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Exploring the Genetics of Amyotrophic Lateral Sclerosis as a Pathway to a Cure

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ABSTRACT: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects nerve cells in the brain and spinal cord, causing progressive weakness of various body muscles, leading to a debilitating fatal outcome. The most common form of ALS, known as sporadic ALS (SALS), occurs in 85%-90% of patients, while familial or inherited ALS (FALS) occurs in 10%-15% of patients. Among FALS patients, 65% have genetic mutations in the four main genes: C9ORF72 (30-40%), SOD1 (10-20%), FUS (5%), and TARDBP (5%) genes. The pathogenesis of ALS is still not fully understood, but several theories have emerged. The pathological hallmark of ALS is TDP-43-positive, ubiquitinated cytoplasmic inclusions, found in nearly 98% of autopsy reports, although few ALS patient actually carry mutations in the TARDBP gene.

KEYWORDS: Cellular Biology; Genetics; Neurodegenerative Disease; Amyotrophic Lateral Sclerosis.

Introduction

Amyotrophic Lateral Sclerosis, ALS is a debilitating fatal disease that affects the brain and spinal cord; more specifically, the neurons controlling movements. The disease initially causes fasciculations (small muscle twitches), weakness in the arms and legs, and slurred speech. As the disease progresses, paralysis of the legs and arms, inability to speak or eat, and inability to breathe independently can occur. Many patients require an artificial feeding tube, tracheostomy, or breathing machine to survive. Although patients lose their motor function, their memory, sensation, and cognition are not affected. Most patients diagnosed with ALS live 2-5 years after they first show symptoms. There are only two FDA-approved treatments: Riluzole and Edaravone, both of which aim to slow down the disease progression by reducing the number of stresses put on the neuron. Although, these treatments do not cure or reverse the symptoms already present.[1]

Epidemiology of ALS :

The incidence of ALS is 0.6-3.8 in every 100,000 persons per year.[2] ALS is slightly more common in males, with the lifetime risk for men being 0.29% and for women 0.25%.[3] The most affected age group consists of individuals aged 50-75. Regionally, it is more common in individuals of European ancestries and less common in individuals of Asian and Native American ancestries.[1]

Non-Genetic Risk Factors:

There are several possible environmental risk factors for ALS, such as diesel exhaust[4] or lead exposure.[5] Some occupations such as agriculture, hunting, forestry, fishing, and construction work showed a weak association with ALS in some studies. In addition, dietary habits such as consumption of red and processed meat, animal protein, sodium, zinc, and glutamic acid are associated with higher risk of ALS in some studies, whereas consuming tea, coffee, whole grain, raw vegetables, and citrus fruits lowers the risk of getting ALS. There is no known association shown with alcohol consumption.[2] There is a correlation between increased blood lipid level,[6] smoking, and physical activity.[7]

Clinical Sub-types of ALS:

ALS is mainly categorized into four major variants, based on clinical presentation and part of the central neural system involved: spinal onset ALS, which mainly involves the cervical spinal cord and reported in about 46% of the patients. Bulbar onset ALS, which mainly involves the brainstem and found in 23% of the patients. Isolated bulbar ALS, which only involves bulbar symptoms, is reported in about 5% of the patients. Concomitant frontotemporal dementia, causes cognitive and behavioral impairments, is found in 5-15% of the patients.[8] Common bulbar symptoms are thought to be due to upper motor neuron (UMN) damage, causing facial weakness, trouble talking, and sometimes difficulty of swallowing. Common spinal symptoms include muscle wasting, cramping, and twitching. Respiratory symptoms can manifest concomitantly or separately, as difficulty with breathing, hypoventilation, and respiratory musculature weakness.[9]

Etiological Sub-types of ALS:

The most common form of ALS, known as sporadic ALS(SALS), occurs in 85%-90% of patients, while familial, or inherited, ALS(FALS) occurs in 15%-10% of patients.[8] Among FALS patients, 65% have genetic mutations in the four main genes; C9ORF72(30-40%), SOD1(10-20%), FUS (5%), and TARDBP(5%) genes.[11] FALS is most commonly inherited as an autosomal dominant allele, meaning that if one parent has the mutated allele, 50% do not carry, and are not at risk, 50% inherit mutant allele, and are likely to develop ALS.[10] It can also be inherited as an autosomal recessive allele, meaning that if two of the parents carry the mutated allele, 25% of the children are at risk, 50% are carriers, and 25% will not carry the allele at all. In rarer cases, an X-linked dominant allele, meaning that if the father has the mutated allele and the mother does not, 100% of female children will carry

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the allele, and 0% of male children will carry the allele.[7] The majority of ALS cases (85-90%) are known as sporadic ALS and do not have a clear family history of ALS due to multifactorial genetic and environmental risk factors involved. Genetic factors have been estimated to contribute to 40-60% of SALS. Due to the rapid expansion of Genome-wide associated studies (GWAS), there are new genes and single nucleotide polymorphisms (SNPs) associations with SALS that have been identified.[11]

Prognosis:

