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Familial amyotrophic lateral sclerosis, a historical perspective

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Amyotrophic lateral sclerosis is a fatal neurodegenerative disease of the upper and lower motor neuron of unknown etiology. Although a familial cause for this disease has been suspected early one, it is only in the past two decades that advances in modern genetics led to the identification of more than 10 genes linked to familial ALS and helped us understand some of the complex genetic and environmental interactions that may contribute to sporadic ALS. In this article, we chronologically summarize the genetic breakthroughs in familial and sporadic ALS and depict how it shaped our understanding of disease pathogenesis and our quest for rational therapies.

Key words: ALS, SOD1, GWAS

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult onset, fatal neurodegenerative disorder, involving the large motor neurons of the brain and the spinal cord. It is characterized clinically by progressive paralysis and eventual death from respiratory failure in three to five years.

ALS is mainly a sporadic disease of unknown etiology. Although it has been first described by Charcot in 1869, a lower motor neuron only form of the disease has been recognized almost two decades earlier by Aran and was called progressive muscular atrophy. Aran was also the first to put forward a familial etiology for this disease based on his description of a 45 years old sea captain with progressive muscular atrophy whose sister and two of his mother's brothers died of similar symptoms (Aran, 1850). By 1880 Sir William Osler recognized that the Farr family of Vermont had a dominantly inherited form of ALS. In 1993, a century later, genetic analysis of ALS kindred including the Farr family, led to the identification of *SOD1* as the first gene to cause familial ALS (1). Since then at least 10 additional genes have been linked to familial ALS/motor neuron disease. The discovery of these genes allowed the engineering of valuable animal models that were instrumental in our understanding of disease pathogenesis and in testing different therapies. More recently, the tools of modern genetic were also applied to sporadic ALS in an attempt to decipher the complex genetic and environmental interactions that lead to ALS.

In this review we will highlight the lessons learned from genetic research in familial and sporadic ALS.

The genetics of familial ALS

Familial ALS represents about 10% of ALS cases. It can be inherited either as an autosomal dominant or recessive trait. Adult onset autosomal dominant inheritance is more common than juvenile onset caused by recessive transmission. X-linked dominant inherited ALS has been reported in one family (2).

Mutations in *SOD1* are the most common cause of familial ALS or ALS1, they are found in about 20% of ALS patients (1). To date more than 150 disease causing mutations have been reported, spread throughout all five exons of the gene. These mutations are mainly missense mutations but small deletions or insertions have also been described (www.alsod.org).

The mode of inheritance is autosomal dominant with age dependent penetrance except for the D90A that is recessive in the Scandinavian population and dominant in others.

The phenotypes largely depend on the mutation with significant intra and inter familial variability. In the US

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the A4V mutation is the most common and is characterized by a limb onset mainly lower motor neuron disease with rapid progression (3, 4). This contrast with the G37R or H46 R mutations that have slower disease progression of at least 10 years (3, 4).

Patients with *SOD1* mutations are usually cognitively intact with rare exceptions (5). To explain the high prevalence of the A4V mutation in the United States, Saeed and colleagues determined that the North American A4V mutation arose from two founders, Native American (82%) and European (18%), about 400-500 years ago at the time of Jamestown and Plymouth landing (6).

Pathologically, *SOD1*-linked familial ALS can be distinguished from sporadic disease by the relative sparing of the motor cortex, slight or mild corticospinal tract involvement that contrast with the severe atrophy of the anterior roots and the degeneration of lower motor neurons. Engel and colleagues in 1959 reported a particular pattern of posterior column demyelination in the "middle root zone" in three patients with a familial syndrome that was clinically indistinguishable from ALS; one of the families reported had a rapid disease progression very suggestive of A4V mutation (7). This particular posterior column involvement has been since reported in autopsy tissue from patients with certain *SOD1* mutations such as A4V, I113T or E100G mutations (8, 9). The surviving motor neurons also harbor SOD1 immunopositive inclusions that have not been detected in the other familial form of ALS or in sporadic disease (10).

