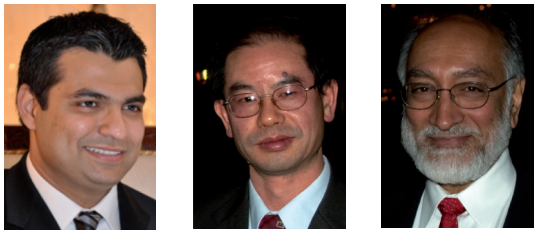


Discovering the connection between familial and sporadic amyotrophic lateral sclerosis: pathology trumps genetics



“...in identifying the pathophysiological connection between FALS and SALS, pathology has trumped genetics.”

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Amyotrophic lateral sclerosis (ALS) is a fatal paralytic disorder caused by the degeneration of motor neurons in the brain and spinal cord. Most cases of ALS are of unknown etiology and appear as sporadic ALS (SALS). Approximately 5–10% of ALS cases have a family history of the condition. Familial ALS (FALS) is genetically heterogeneous. The penetrance of genetic mutation-linked FALS may vary substantially, ranging from a classic Mendelian pattern to an apparently sporadic pattern depending on the effect of the mutations. Mutations in *SOD1* account for approximately 20% of FALS and 1% of SALS cases, which represents the most prevalent known cause of ALS [1,2]. Biophysical, biochemical and animal model studies of the ALS-linked *SOD1* mutations have provided a major body of data for understanding the pathophysiology of the disease to date [3]. Mutations in several other genes, including *DCTN1*, *ALS2*, *SETX*, *VAPB*, *ANG*, *SPG11* and *FIG4*, have been linked to rare instances of ALS or ALS-like syndromes [3,4], while mutations in *TARDBP*, *FUS*, the *PONs* and *OPTN* have been more robustly implicated in the etiology of ALS [5–7].

Where SALS is concerned, the paradigm has shifted to dissecting its complex nature by studying gene–gene and gene–environment interactions. However, several whole-genome association studies and candidate-gene studies have failed to identify a robust genetic contribution to ALS in the same league as *APOE4* for Alzheimer disease (AD) [8]. Lack of replication and the small effects of the associated alleles are obvious problems in the understanding of complex disorders such as SALS, but a clearer picture may become apparent after a combined

analysis of all the available genome-wide association data is carried out. However, if the odds ratio remains small, a large number of risk genes might be implicated. To date, the success of genome-wide studies in SALS has been minor, and so a hard look at novel causes for SALS is needed. Epigenetic factors and post-translational changes that cause the conversion and amplification of pathological protein conformations should be investigated.

Since FALS and SALS can be clinically indistinguishable, it has long been speculated that they may, at least in part, share the same pathogenic pathway. Indeed, recent studies of TDP-43, FUS and optineurin have provided important lines of evidence supporting the hypothesis that FALS and SALS do share elements of a common pathogenic pathway (FIGURE 1).

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In 2006, TDP-43 was shown to co-localize with ubiquitinated neuronal inclusions in patients with frontotemporal lobar degeneration (FTLD) or ALS [9]. A phosphorylated 25-kDa C-terminal fragment of TDP-43 is enriched in detergent-resistant fractions of ALS tissue and neurons with cytoplasmic inclusions show a marked loss of nuclear TDP-43 [9,10]. A later study showed that TDP-43 inclusions are present in *SOD1*-negative FALS, SALS and ALS with dementia, but not in *SOD1*-linked ALS [11]. These discoveries raise

Keywords

- amyotrophic lateral sclerosis
- FUS ■ neurodegeneration
- optineurin ■ protein aggregation ■ *SOD1* ■ TDP-43
- ubiquitin

