



Unravelling the Genetic Factors In the Pathogenesis of Amyotrophic Lateral Sclerosis (ALS)

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ABSTRACT

Progressive degeneration of the large motor neurons in the brain and spinal cord is a common finding in Amyotrophic Lateral Sclerosis (ALS). Several factors considered to underlie the disease have been proposed including genetic and environmental factor. Genetic factor have been well known to be highly implicated in the pathogenesis of ALS. Today, more than 20 genes contribute to the development of ALS. The pathologic hallmark of ALS is the accumulation of ubiquitinated protein aggregates. TDP-43 (TAR DNA binding protein, 43 kD) encoded by *TARDBP* gene was found to be the major component of these aggregates in most patients. Disruption of RNA binding capacity of TDP-43, either in the presence or absence of mutations will lead to the dysregulation of its targeted RNAs. Misregulation of RNAs regulated by TDP-43 is possibly a major cause of the disease. **Muthmainah. Unravelling the Genetic Factor in the Pathogenesis of Amyotrophic Lateral Sclerosis (ALS).**

Keywords: ALS, genetic, TDP-43 protein

ABSTRAK

Degenerasi progresif dari neuron motorik di otak dan sumsum tulang adalah karakteristik dari penyakit ALS. Beberapa faktor yang dianggap menjadi penyebab ALS antara lain adalah faktor genetik dan faktor lingkungan. Faktor genetik diketahui berperan penting dalam patogenesis ALS. Saat ini, lebih dari 20 gen telah teridentifikasi dan terlibat dalam perkembangan penyakit ini. Penanda patologis kelainan ini adalah adanya agregat protein yang tersebar. Komponen utama dari agregat ini adalah protein TDP-43 yang diatur oleh gen *TARDBP-43*. Gangguan dalam kemampuan mengikat protein dari TDP-43 akan menyebabkan kelainan regulasi RNA yang menjadi target TDP-43. Gangguan regulasi RNA-RNA yang terikat pada protein ini diperkirakan menjadi penyebab utama penyakit ini. **Muthmainah. Mengungkap Latar Belakang Genetik dalam Patogenesis Penyakit Amyotrophic Lateral Sclerosis (ALS).**

Kata kunci: ALS, genetik, protein TDP-43

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is characterised by the progressive degeneration of the large motor neurons in the brain and spinal cord. Symptoms of lower motor neuron degeneration include weakness, cramps, twitching of muscles, incoordination and fatigue. Stiffness, weakness of muscles and incoordination are the main clinical features of upper motor neuron degeneration. Patients may become paralysed and most patients die 2-5 years after symptom onset due to respiratory failure.^{1,2} Several factors underlying this disease have been proposed including the genetic and environmental causes.^{2,3}

Etiology of ALS

Among all ALS cases, around 10% are associated with family history of the disease. Mutations in the *SOD1* gene encoding

superoxide dismutase 1 account for one fifth of all the familial ALS cases.⁴ Mutations in more than 20 other genes have been implicated in ALS but the cause remains unknown in 32 % of familial cases.⁵ Transgenic *SOD1* mouse models have been used widely to study the mechanism of the disease and to develop the potential therapies for the patients. However, these models have not been able to provide a comprehensive understanding on the pathogenesis of ALS.

Environmental causes such as heavy metal exposure and viral infection are considered to contribute to the development of ALS. Many neurologists suspected that mercury, lead and arsenic exposure may cause ALS but there has been no convincing evidence that these substances play a role in the course of the disease.² Motor neuron disorder

symptoms were also detected in patients with enteroviral and retroviral infection. However, the correlation of the infection with ALS is not fully understood.^{6,7} Furthermore, the association between enterovirus infection and ALS was not confirmed.⁸ Sensitive Reverse Transcription Polymerase Chain Reaction (RT-PCR) method also failed to detect the virus in spinal cord of ALS patients.⁹

State of the Art on the Role of Genetic Factor in ALS

Genetic factor have been well known to be highly implicated in the pathogenesis of ALS. The cellular mechanism of motor neuron degeneration can be analysed by assessing the genes involved in the disorder. Thus, unravelling the genetic factor can help facilitate disease modelling as well as design a better therapeutic approach. List of genes that



have been implicated in the development of ALS is presented in the following tables.⁵

The Role of *TARDBP* Gene Encoding TDP-43 protein in ALS

TDP-43 (TAR DNA binding protein, 43 kD) is an RNA binding protein that is structurally similar to the heterogenous nuclear ribonuclear protein (hnRNP) family of RNA binding proteins. Similar to other members of the hnRNP family, TDP-43 plays various roles in the regulation of gene expression, including pre-mRNA splicing, microRNA processing, mRNA transport and translation.¹⁰ It is also involved in stress granule formation.¹¹ TDP-43 is encoded by the *TARDBP* gene and is normally located inside the nucleus.¹²