The role of *SOD1* mutations in familial ALS continue to be investigated. Significant insight into the pathogenesis of *SOD1*-linked familial ALS was gained from the study of the transgenic rodent models over expressing mutant *SOD1* and in particular from the mouse model over expressing $SOD1^{G93A}$ mutation originally developed by Gurney et al. in 1994 (11). Several studies have revealed that mutant SOD1 protein is misfolded and prone to aggregate. These aggregates have been shown to recruit wild type protein (12), disrupt mitochondrial function (12) or axonal transport (13) and can induce ER stress (14). The beneficial effect of lowering *SOD1* expression in the animal model is now being explored as a therapeutic option in human.

A little less than ten years after the discovery of *SOD1* mutations, *ALSIN* gene mutations were found to cause a rare, recessive, juvenile onset ALS or ALS2, *ALSIN* gene encodes a ubiquitously expressed protein named ALSIN. It localizes to the cytosolic face of endosomal membrane and acts as an exchange factor for Rab5 and other small GTPases and may be important for endosomal trafficking and axonal outgrowth (15). Few years' later, an atypical form of ALS or ALS 8 in a Brazilian family was linked to a missense mutation in vesicle-associated membrane protein -associated protein B gene (*VAPB*) (16), emphasizing the role of vesicular transport in motor neuron survival. VAPB is also involved in the unfolded protein response and interacts with lipid binding proteins, linking lipid metabolism dysfunction to the pathogenesis of ALS.

More recently the identification of TDP43 as the major disease protein in the ubiquinated inclusions in ALS and ALS /FTD led to the discovery of *TDP43* mutations in a subgroup of familial ALS patients or ALS10 and in rare families with FTD or FTD/ALS (17). TDP43 is a nuclear protein that is involved in many biological functions such as transcription and splicing regulation, mRNA stability (18) and micro RNA processing (19). In autopsy tissue as well as in animal or cell models, mutant protein form aggregates where TDP43 is phosphorylated, cleaved and translocated to the cytoplasm with sometimes loss of nuclear staining. *FUS* gene encodes a DNA/RNA binding protein of similar function to *TDP43*. Mutations in *FUS* gene have emerged as the second most common cause of familial ALS or ALS6 (20, 21). Although phenotypically they are indistinguishable from sporadic ALS, in our series, we found that when compared to *SOD1* patients they have an earlier age of onset, more frequent bulbar disease and a more rapid disease progression (22). Similar to TDP43, FUS inclusions were found to co-localize with P62, ubiquitin and TDP43 in autopsy tissues of patients with sporadic ALS, *TDP43* familial ALS, familial ALS with dementia and non-*SOD1* familial ALS (10) suggesting that sporadic disease and non-SOD1 familial ALS share similar pathogenic mechanisms.

TDP43 and *FUS* are not the only motor neuron causing genes involved in DNA/RNA metabolism, ALS4 linked gene, Senataxin (*SETX*), spinal musclar atrophy gene *SMN* and spinal muscular atrophy with respiratory distress gene *IGHMBP2* are also important for RNA processing. These findings have recently shifted the focus of research to the role of DNA and RNA in motor neuron degeneration and it can be expected that more DNA/RNA interacting proteins important for motor neuron viability will be discovered.

Finally the latest genetic research revealed two additional ALS associated genes: optineurin (*OPTN*) and valosin –containing Protein gene (*VCP*), both genes involved with Paget's disease of bone. *OPTN* mutations also cause hereditary glaucoma and *VCP* causes inclusion body myopathy with frontotemporal dementia and Paget's disease. The common glaucoma causing mutation in *OPTN* was shown to disrupt the ubiquitin-proteasome pathway and induces autophagy (23) whereas *VCP* is thought to be important for the coordination of protein degradation by both the ubiquitin-proteasome system and autophagy and mutations in *VCP* disrupt its function in protein degra-

dation (24). These genes underscore the role of protein homeostasis and the interface between proteasomal system and autophagy in the pathogenesis of ALS.