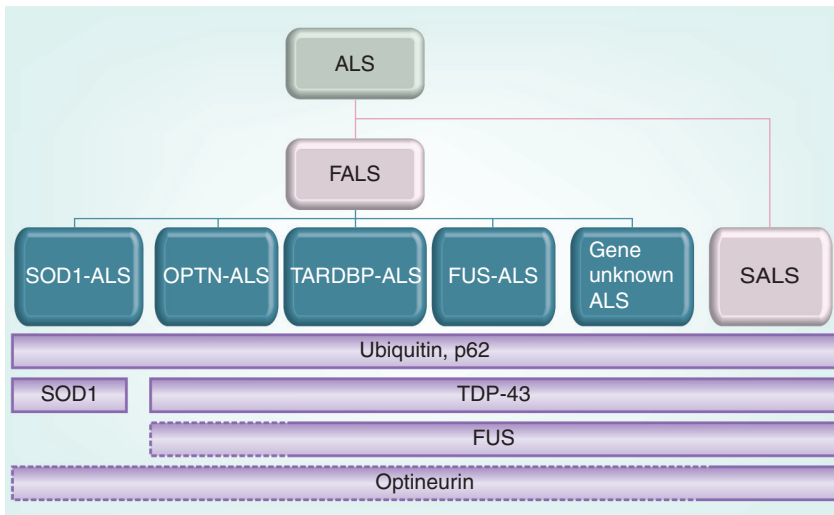


Figure 1. Pathological signature of different forms of amyotrophic lateral sclerosis, highlighting many commonalities. Various proteins that have been identified in neuronal cytoplasmic inclusions in different forms of amyotrophic lateral sclerosis are highlighted by the full purple bars. Dashed bars represent conditions in which the respective protein has not yet been tested or validated. ALS: Amyotrophic lateral sclerosis; FALS: Familial amyotrophic lateral sclerosis; SALS: Sporadic amyotrophic lateral sclerosis.

the possibility that cytoplasmic inclusions of TDP-43 might play a mechanistic role in neurodegeneration in ALS. Subsequent to these discoveries, mutations in *TARDBP*, the gene encoding TDP-43 were reported by several groups in approximately 1–4% of FALS and some SALS patients [12,13]. Interestingly, these findings followed reports of negative genetic associations between *TARDBP* and ALS [14].

In 2009, mutations in *FUS* were found in a subset of FALS [15,16]. Similarly to TDP-43, *FUS* is usually a nuclear protein, but when mutated, it is mislocalized to the cytoplasm [15,16]. Immunoblot experiments have shown an enrichment of full-length *FUS* in cytoplasmic fractions, but biochemical abnormalities involving post-translational modifications such as phosphorylation have not been found [15,16]. Although mutations in *FUS* account for only 4–5% of FALS, it was recently shown that pathological *FUS*-immunoreactive inclusions are present in virtually all of the SALS and ALS/dementia cases [17]. *FUS*-immunoreactive inclusions are also present in most but not all cases of FALS, including those with mutations in *FUS* or *TARDBP* [17]. However, the FALS cases with *SOD1* mutations appeared to be an exception in which *FUS* inclusions were not detected [17]. The *FUS*-containing inclusions are also immunoreactive, with antibodies to TDP-43, p62 and ubiquitin [17]. It is not immediately evident whether these *FUS*/TDP-43/ubiquitin/p62-positive inclusions are toxic to the motor neurons

or whether sequestration of these proteins in inclusions deprives the cells of these and other essential proteins and RNA transcripts. However, these findings indicate that these proteins may be involved in a common pathogenic pathway shared by both SALS and FALS, but the *SOD1*-linked FALS is likely to have a pathway that is distinct from the other forms of ALS.

TDP-43 and *FUS* are both RNA/DNA-binding proteins with many structural and functional similarities. They have both been implicated in transcription, splicing, transport of RNA and protein translation [18]. Given their causal role in ALS, research efforts need to be directed towards understanding the role of RNA processing and protein aggregation pathways in the pathophysiology of ALS.

Recently, mutations in *OPTN*, the gene encoding optineurin, previously reported to be a causative gene for primary open-angle glaucoma, were linked to ALS [7]. Immunohistochemistry revealed an increased cytoplasmic staining with optineurin antibody in a patient with a mutation in this gene. Optineurin was found in skein-like inclusions of SALS and could be co-localized with antiubiquitin and anti-TDP-43 antibodies. Moreover, *SOD1*-immunoreactive Lewy-body-like hyaline inclusions of *SOD1*-FALS were also reactive with the optineurin antibody [7]. We have not observed co-localization of optineurin with skein-like inclusions of *SOD1*-FALS, which is the prominent type of inclusion observed in this form of ALS [SIDDIQUE T ET AL., UNPUBLISHED DATA]. This implies that optineurin might be involved in a broader pathogenesis of ALS and may provide a bridge, based on pathology, between *SOD1*-ALS and other forms of ALS. However, whether the more characteristic skein-like inclusions in *SOD1*-ALS are also immunoreactive with optineurin antibodies remains to be verified in future studies.

“Since (familial amyotrophic lateral sclerosis) and (sporadic amyotrophic lateral sclerosis) can be clinically indistinguishable, it has long been speculated that they may, at least in part, share the same pathogenic pathway.”