Recent findings show that the key feature of the pathology of several neurodegenerative diseases, including ALS, is the accumulation of ubiquitinated, protein aggregates. In ALS, TDP-43 was found to be a major component of these aggregates in most patients.¹³ TDP-43 positive inclusions are mislocalised in the cytoplasm and in neurites, and occasionally in the nucleus.¹⁴

The fact that TDP-43 is the major component of the inclusions has led to further investigation about the correlation of TDP-43 and ALS. Dominant mutation in the *TARDBP* gene encoding TDP-43 is responsible for the development of up to 4% of familial ALS cases.¹⁴ Eight mis-sense mutations in *TARDBP* gene have been found in patients with sporadic and familial ALS.¹⁵ To date, 48 mutations in *TARDBP* gene have been identified in familial and sporadic ALS.¹⁶ *TARDBP* is located on Chromosome 1 in band 1p36. Mutations in the highly conserved C-terminal region of *TARDBP* were confirmed to cause ALS.¹⁷ A mouse model in which mutant TDP-43 is overexpressed in neurons led to splicing alterations accompanying adult onset motor neuron disease without the presence of TDP-43 inclusion or nuclear clearing.¹⁸ A study revealed that *Drosophila* lacking TDP-43 showed normal appearance externally but developed locomotive behaviours deficit and reduced life span.¹⁹

It is reported that a transgenic mice over-expressing mutant human TDP-43 developed motor neuron degeneration in the absence of cytoplasmic aggregates. This result shows

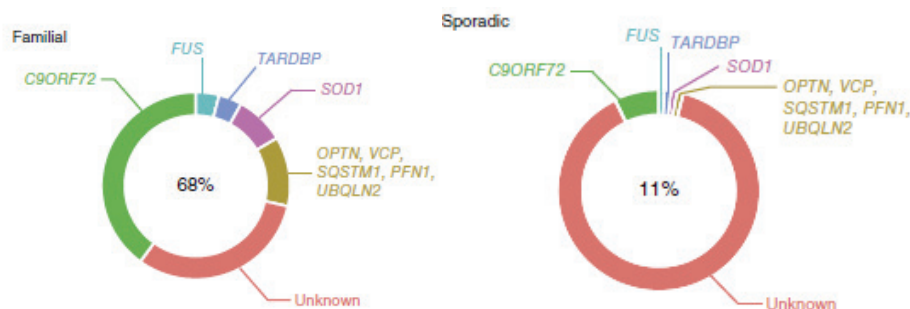


Figure 1. Genes identified to contribute to the development of ALS. The unknown causes of familial ALS account for 32% of total cases while the unknown causes in sporadic ALS have not been defined yet.⁵

that toxic aggregation of TDP-43 is not the principal cause of the disease as previously assumed.²⁰ In addition, over-expression of mutant human *TARDBP* in zebrafish embryos (*Danio rerio*) resulted in neurotoxicity leading to behavioural motor deficits characterised by shortening of motor neuronal axons, swimming deficits, and premature and excessive branching of the neuron. Furthermore, knock-down of zebrafish *tardbp* gave rise to the same phenotype. This indicates that mutations of *tardbp* cause motor neuron defects.^{21,22}

Structure of TDP-43

TDP-43 consists of 414 amino acids with two RNA recognition motifs (RRM1 and RRM2) that are the RNA binding domain. Both RRM1 and RRM2 play a role in the DNA/RNA interaction.²³ TDP-43 has the propensity to bind with TG/UG sequences through the highly conserved phenylalanine residues in RRM1 and the affinity of this binding increases with the number of repeats. RRM1 is crucial for RNA binding, whereas RRM2 facilitates the interactions with ssDNA and TDP-43 self association.^{24,25}

Table 1. List of genes confirmed to have ALS-causing mutations⁵

No	Gene	Location	Inheritance	Protein Function
1.	TARDBP	1p36	AD	RNA metabolism
2.	SQSTM1	5q35	AD	Ubiquitination; autophagy
3.	C9ORF72	9p21	AD	DENN protein
4.	VCP	9p13	AD	Proteasome; vesicle trafficking
5.	OPTN	10p13	AR and AD	Vesicle trafficking
6.	FUS	16p11	AD and AR	RNA metabolism
7.	PFN1	17p13	AD	Cytoskeletal dynamics
8.	SOD1	21q22	AD and AR	Superoxide metabolism
9.	UBQLN2	Xp11	XR	Proteasome

AD, autosomal dominant; AR, autosomal recessive; XD, X-linked dominant; DENN, differentially expressed in normal and neoplasia.

