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Multi-Ethnic Comparison of the Characteristics of Amyotrophic Lateral Sclerosis-Related TBK1 Gene Variants

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Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease, and the etiology of sporadic ALS is unknown. The TANK-binding kinase 1 (*TBK1*) gene identified as an ALS gene, contributes towards a predisposition for ALS. In this review, we analyzed variants of *TBK1* found in ALS cohort studies belonging to various regions and ethnic populations. *TBK1* variants tend to be enriched in patients with ALS compared to patients without ALS. The frequency of *TBK1* variants is more in the familial Caucasian than that in the Asian population. However, loss of function (LoF) variant associated with sporadic ALS is almost similar among the Asian group, including Japanese population. LoF variants were frequently reported to be associated with the TBK1 biology. These findings indicate that *TBK1*- LoF variants are pathogenic for ALS, regardless of race or region.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive upper and lower motor neuron loss, which causes spasticity and muscle atrophy. Moreover, it leads to general weakness of the skeletal muscles and death within 2-5 years. Approximately 5-10% of ALS patients have a positive family history, and the rest of them are detected with sporadic ALS[1].

More than 30 causative genes of familial ALS, including *SOD1*, *FUS*, *TARDBP*, *OPTN*, and *C9ORF72* have been identified. Mutations in these genes can be found in more than 50% of familial ALS patients, and in a small proportion of sporadic ALS patients[2]. In Japan, a genetic screening study reported that 3% of sporadic ALS patients have a known familial ALS-related pathogenic gene mutation[3]. To reveal the pathophysiology and develop therapeutics particularly for sporadic ALS, the gene variants and their roles in neurodegeneration in the cohort of sporadic ALS needs to be studied.

TBK1 is a ubiquitously expressed molecule, which plays important roles in multiple cell signaling pathways implicated in autophagy[4], insulin signaling[5], and cellular proliferation. TBK1 phosphorylates multiple substrates including optineurin and SQSTM1[6], which are genes related to ALS. *TBK1* was first identified as an ALS related gene through exome sequencing in two independent studies[7,8]. Moreover, the variants of the *TBK1* gene in ALS has been reported in the cohort studies of Europe[7,8], Australia[9], Taiwan[10], Belgium[11], France[12], China[13], Korea[14], Sweden[15], Germany[16], Italy[17], and Japan[18]. In this review, we summarize the *TBK1* variants of each cohort study that has been reported, and their pathogenic significance in ALS.

TBK1 Structure and Function

TBK1 is a multifunctional kinase, involved in multiple cellular processes, such as autophagy, cell proliferation, native immune response, and inflammation. TBK1 was initially identified as a kinase mediating the ability of TANK to activate NF-kappa-B (NF-κB)[19]. It is an important member of the I-kappaB (IκB) kinase (IKKs) family and is involved in the regulation of the Interferon regulatory factor 3 (IRF3) and NFκB signaling. The role of TBK1 in selective autophagy has been extensively studied in *Salmonella*[20]. TBK1 also phosphorylates the ubiquitin-associated (UBA) domain of optineurin on serine-177, enhancing the binding affinity of microtubule-associated protein light chain 3 (LC3) and autophagic clearance of cytosolic *Salmonella*[21, 22]. TBK1 is also involved in the autophagic clearance of *Mycobacterium tuberculosis* in mouse macrophages and phosphorylates the autophagic receptor SQSTM1 to enhance its binding to polyubiquitinated bacteria[23]. Moreover, TBK1 is particularly important for the maturation of autophagosomes to hydrolytic autophagosomes, which results in degradation of SQSTM1 and its associated cargo[5].

TBK1 has 729 amino acids, a serine/threonine kinase domain, a ubiquitin-like domain (ULD), and two c-terminal coiled-coil domains (CCD1 and CCD2). The serine/threonine kinase domain phosphorylates various substrates such as the interferon regulatory factor 3[24]. The ULD region of the TBK1 regulates the kinase activity and interaction with other molecules in the TBK1 pathway[25]. The CCD region of TBK1 binds to optineurin and phosphorylates SQSTM[6, 8].

TBK1 variants in the ALS and Control Cohorts

Racial frequency of *TBK1* variants

TBK1 was first reported as a causative ALS gene by two independent studies[7, 8]. Cirulli et al. analyzed *TBK1* variants using a number of inheritance models by performing exome sequencing on 2869 ALS patients and genetically on Caucasian ethnic healthy controls[7]. This study confirmed several previously identified ALS genes and reported *TBK1* as a new ALS-related gene. Freischmidt et al. identified *TBK1* as an ALS gene using the exome sequencing method and the target variant screen in Swedish population[8]. Moreover, the study conducted on an Australian cohort of familial ALS patients identified a novel *TBK1* LoF variant in a family of Chinese origin[9]. Subsequently, the *TBK1* LoF variant was found to be a rare cause of ALS in the Taiwanese[10] and Chinese cohort[13], as well as in Sardinian ALS patients[15]. *TBK1* LoF variants have been reported in more than 10 cohorts so far (Table 1), including approximately 1.6% patients with familial ALS and 0.4% patients with sporadic ALS. Patients with familial ALS have more *TBK1* LoF variants than sporadic ALS patients, probably because they include family members with LoF variants in the cohorts examined as well. In our previous study, we analyzed the *TBK1* gene by exome sequencing in a large Japanese cohort of 713 sporadic ALS patients and 800 controls[18]. All cases were registrant of Japanese Consortium for Amyotrophic Lateral Sclerosis Research (JaCALS) [3, 26]. We identified LoF variants of *TBK1* in 0.42% (3/713) sporadic ALS patients in our cohort.

