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

Genki Tohnai1, Ryoichi Nakamura1, Masahiro Nakatochi2, Naoki Atsuta1, Masahisa Katsuno1, Gen Sobue1,3*

1Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan
2Statistical Analysis Section, Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan
3Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine, Nagoya, Japan

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 ALS1.

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 patients2. In Japan, a genetic screening study reported that 3% of sporadic ALS patients have a known familial ALS-related pathogenic gene mutation3. 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 autophagy4, insulin signaling5, and cellular proliferation. TBK1 phosphorylates multiple substrates including optineurin and SQSTM16, which are genes related to ALS. TBK1 was first identified as an ALS related gene through exome sequencing in two independent studies7,8. Moreover, the variants of the TBK1 gene in ALS has been reported in the cohort studies of Europe9, Australia10, Taiwan11, Belgium12, China13, Korea14, Sweden15, Germany16, Italy17, and Japan18. 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, innate immune response, and inflammation. TBK1 was initially identified as a kinase mediating the ability of TANK to activate NF-kappa-B (NF-kB)9,10. It is an important member of the I-kappaB 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 Salmonella21-23. 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 Salmonella24. TBK1 is also involved in the autophagic clearance of Mycobacterium tuberculosis in mouse macrophages and phosphorylates the autogapic receptor SQSTM1 to enhance its binding to polyubiquitinated bacteria25. Moreover, TBK1 is particularly important for the maturation of autophagosomes to hydrolytic autophagosomes, which results in degradation of SQSTM1 and its associated cargo26.

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 324. The ULD region of the kinase domain phosphorylates various substrates such as the interferon regulatory factor 324. The CCD region of TBK1 regulates the kinase activity and interaction with SQSTM1 and its associated cargo5.

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 studies7,8. 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 controls7. 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 population6. Moreover, the study conducted on an Australian cohort of familial ALS patients identified a novel TBK1 LoF variant in a family of Chinese origin8. Subsequently, the TBK1 LoF variant was found to be a rare cause of ALS in the Taiwanese10 and Chinese cohort13, as well as in Sardinian ALS patients15. 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 controls18. All cases were registar 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 Asians27. 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 Asians7. 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

<table>
<thead>
<tr>
<th>Population</th>
<th>Missense variants</th>
<th>LoF variants</th>
<th>Total ALS patients</th>
<th>Patients</th>
<th>Cohort</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Caucasian</td>
<td>-</td>
<td>8</td>
<td>12</td>
<td>0.28%</td>
<td>European</td>
<td>2015</td>
<td>7</td>
</tr>
<tr>
<td>Caucasian</td>
<td>10</td>
<td>33</td>
<td>12</td>
<td>0.28%</td>
<td>European</td>
<td>2015</td>
<td>8</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0</td>
<td>7</td>
<td>12</td>
<td>0.28%</td>
<td>Australian</td>
<td>2015</td>
<td>9</td>
</tr>
<tr>
<td>Caucasian</td>
<td>5</td>
<td>1</td>
<td>14</td>
<td>1.2%</td>
<td>European</td>
<td>2015</td>
<td>12</td>
</tr>
<tr>
<td>Caucasian</td>
<td>11</td>
<td>5</td>
<td>16</td>
<td>1.75%</td>
<td>Belgian</td>
<td>2015</td>
<td>11</td>
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<tr>
<td>Asian</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1.2%</td>
<td>Chinese</td>
<td>2016</td>
<td>13</td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1.2%</td>
<td>Taiwanese</td>
<td>2016</td>
<td>10</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>1.2%</td>
<td>Korean</td>
<td>2016</td>
<td>14</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>1.2%</td>
<td>Italian</td>
<td>2017</td>
<td>17</td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>1.2%</td>
<td>Japanese</td>
<td>2018</td>
<td>18</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>1.2%</td>
<td>German</td>
<td>2018</td>
<td>16</td>
</tr>
</tbody>
</table>

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

The pathogenicity of the missense variants can be predicted by in silico analysis using several algorithms (e.g., SIFT (http://sift.jcvi.org/), PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2/), and CADD etc.). Freischmidt et al., suggested that TBK1 missense variants can cause TBK1 dysfunction in vitro, 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. 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.

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

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

TPPV: Tracheostomy positive pressure ventilation
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, primary lateral sclerosis, progressive supranuclear palsy (PSP)-like syndrome, and cerebellar ataxia. 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. 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-positive and p62-positive neuronal cytoplasmic inclusions, as well as ALS patients without TBK1 LoF variants. 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.

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 variant. 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.

Acknowledgments

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References


