomics still have to play in our understanding of ALS pathogenesis and in developing rational therapies.

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