The Genetics of Sporadic ALS

The tremendous progress accomplished in modern genetics has allowed us to study the genetic susceptibility of complex diseases like sporadic ALS through genome wide association studies (GWAS), comparing cases to population based or family based controls.

Several GWAS and candidate genes association studies have been published in the past years with conflicting results. It was soon discovered that the interpretation of GWAS results is challenging. The results depend on strong GWAS design that requires a large sample size and adequate population stratification. Most results published did not survive statistical correction for multiple testing and were not replicated in different population.

Recently several studies have established the 9p21 locus as a susceptibility locus for ALS (25-27). In the study that used familial and sporadic cases the association signal was mainly driven by the familial cases (27). This fact emphasizes the importance of case selection in GWAS design. Extra care should be taken in identifying and excluding familial cases.

Finally the paraoxonase (PON) gene cluster on chromosome 7q21 has been extensively examined in the past years and has emerged as the most robust genetic risk factor for ALS. PON proteins play a role in preventing lipid oxidation and detoxifying organophosphates. Variants in PON genes were found to be associated with sporadic ALS in North American (28), Polish (29), Irish (30), French, French Canadian and Swedish populations (31).

Direct sequencing of the PON genes revealed at least seven mutations in familial and ALS patients that were predicted to alter PON function (32) implicating PON in sporadic and familial ALS.

Conclusion

The recent advances in the genetic of familial ALS made a significant contribution to our understanding of the pathogenesis of this fatal disease. The different genes discovered share similar biological functions that allowed us to identify common molecular pathway that lead to motor neuron degeneration. These pathways are also involved in other hereditary neurodegenerative disease such as spinal muscular atrophy, spastic paraplegia and hereditary motor and sensory neuropathies. Developing specific therapies targeting these molecular mechanisms will not only be important for ALS but for a wide array of neurodegenerative diseases.

Despite all the progress achieved, the large majority of ALS genes remain unknown, and with the tools of modern genetics one can only expect that the number of genes involved with familial ALS will continue to increase.

The discovery of additional susceptibility genes that increase the risk for sporadic ALS, like the PON gene cluster, may help us better understand the complex environment interaction and may improve our modeling of sporadic disease.

References

1. Rosen DR, Siddique T, Patterson D, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993;362:59-62.
2. Hong SBB ST. X-linked dominant locus for late-onset familial amyotrophic lateral sclerosis (Abstract). Soc Neurosci 1998;24:478.
3. Cudkowicz ME, McKenna-Yasek D, Sapp PE, et al. Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Ann Neurol 1997;41:210-21.
4. Juneja T, Pericak-Vance MA, Laing NG, et al. Prognosis in familial amyotrophic lateral sclerosis: progression and survival in patients with glu100gly and ala4val mutations in Cu,Zn superoxide dismutase. Neurology 1997;48:55-7.
5. Lopate G, Baloh RH, Al-Lozi MT, et al. Familial ALS with extreme phenotypic variability due to the I113T *SOD1* mutation. Amyotroph Lateral Scler 2010;11:232-6.
6. Saeed M, Yang Y, Deng HX, et al. Age and founder effect of *SOD1* A4V mutation causing ALS. Neurology 2009;72:1634-9.
7. Engel WK, Kurland LT, Klatzo I. An inherited disease similar to amyotrophic lateral sclerosis with a pattern of posterior column involvement. An intermediate form? Brain 1959;82:203-20.
8. Ince PG, Shaw PJ, Slade JY, et al. Familial amyotrophic lateral sclerosis with a mutation in exon 4 of the Cu/Zn superoxide dismutase gene: pathological and immunocytochemical changes. Acta Neuropathol 1996;92:395-403.
9. Ince PG, Tomkins J, Slade JY, et al. Amyotrophic lateral sclerosis associated with genetic abnormalities in the gene encoding Cu/Zn superoxide dismutase: molecular pathology of five new cases, and comparison with previous reports and 73 sporadic cases of ALS. J Neuropathol Exp Neurol 1998;57:895-904.
10. Deng HX, Zhai H, Bigio EH, et al. *FUS*-immunoreactive inclusions are a common feature in sporadic and non-*SOD1* familial amyotrophic lateral sclerosis. Ann Neurol 67:739-48.
11. Gurney ME, Pu H, Chiu AY, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 1994;264:1772-5.
12. Deng HX, Shi Y, Furukawa Y, et al. Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of *SOD1* in mitochondria. Proc Natl Acad Sci U S A 2006;103:7142-7.
13. Strom AL, Gal J, Shi P, et al. Retrograde axonal transport and motor neuron disease. J Neurochem 2008;106:495-505.
14. Wang L, Popko B, Roos RP. The unfolded protein response in familial amyotrophic lateral sclerosis. Hum Mol Genet 2010;20:1008-15.
15. Yang Y, Hentati A, Deng HX, et al. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat Genet 2001;29:160-5.