The survival of ALS patients is variable. About 10% of ALS patients have slow progression and have a high likelihood of survival of 10 years or longer, yet most ALS patients only survive 2-5 years after the onset of symptoms. Longer survival is associated with male sex, spinal onset, the younger onset of disease, higher body mass index, and weight since diagnosis.[2] Bulbar onset, respiratory onset, higher age of onset, smoking, and weight loss since diagnosis associated with shorter survival.[1,2] Persons who get a tracheotomy have a longer survival independent of the above factors.[2]

Results and Discussion

Pathogenesis of ALS:

The pathogenesis of ALS is complex and still not fully understood yet, but there are several theories that have some support. The pathological hallmark of ALS is TDP-43-positive, ubiquitinated cytoplasmic inclusions found in nearly 98% of autopsy reports. In most cases, however, these are not associated with mutations in TARDBP, the gene encoding TDP-43.[7] In general, it is believed that an interplay between genetic and environmental factors contributes to developing ALS. An alternative theory is that all ALS cases are primarily due to complex genetic factors. There is heterogeneity in genetic causes in ALS, but familial and sporadic ALS have similarities in their disease process and clinical features, suggesting similarities in cellular and molecular events that lead to motor neuron degeneration. The pathogenesis of ALS is explained in a multistep process. ALS genes are grouped into multiple cellular and tissue level categories. At the level of nuclear function, these categories include genes involved in RNA metabolism, DNA repair, or nuclear export. There are four groups at cytoplasm and mitochondrial level: genes involved in protein hemostasis, mitochondrial function, vesicular transportation, or oxidative stress. There are three sub-groups at the cytoskeletal and neuronal axon levels: gene groups involved in glial function, Nerve excitability, and Axonal transportation.[12]

Each category leads to multiple cellular abnormalities, as seen in Figure 1[12], such as the deposition of intranuclear and cytosolic protein and RNA aggregates, disturbances of protein breakdown mechanisms, mitochondrial malfunction, endoplasmic reticulum stress, defective transportation from the nucleus to the cytoplasm, changes in neuronal excitability, and changes in axonal transportation.

Genes associated with ALS:

With the advancement of gene mapping and DNA analysis, more than 120 genetic variants, and about 300 single nucleotide polymorphisms (SNPs) (GWAS catalog) have shown associations with ALS, and nearly 30 genes are suspected to

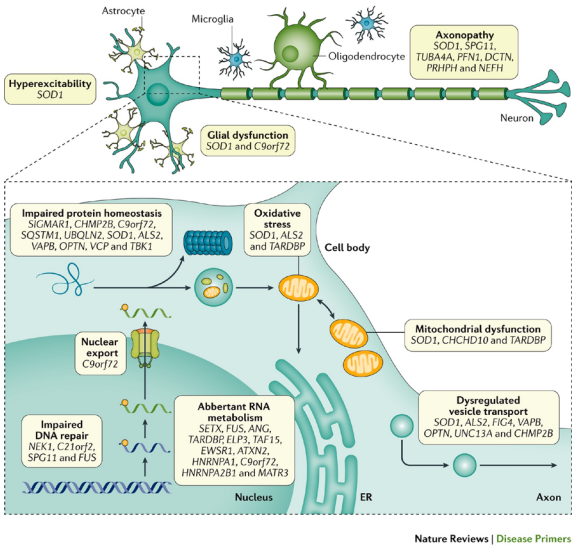

Figure 1: Pathogenesis of ALS.[12]

cause ALS (Figure 2). In 1993, the first ALS gene SOD1 was identified and subsequently many more genes have been identified.[3] There are some correlations between genetic variants and different clinical types identified such as age onset, site of onset, and survival or rate of progression. Gene FUS-P525L and SOD1/SOD1 are associated with early onset disease, and SNP rs3011225-1p34 is associated with late-onset disease. Gene C9ORF72 primarily leads to bulbar ALS, and variants PFN1 and SOD1-A4V are associated with rapid onset, and mainly affect the lower motor neurons. Also, lower expression of a gene that encodes enzyme ephrin A4(EPHA4) is associated with prolonged survival. In contrast, AV4 mutation of SOD1 and P525L mutation of FUS/TLS genes may lead to early onset ALS[13].

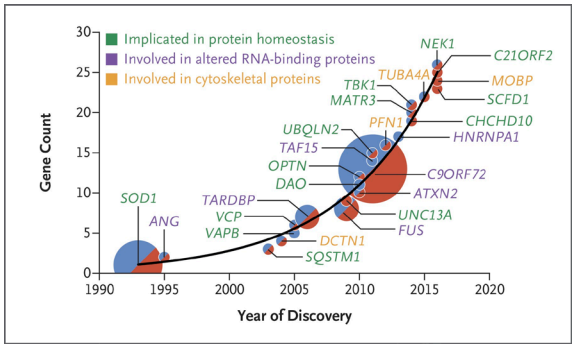

Figure 2: Above diagram shown the timeline of gene discovery in ALS. The size of each circle represents the proportion of familial ALS cases associated with that gene (e.g., *C9ORF72* 45% and *SOD1* 20%). Blue circles indicate that genes are only associated with familial ALS, red circles indicate genes associated only with sporadic ALS, and half red and half blue indicates these genes associated with both familial and sporadic ALS. (Brown 2017 NEJM)[13].