A meta-analysis by Cui et al., revealed that the frequency of *TBK1* variants is higher in Caucasian patients than in Asians[27]. Therefore, we summarized the data of *TBK1* variants in each cohort including recent studies (Table 1). The frequency of missense variants in the *TBK1* gene was higher in Caucasian sporadic ALS patients than in Asians[7]. However, there was no racial difference in the frequency of *TBK1* LoF variants in cohort studies with sporadic ALS (Caucasian sporadic ALS patients: 0.44%; Asian patients: 0.39%). On the contrary, the *TBK1* LoF variants in the familial ALS patients were reported only in Caucasian patients (Caucasian familial ALS patients: 1.69%; Asian patients: 0%).

Table 1. Characteristics of TANK-binding kinase 1 (TBK1) variants in the cohort

			Frequency of LoF variants						
Population	LoF variants	Missense variants	fALS patients	sALS patients	Total ALS patients	Patients	Cohort	Year	Reference
Caucasian	8	33	-	-	0.28%(8/2874)	2874 (fALS105/sALS1563)	European	2015	7
Caucasian	12	100	3.5% (9/252)	0.28% (3/1010)	0.95%(11/1262)	1262(fALS252/sALS1010)	European	2015	8
Caucasian	1	0	0.79%(1/127)	-	0.79%(1/127)	127(fALS)	Australian	2015	9
Caucasian	5	5	-	-	1.2%(5/416)	416	French	2015	12
Caucasian	11	5	-	-	1.75%(11/629)	629(fALS154/sALS475)	Belgian	2015	11
Asian	0	2	0(0/23)	0(0/271)	0(0/294)	294(fALS23/sALS271)	Chinese	2016	13
Asian	1	2	0(0/32)	0.57%(1/175)	0.48%(1/207)	207(sALS175)	Taiwanese	2016	10
Asian	1	3	-	0.78%(1/129)	0.78%(1/129)	129(sALS)	Korean	2016	14
Caucasian	1	2	-	-	0.54%(1/186)	186	Sardinian	2016	15
Caucasian	2	5	0(0/30)	1.6%(2/124)	1.3%(2/154)	154(fALS30/sALS124)	Italian	2017	17
Asian	3	6	-	0.42%(3/713)	0.42%(3/713)	713(sALS)	Japanese	2018	18
Caucasian	2	2	0.66%(2/301)	-	0.66%(2/301)	301(fALS)	German	2018	16

Key: ALS: amyotrophic lateral sclerosis, sALS: sporadic ALS, fALS: familial ALS, LoF: loss-of-function

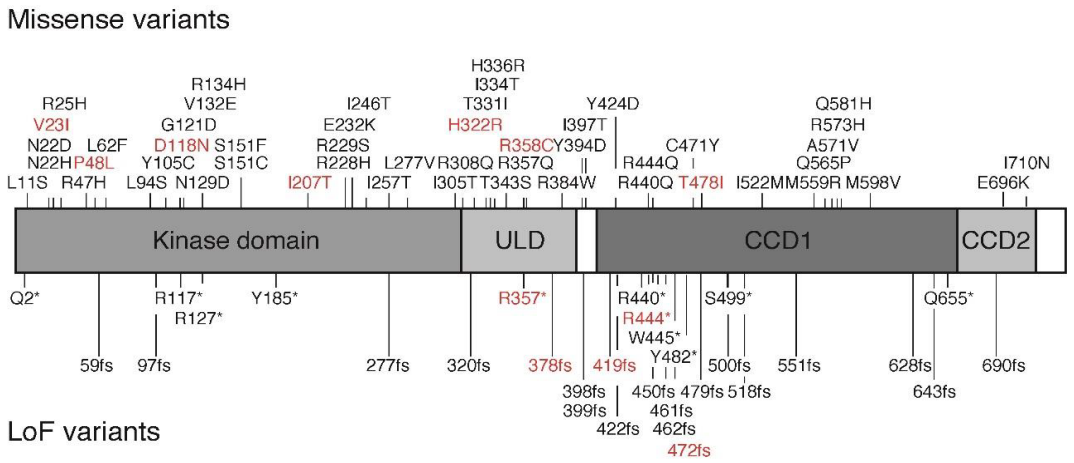

Figure. Schematic diagram showing the location of TBK1 variants

Schematic representation of the location of missense variants (above) and LoF variants (below). Red letters indicate variants found in East Asia and black letters indicate those found in Europe. Abbreviations: CCD, C-terminal coiled-coil domain; ULD, ubiquitin-like domain. *: a terminal codon, fs: frameshift (leading to a premature terminal codon)