Table 2. List of other genes implicated in the pathogenesis of ALS⁵

No	Gene	Location	Inheritance	Protein Function
1.	DCTN1	2p13	AD	Axonal transport
2.	ALS2	2q33	AD	Vesicle trafficking
3.	CHMP2B	3p11	AD	Vesicle trafficking
4.	FIG4	6q21	AD and AR	Vesicle trafficking
5.	HNRNPA2B1	7p15	AD	RNA metabolism
6.	ELP3	8p21	Undefined	RNA metabolism
7.	SETX	9q34	AD	RNA metabolism
8.	HNRNPA1	12q13	AD	RNA metabolism
9.	ATXN2	12q24	Undefined	Endocytosis; RNA translation
10.	ANG	14q11	AD	Angiogenesis
11.	SPG11	15q14	AD	DNA damage repair
12.	VAPB	20q13	AD	Vesicle trafficking
13.	NEFH	22q12	AD	Axonal transport

AD, autosomal dominant; AR, autosomal recessive



TDP-43 also has a C-terminal domain rich in glycine residues which is essential for interaction with other hnRNPs (protein-protein interaction) and plays an important role in various kind of RNA splicing and metabolism. Mutations in this region have been identified in ALS cases. In addition, TDP-43 contains a nuclear localisation signal (NLS) and a nuclear export signal (NES). The NES facilitates protein shuttling between the nucleus and cytoplasm. The NLS and the C-terminal region of TDP-43 are crucial for nuclear localization. Mis-localisation of TDP-43 can happen if loss of either one of them occurs.²⁶

Functions of TDP-43

Initially, TDP-43 was identified as the transcriptional repressor of HIV-1 genome through binding with the TAR DNA segment. Later, it was reported that TDP-43 also mediates transcriptional repression during spermatogenesis. These describe the role of TDP-43 in transcriptional regulation. TDP-43 interacts with various kinds of splicing factors.²⁶ Over-expression of TDP-43 increased exon 9 skipping resulting in non-functional cystic fibrosis trans-membrane conductance regulator (CFTR) protein.²⁷ TDP-43 binds to the splicing enhancer element of SMN2 gene and promotes inclusion of exon 7.²⁸

TDP-43 increases the stability of mRNA through binding to the 3' UTR.²⁹ It also regulates microRNA biogenesis by binding to their precursor elements. Knockdown of TDP-43 impaired the expression of these miRNA's target transcript.³⁰ TDP-43 has been shown to regulate the transport of mRNA to the dendrites and regulated their translation



Figure 2. Schematic representation of TDP-43 depicts a glycine rich C terminal region, two RNA recognition motifs (RRM), a nuclear localization signal (NLS) and a nuclear export signal (NES).²²

locally at synapses.³¹ Another report has shown that TDP-43 is transported in live cultured motor neuron axons. Additionally, overexpression of mutant TDP-43 resulted in the severely impaired axonal growth.³² Therefore, TDP-43 has been shown to play a role in many aspects of RNA regulation.

Potential Significance of TDP-43 Target Genes in the Development of ALS

To date, 1839 potential gene targets of TDP-43 have been identified. These are comprised of genes involved in signal transduction, synaptic vesicle associated proteins, and enzyme activity.²² A study using transgenic animals which overexpress human TDP-43 carrying ALS causing mutation revealed 154 genes which are aberrantly spliced suggesting the role of TDP-43 in the splicing regulation of its targeted mRNA.²²

Defects in RNA metabolism have been heavily implicated in ALS, largely due to the fact that TDP-43 aggregates are found in most ALS patients whether or not they have mutations in *TARDBP*. Evidence showing that ALS is a disorder of RNA metabolism is increasing. Disruption of RNA binding capacity of TDP-43, either in the presence or absence of

mutations will lead to the dysregulation of its targeted RNAs.^{20,22} Misregulation of RNAs regulated by TDP-43 is possibly a major cause of the disease.

In addition to its role in splicing regulation, TDP-43 also regulates RNA transport. Motor neurons have long axon and thus are more sensitive to disruptions in mRNA transport. In the mouse neuromuscular junction, TDP-43 can be found at the presynaptic membrane of axon terminals. More than 100 RNAs correlated with synaptic function are the binding targets of TDP-43. It is likely that decreased capacity of TDP-43 to transport these synaptic mRNAs into distal processes is responsible for the loss of synapse protein giving rise to motor neuron death in ALS.^{22,36}

Conclusion

More than 20 genes have been identified to contribute to the pathogenesis of ALS. However, the causes of other familial and sporadic ALS cases remain unknown. This article provides state of the art on the role of the genetic factor in the pathogenesis of ALS. Better understanding of the etiology will lead to a more comprehensive disease modelling and therapeutic designing.

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