Parallels can be drawn between TDP-43, *FUS* and optineurin and other proteins involved in neurodegeneration such as APP, α -synuclein and tau. Mutations in the genes encoding APP or α -synuclein are known to cause AD or Parkinson disease (PD) in a small number of

families. However, extracellular plaques containing β -amyloid and Lewy bodies containing α -synuclein appear to be the most prominent pathology in virtually all AD and PD, respectively. Similarly, a common feature of TDP-43, FUS and optineurin is that they aggregate in SALS, even though mutations are present in only a very small minority of ALS cases. In addition, it is noteworthy that whereas TDP-43 and/or FUS inclusions are present in the pathology of diverse diseases such as ALS, dementias, polyglutamine diseases, Parkinsonism and myopathies, mutations in these genes are very specific to causing ALS and, rarely, FTLD [18]. In this respect, these proteins are similar to tau, which is present in the neurofibrillary tangles observed in AD pathology, but mutations in its gene cause syndromes that are more consistent with FTLD. Such rare but pathogenic mutations provide a novel approach where the gene and its product can be investigated in molecular pathways at epigenetic, genetic and post-translational levels. Hence, discovery of mutations in TDP-43, FUS and optineurin should point to the appropriate directions and provide novel targets to explore the pathogenic pathways not only for a specific form of FALS, but also for the most common form of the disease – SALS. Sometimes the discovery of pathological post-translational mechanistic involvement (as was the case with TDP-43) precedes the discovery of genetic linkage showing causal involvement (as was the case with FUS and optineurin). These discoveries may also unravel the role of these proteins in the common pathophysiology of neurodegeneration by highlighting unifying pathways and mechanisms. It appears that in identifying the pathophysiological connection between FALS and SALS, pathology has trumped genetics. The pendulum has swung towards SALS; we are now where research into more common diseases such as AD and PD was several years ago.

These recent discoveries have illuminated the strong connection between SALS and FALS with many implications for future research. Using genomic and proteomic tools, many new agents responsible for pathology will be found in ALS in the future. To date, the most commonly used models of ALS are transgenic mice overexpressing mutant SOD1, especially SOD1-G93A [19]. These mice are widely accepted as the most phenotypically proximate animal models for basic and translational ALS research. A large number of potentially therapeutic agents have been screened using these mutant SOD1 transgenic mice, based at least in part on the assumption

that the disease mechanism(s) in SALS is similar to that in SOD1-ALS. However, this assumption may not be appropriate. Although motor neuron degeneration is a shared downstream pathway in every type of ALS, the upstream pathway of SALS is probably different from that of SOD1-ALS based on the protein composition of the intracellular inclusions. Therefore, therapeutics for the upstream pathway of SOD1-ALS may not be effective for SALS.

“...SOD1-linked (familial amyotrophic lateral sclerosis) is likely to have a pathway that is distinct from the other forms of (amyotrophic lateral sclerosis).”

Recently, several studies have shown that overexpression of either wild-type or mutant TDP-43 in rodents, flies and worms leads to the features of TDP-43 proteinopathy observed in ALS [20–24]. Several groups are currently developing FUS transgenic models. A previous study has already demonstrated that FUS deficiency leads to perinatal death in mice, whereas hemizygous mice appear to have a normal phenotype [25]. These findings imply that mutations in TDP-43 and FUS may cause disease through a gain of function mechanism. However, because *FUS*- and *OPTN*-linked ALS may follow a recessive inheritance pattern, a loss of function mechanism cannot be excluded, especially because extensive histopathological analysis has not been possible in these recessive families owing to a lack of autopsy material. In the recessively linked ALS families described for these genes, heterozygous individuals have been disease-free. Development of new ALS models targeting these genes should rapidly accelerate the discovery of new drugs that can regulate accumulation of these proteins or their downstream consequences. For instance, a high throughput approach to find novel drugs that restore the localization of TDP-43 and FUS may not only help in treating FALS caused by mutations in these genes, but also SALS in which these proteins are mislocalized. These drugs may be more relevant to the treatment of the vast majority of ALS patients.

Future perspective

Familial and sporadic ALS are clinically indistinguishable, and it has long been speculated that they may share elements of the same pathogenic pathway. This hypothesis has been supported by recent studies of TDP-43, FUS and optineurin providing implications for future

research. Given the causal role of TDP-43 and FUS, future research efforts need to be directed towards understanding the role of RNA processing pathways in the pathophysiology of ALS. At the same time, future effort should be directed towards understanding the role of protein degradation pathways, since ubiquitin and p62 immunoreactivity is a common feature of protein aggregates observed in ALS. Using genomic and proteomic tools, many new agents will be found in the future. Development of new ALS models targeting these agents should rapidly accelerate the discovery of new drugs using a high-throughput approach. These drugs may

be more relevant to the treatment of the vast majority of ALS patients by regulating protein aggregation or its downstream consequences.

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