Table 2. Clinical characteristics of Asian ALS patients with LoF variant of the TBK1 gene

Variant	Sex	Age at onset	Site and symptoms of onset	Cognitive impairment	TPPV free survival duration (Month)	Population
p.Arg357Term	Male	49.0	Dysarthria	-	8	Japanese
378fs	Female	55.9	Dysarthria	-	>20	Japanese
419fs	Male	62.2	Lower Limb	-	>10	Japanese
p.Arg444Term	Female	55.0	Upper Limb + Behaviour disorder	+	Died at 59 years old	Taiwanese
472fs	Male	53.0	Dysarthria+Dysphagia	-	Gastrostomy at 46 months after onset	Korean

TPPV; Tracheostomy positive pressure ventilation

Distribution of variants in the TBK1 molecule, and their pathogenicity

TBK1 mutation has been found in the *TBK1* gene from various patients. Thus, we evaluated the location of the *TBK1* variants including the LoF variants in *TBK1* gene among Caucasian and Asian patients (Figure). We could not find remarkable racial differences in *TBK1* missense variants between Asian and Caucasian ALS patients. The LoF variants of *TBK1* were distributed throughout the gene in Caucasian ALS patients. On the contrary, LoF variants of *TBK1* were present around the ULD domain in Asian ALS patients. The clinical manifestation of Asian ALS patients with *TBK1* LoF variant varies in age and site of onset, neuropsychological abnormalities, and survival (Table 2). Freischmidt et al. identified eight LoF variants, seven of which were linked to the loss of expression of *TBK1* and were therefore reported as a causative via haploinsufficiency[8]. *TBK1* variants were enriched in the cohorts compared to controls. In our study, the frequency of *TBK1* variants was higher in ALS patients than in healthy controls (OR = 10.2; p = 0.008, 95% confidence interval = 1.67 - 62.47). Moreover, patients with variants of *TBK1* had an earlier onset age than those without *TBK1* variants in our study[18]. A majority of currently identified ALS-related *TBK1* variants are unknown pathogenic missense variants[28]. However, many of the missense variants have not been elucidated for their pathogenicity. The pathogenicity of the missense variants can be predicted by *in silico* analysis using several algorithms (eg. SIFT (http://sift.jcvi.org/), PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2/), and CADD[29] etc.). Freischmidt et al., suggested that *TBK1* missense variants can cause *TBK1* dysfunction *in vitro*[8], demonstrating that variants in the CDD domain hindered the binding of *TBK1* to optineurin. Xu et al, reported that decreased myeloid TAK1 (transforming growth factor-β-activated kinase 1) expression in TBK1 knockdown mice promotes ALS / FTD features including neuroinflammation, TDP-43 aggregation, axonal degeneration, neuronal loss, and behavioral disturbance[30]. This result indicates that reduction of TAK1 expression in the brain due to aging and mutation of TBK1 are sufficient to produce important features of ALS / FTD. However, only a few missense variants were verified for their pathogenicity. Further evidence is needed to classify the missense variants as pathogenic or non-pathogenic.

Clinical manifestation of *TBK1* variants

The clinical manifestation of ALS patients with *TBK1* LoF variant varies in age and site of onset, neuropsychological abnormalities, and survival. The age of onset ranges from 30 to 80 years, and survival ranges from a few months to more than 10 years. Some patients show extrapyramidal symptoms such as positional tremor, bradykinesia[31], primary lateral sclerosis[32], progressive supranuclear palsy (PSP)-like syndrome, and cerebellar ataxia[33]. Brain MRI findings of ALS patients with the mutation of the *TBK1* gene are variable, although the temporal lobe atrophy is the most frequently involved[31]. The fluorodeoxyglucose positron emission tomography (FDG-PET) in ALS-FTD patients indicated that glucose metabolism decreased in the frontal and temporal lobe. Immunohistochemistry of the brain tissue and spinal cord from patients with a *TBK1* LoF variant showed TDP-43-positve and p62-positive neuronal cytoplasmic inclusions, as well as ALS patients without *TBK1* LoF variants[11,31,34]. Here, one patient carrying the *TBK1* missense variant was diagnosed with ALS-FTD, and the patient showed alterations of behavior preceding the appearance of motor symptoms and atrophy of the temporal lobes[18].

Conclusion

TBK1 variants tend to be enriched in the cohort compared to the control. There is no racial difference in the frequency of *TBK1* LoF variants analyzed in the cohort studies for sporadic ALS patients. Variants of *TBK1* were scattered throughout the genes of ALS patients, and there were no racial differences in the missense variants. However, in Asian patients, accumulation of LoF variants around the ULD region was observed. The accumulation may be due to the small number of cases as compared with those of Caucasian cohorts. In the future, as research progresses, a similar result might be observed in Caucasian patients. The LoF variants of *TBK1* are likely pathogenic for sporadic ALS, regardless of race or region, however, the pathogenicity of missense variants of *TBK1* is somewhat difficult. Further studies are necessary to investigate pathogenicity of individual missense variants through molecular biological techniques.

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