**Title:**

Screening for *TARDBP* Mutations in Japanese Familial Amyotrophic Lateral Sclerosis

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**Abstract**

TAR-DNA-binding protein 43 (TDP-43), encoded by the *TARDBP* gene on chromosome 1p36.22, has been identified as the major pathological protein in abnormal inclusions in neurons and glial cells in sporadic amyotrophic lateral sclerosis (SALS), *SOD1*-negative familial ALS (FALS) and frontotemporal lobar dementia (FTLD). Twenty mutations of *TARDBP* in *SOD1*-negative FALS and SALS cases have been reported so far. To investigate the presence and frequency of *TARDBP* mutations in Japanese *SOD1*-negative FALS patients, we performed mutational screening of *TARDBP* in 30 *SOD1*-negative FALS patients. An N352S mutation was found in one case of FALS, but no *TARDBP* mutations were found in cases of SALS. It was thought that this mutation increases TDP-43 phosphorylation. This might lead to impaired nuclear cytoplasmic transport or protein-protein interaction, thereby leading to TDP-43 accumulation.

**Keywords:** *TARDBP* mutation, TDP-43, Amyotrophic lateral sclerosis, ALS, familial

## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease. It is a

progressive disorder that involves degeneration of upper and lower motor neurons at all levels of the motor system, from the cortex to the anterior horn of the spinal cord. The clinical features of ALS can be considered in relation to neurological regions or levels (bulbar, cervical and lumbar). The disorder is characterized by dysarthria, dysphagia, brisk reflexes, pyramidal signs, fasciculation, and progressive atrophy and muscle weakness. The mean duration of survival is three to five years from onset without intensive physiological support (e.g., a ventilator) [1, 2]. About 10% of cases of ALS are familial (FALS), and the others are thought to be sporadic (SALS) [3-5]. Various genes that cause FALS, including copper/zinc superoxide dismutase-1 (*SOD1*) [6], dynactin 1 [7-9], alsin [10], senataxin [11], vesicle-associated membrane protein B [12] and angiogenin [13, 14], have been identified, but the frequency of their mutation is low.

TAR-DNA-binding protein 43 (TDP-43) has recently been identified as the major pathological protein in abnormal inclusions in neurons and glial cells in SALS, *SOD1*-negative FALS and frontotemporal lobar dementia (FTLD) [15-17]. Some reports suggest clinical and pathological overlap between ALS and FTLD [18-20]. TDP-43 is encoded by *TARDBP* on chromosome 1p36.22, and its structure is evolutionarily conserved, consisting of two RNA recognition motifs and a glycine-rich domain. It was originally identified as a transcriptional receptor that binds to the TAR-DNA element of

human immunodeficiency virus type 1 (HIV-1) [21]. TDP-43 is involved in the regulation of expression and splicing, and it is part of a complex that splices the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) [21-25]. In ALS, 20 mutations of *TARDBP* have been reported not only in *SOD1*-negative FALS cases (G290A, G298S, A315T, M337V, Q343R, N345K, N352S, A382T, I383V) but also in SALS cases (D169G, G287S, G294A, Q331K, G348C, R361S, P363A, Y374X, A382P, N390D, N390S) [26-33].

In this study, in order to investigate the presence and frequency of *TARDBP* mutations in Japanese *SOD1*-negative FALS patients, we performed mutational screening of *TARDBP* in *SOD1*-negative FALS patients, SALS patients and healthy control subjects.

## 2. Materials and methods

The subjects included 30 *SOD1*-negative FALS patients from 30 unrelated families (mean age at onset, 60.2 years; age range, 33-76 years), 220 (including 12 autopsy-confirmed) SALS patients (mean age at onset, 58.6 years; age range, 23-84 years), and 105 healthy control subjects (mean age, 63.9 years; age range, 40-96 years). All of the subjects were Japanese. Informed consent for participation in this study was obtained from all subjects.

Genomic DNA was extracted from peripheral blood leukocytes or frozen brain sections

using standard methods. In cases of FALS, the entire coding region of the *TARDBP* gene (accession number NM_007375), consisting of exons 2-5 and the first 531 nucleotides of exon 6, was amplified with primers designed using Primer3 software. In SALS patients and healthy control subjects, only the first 531 nucleotides of exon 6 were amplified because exon 6 seems to be a hotspot for ALS-linked *TARDBP* mutations [26-33]. Each PCR product was sequenced using Applied Biosystems BigDye terminator v3.1 sequencing chemistry and run on an ABI PRISM 3130 Genetic Analyzer.

## 3. Results

A c.1055 A>G mutation, predicted to substitute asparagine for serine at codon 352 (p.N352S), was identified in one case of FALS (Fig. 1). This mutation was not found in any of the 200 SALS patients or the 105 healthy subjects. The patient with this mutation first showed clinical signs at the age of 55 years, beginning with weakness of the right hand. The symptom was progressive. Gait disturbance, bulbar signs and respiratory impairment appeared 2 years later. Cognitive function was normal. Electromyography showed acute and chronic changes in the upper and lower limbs and cranial lesions. The patient's older sister had been bedridden with respiratory impairment and died of ALS at the age of 42 years. The patient's father died of an accident and her mother died of stroke.

We also found a c.1098C>G variation (p.A366A) in 16 cases of SALS. However, this variation was silent and was thought to be a benign polymorphism as it was also found in seven control subjects.

## 4. Discussion

In this study, we found an N352S missense mutation in *TARDBP* in a patient with *SOD1*-negative FALS. This mutation was previously reported in a German family [27]. The frequency of the *TARDBP* mutation was 3.3% (1 of 30 patients). The frequency of the *TARDBP* mutation in *SOD1*-negative FALS patients in previous studies was 0.6% to 6.5% [26-32]. In Japan, Yokoseki et al reported one missense mutation (p. Q343R) in 16 *SOD1*-negative FALS patients [31]. Combining the number of *TARDBP* mutations in Japanese *SOD1*-negative FALS yields a rate of 2 in 46 (4.3%). Our identified mutation was not present in our SALS and healthy control subjects.