Chromosome 9 open reading frame 72(C9ORF72):

The *C9ORF72* is the most commonly mutated gene in ALS, found in 40% of Familial ALS, and 6-8% of sporadic ALS. *C9ORF72* is also associated with a dopa non-responsive Parkinson's disease and Huntington's disease phenotype.[14] The *C9ORF72* protein has an important role in nuclear and endosomal membrane transportation and autophagy. This gene

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has a noncoding stretch of six nucleotides that repeats up to 30 times in healthy people. In familial ALS and frontotemporal dementia this segment expands up to a hundred to a thousand times. This expansion is sometimes leading to sporadic ALS. This abnormal repeat expansion causes neurotoxicity by several mechanisms, such as leading to defective RNA, and abnormal protein synthesis that generate several neurotoxins. In addition, reduced normal *C9ORF72* protein levels contribute to neurotoxicity.[13]

Superoxide dismutase 1 (SOD1) :

SOD1 on chromosome 12q12.[1] was the first identified ALS gene. Normal *SOD1* protein catalyzes the reduction of superoxide to hydrogen peroxide. A point mutation in the *SOD1* gene, leads to disease variants that frequently form intracellular aggregates. Genes that encode the adaptor proteins such as valocin containing protein (VCP), Protein optineurin (OPTN), TANK-binding kinase 1 (TBK1), and sequestosome1 (SQSTM1/p62) involve in protein maintenance and degradation are also affected.[13] *SOD1* gene is involved in about 12% of patients with familial ALS and 1–2% of sporadic ALS. The *SOD1* gene has an autosomal dominant inheritance, except the *SOD1* D19A variant has autosomal recessive inheritance. About 160 pathogenic *SOD1* variants have been identified and cognitive impairment is rare among all the SOD1 variants. Phenotypes of *SOD1* ALS include classical ALS and progressive motor atrophy, often with asymmetrical lower limb onset; when upper motor neuron signs are missing, and lower motor neuron signs tend to predominate. *SOD1* ALS has an average age of onset of forty, and disease duration is variable. However, age at onset and severity may vary significantly depending upon the variant involved , and within families for some variants such as *SOD1* I114T, and penetrance may be less than 100%.[14]

Transactive response DNA binding protein 43 (TARDBP) :

The TARDBP gene provides signals for making a protein called the transactive response DNA binding protein 43 kDa (TDP-43). The TDP-43 protein attaches to the DNA and regulates transcription, the process by which mRNA is produced from the template strand of DNA. The TDP-43 protein is associated with the processing of RNA and is one of the many proteins which aid in the splicing of mRNA.[15] By helping to cut and rearrange mRNA molecules in various ways, the TDP-43 protein regulates the production of different versions of specific proteins. This protein can also bind to mRNA to ensure its stability. A mutated TDP-43 may thereby allow some sections of the genetic code which were meant to be spliced, to code proteins. The A382T variant in the *TARDBP* gene, which has an association with both familial and sporadic ALS, has been identified in individuals with frontotemporal lobe dementia with or without motor neuron disease.[14]

Fused in Sarcoma (FUS):

Abnormalities of the *FUS* gene are found in around 5% of patients with familial ALS and 1% of sporadic ALS patients.[14] *FUS* gene is functionally similar to TDP-43, which regulates DNA and RNA transcription, splicing, and processing. The reason why the mutated *FUS* gene causes ALS is still not clear.[13] One presumption is that the alteration of gene sequence variance leads to protein aggregates within the cell causing neurotoxicity and neurodegeneration. About 85 *FUS* gene mutations associated with ALS have been identified.[16] There are notable *FUS* variations such as the P525L mutation which causes severe childhood-onset ALS, which causes a shorter lifespan.[13]

Conclusion

There are only two Food and Drug Administration-approved medications available for ALS treatment. Riluzole is a pill administered twice daily. Its mechanism of action is unclear but presumed to reduce glutamatergic neurotransmission and suppress excessive firing of neurons.[9] Edaravone, is a daily injection, that acts by reducing oxidative stress and slowing down disease progression. However, it has not shown reversal of weakness or been able to be used to cure the disease. Other options are supportive care such as artificial feeding, physical therapy, tracheostomy, aspiration prevention, and breathing support with a mechanical ventilator.[13]

The high heterogeneity of the disease and multiple gene involvement is the greatest challenge to finding a treatment for ALS. Therefore, multiple targeted treatment approaches will be the best option. Recent developments in gene-targeted therapeutics with increasing success in similar diseases such as Huntington's disease and Spinal muscular atrophy create great hope in FALS treatment. One approach is to find gene-targeted treatment for *C9ORF72* and SOD1 associated with more than 60% of FALS and identify gene-specific biomarkers which will help to evaluate the success of the treatment. Another approach is repairing the defective gene using gene-editing technology such as CRISPR,[11] which is currently being tested in Thalassemia and Sickle Cell Anemia.[17]

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