16. Nishimura AL, Mitne-Neto M, Silva HC, et al. A mutation in the vesicle-trafficking protein *VAPB* causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am J Hum Genet 2004;75:822-31.
17. Sreedharan J, Blair IP, Tripathi VB, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008;319:1668-72.
18. Buratti E, Baralle FE. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. Front Biosci 2008;13:867-78.
19. Buratti E, Baralle FE. The multiple roles of TDP-43 in pre-mRNA processing and gene expression regulation. RNA Biol 2010;7:420-9.
20. Vance C, Rogelj B, Hortobagyi T, et al. Mutations in *FUS*, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 2009;323:1208-11.
21. Kwiatkowski TJ Jr., Bosco DA, Leclerc AL, et al. Mutations in the *FUS*/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 2009;323:1205-8.
22. Yan J, Deng HX, Siddique N, et al. Frameshift and novel mutations in *FUS* in familial amyotrophic lateral sclerosis and ALS/dementia. Neurology 2010;75:807-14.
23. Shen X, Ying H, Qiu Y, et al. Processing of optineurin in neuronal cells. J Biol Chem 2011;286:3618-29.
24. Weihl CC. Valosin containing protein associated fronto-temporal lobar degeneration: clinical presentation, pathologic features and pathogenesis. Curr Alzheimer Res 2011;8:252-60.
25. van Es MA, Veldink JH, Saris CG, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. Nat Genet 2009;41:1083-7.
26. Shatunov A, Mok K, Newhouse S, et al. Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: a genome-wide association study. Lancet Neurol 2010;9:986-94.
27. Laaksovirta H, Peuralinna T, Schymick JC, et al. Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: a genome-wide association study. Lancet Neurol 2010;9:978-85.
28. Saeed M, Siddique N, Hung WY, et al. Paraoxonase cluster polymorphisms are associated with sporadic ALS. Neurology 2006;67:771-6.
29. Slowik A, Tomik B, Wolkow PP, et al. Paraoxonase gene polymorphisms and sporadic ALS. Neurology 2006;67:766-70.
30. Cronin S, Greenway MJ, Prehn JH, et al. Paraoxonase promoter and intronic variants modify risk of sporadic amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2007;78:984-6.
31. Valdmanis PN, Kabashi E, Dyck A, et al. Association of paraoxonase gene cluster polymorphisms with ALS in France, Quebec, and Sweden. Neurology 2008;71:514-20.
32. Ticozzi N, LeClerc AL, Keagle PJ, et al. Paraoxonase gene mutations in amyotrophic lateral sclerosis. Ann Neurol 2010;68:102-7.