In previous studies, the clinical phenotype of *TARDBP* mutation cases consisted mainly of spinal onset and absence of cognitive impairment [26-32]. The clinical phenotype in our case was similar. This clinical phenotype does not allow for separating *TARDBP* mutation cases from other forms of ALS, with similar features being reported in SALS and in *SOD1* FALS [34].

TDP-43-positive FALS is thought to be an autosomal dominant trait. In this family,

the parents did not show ALS symptoms. The father or mother might have had the same mutation but died before ALS onset, or the mutation might have had low penetrance. De novo mutation is also a possibility. However, this seems unlikely since the patient's older sister also had ALS.

Except for the D169G mutation, all other *TARDBP* mutations are located in exon 6 encoding for the C-terminus of TDP-43. Mutations of the C-terminus region of TDP-43 may impair the function or transport of TDP-43 by influencing protein-protein interaction, transport through the nuclear pore, or exon skipping and splicing inhibitory activity. These *TARDBP* mutations may also cause a toxic gain of function through novel protein interactions or intracellular accumulation of TDP-43 fragments, leading to apoptosis [26-32].

The N352S mutation is localized to a highly conserved region of the C-terminus of TDP-43 that is known to be involved in protein-protein interaction. Asparagine at codon 352 is conserved across all mammals examined so far, as well as in Gallus gallus [27]. Kuhnlein et al. predicted that the most likely effect of the N352S mutation might be an increase in TDP-43 phosphorylation and that the N352S mutation might not only introduce a new serine residue at position 352 but also lead to an increase in the phosphorylation prediction score for serine residues at positions 347 and 350 of TDP-43

using a network service [27]. This might lead to impaired nuclear cytoplasmic transport or protein-protein interaction, resulting in TDP-43 accumulation.

In conclusion, we identified one *TARDBP* mutation in *SOD1*-negative FALS. The frequency of *TARDBP* mutations in FALS may not be high compared with the frequency of SOD1 mutations, but the function analysis of *TARDBP* mutations may contribute to understanding the cause of ALS because TDP-43 is the major pathological protein in the abnormal inclusions of ALS. The identification of rare familial mutations in the ß-amyloid precursor protein in Alzheimer's disease and in α-synuclein in Parkinson's disease has dramatically advanced studies aimed at elucidating the pathogenesis of predominantly sporadic diseases. Further studies, including studies using transgenic animal models, are needed to elucidate the links between TDP-43 amino acid change, TDP-43 neuropathology, and ALS neurodegeneration.

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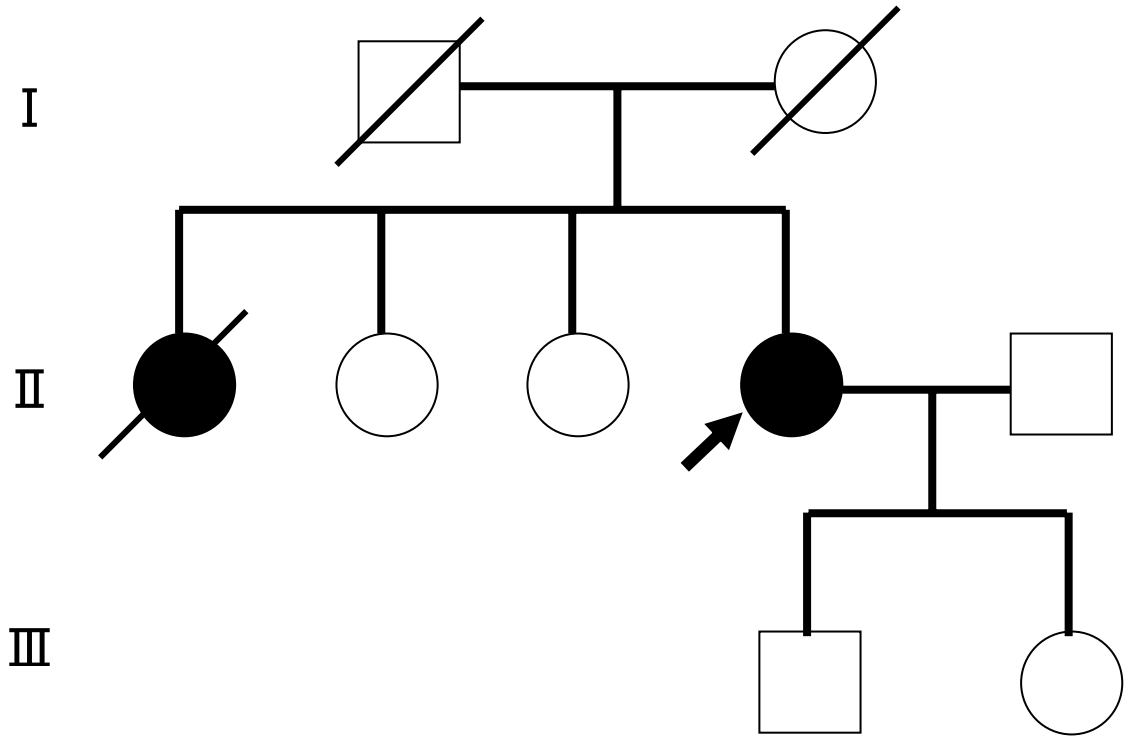
Fig.1.




Pedigrees of Japanese familial ALS with N352S TARDBP mutations.

Black symbols represent patients affected with ALS.

White symbols represent unaffected individuals.