Letters to the Editor

**TARDBP** p.G376D mutation, found in rapid progressive familial ALS, induces mislocalization of TDP-43

**A R T I C L E  I N F O**

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- Amyotrophic lateral sclerosis (ALS)
- TDP-43 mislocalization
- TARDBP gene
- Cellular model

**Dear Editor:**

Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease which causes the progressive weakness and atrophy of limbs and respiratory muscles, resulting in death within 3–5 years of onset. Targeted next-generation sequencing has enabled a comprehensive analysis of ALS disease-causing genes [1]. However, a method to confirm the pathogenicity of variants of unknown significance (VUS) remains to be elucidated. The previously reported variants require validation if they are not isolated in the designated family members. For example, we have reported the first Asian familial ALS case with TARDBP p.G376D mutation [1], which was previously reported as a familial ALS-linked disease among the family members. Medical records, except for records of Patients 1 and 2, were not available.

1. **Cases**

A 36-year-old male (Patient 1) suffered from progressive head drop and muscle weakness in his upper extremities. Physical examination findings revealed muscle atrophy and weakness in his tongue and distal dominant upper extremities. Fasciculation was observed in his tongue and right upper extremity. Hyperreflexia was observed in four extremities without Babinski response. Needle electromyography showed reduced interference patterns in the upper extremities. Denervation potentials and high-amplitude polyphasic motor unit potentials were detected in the biceps brachii. Routine blood analysis, cerebrospinal fluid analysis, and brain and spinal MRIs revealed no abnormalities. In accordance with the revised El Escorial criteria [4], we diagnosed him with clinically possible ALS. One year after onset, he died from respiratory failure.

Patient 1’s paternal grandmother (Patient 2) was diagnosed with clinically probable ALS in our hospital at the age of 52 years, presenting with weakness in her right-side extremities. Four months after onset, physical examination revealed dysarthria and muscle atrophy and weakness with hyperreflexia in four extremities. She died from the disease one year later. Other family members (Patient 2’s mother, her maternal aunt and cousins) were also considered as suffering from ALS and died around in their fourth and fifth decades. We clarified the diagnosis of Patient 1 as familial ALS because of the high prevalence of disease among the family members. Medical records, except for records of Patients 1 and 2, were not available.

2. **Gene and cell analysis**

To confirm mutations detected by targeted next-generation sequencing, we analyzed the variant sites using Sanger sequencing and also investigated chromosome 9 open reading frame 72 (C9orf72) gene by repeat primed PCR as described in our previous reports [1,5]. We identified a heterozygous c.1127G > A missense mutation located in exon 6 of TARDBP in Patient 1. This mutation was not identified in healthy controls from the Human Genetic Variant Database. In the Exome Aggregation Consortium (ExAC) browser (http://exac.broadinstitute.org/), the frequency of the c.1127G > A mutation have not been shown. This mutation results in an amino acid substitution of glycine with aspartic acid (p.G376D) in glycine-rich regions, where most of the reported TARDBP mutations were accumulated [6]. Different in silico pathogenicity prediction software programs indicated different results about p.G376D variant. Two programs (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/; and SIFT, http://sift.jcvi.org/) indicated p.G376D as benign or tolerated change [1]. Other program (PATHMM, http://fathmm.biocompute.org.uk/) indicated a damaging score (p.G376D − 2.08; p.M337V − 3.45; p.A315T − 1.87). However, p.G376D was not verified to be segregated in the ALS family members in our study, and its pathogenicity remained unclear. Subsequently, we analyzed cellular phenotypes of TDP-43, encoded by TARDBP, with the p.G376D mutation.

Reports show that ALS-linked mutant TDP-43 mislocalized from the nucleus to the cytoplasm [7,8], whereas wild type (WT) TDP-43 localizes in the nucleus. We generated GFP-tagged mutant TDP-43 expression plasmid vectors and analyzed their localization in SH-SY5Y cells. Western blotting was performed to detect the GFP-tagged TDP-43 expression (approximately 70 kDa, an arrowhead, Fig. 1A) and also endogenous TDP-43 (43 kDa, an arrow, Fig. 1A). We noted that p.G376D mutant TDP-43 showed cytoplasmic mislocalization as exhibited in p.A315T and p.M337V (Fig. 1B, C), which were experimentally proven to be ALS-linked mutation [6]. This result suggests the pathogenicity of TARDBP p.G376D mutation.

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3. Discussion

To the best of our knowledge, this familial case study is the first Asian familial ALS case report with a TARDBP p.G376D mutation, in which the cellular phenotype was similar to the previously reported familial ALS linked to European population cases (Fig. 1D) [2,3]. Onset symptoms included limb muscle weakness in almost all the symptomatic family members of the present and reported p.G376D pedigrees. The rapid progression and the short duration after onset are also comparable with the reported data (from 0.5 years to 1.5 years) [3]. There is another base changed variant c.1127G > C p.G376A in the TARDBP previous report of familial ALS pedigree with a TARDBP p.G376D mutation, in which the clinical phenotype was similar to the previously reported familial ALS linked to European population cases (Fig. 1D) [2,3]. Onset symptoms included limb muscle weakness in almost all the symptomatic family members of the present and reported p.G376D pedigrees. The rapid progression and the short duration after onset are also comparable with the reported data (from 0.5 years to 1.5 years) [3].

In the present family, the father of Patient 1 was not affected. In a previous report of familial ALS pedigree with a TARDBP p.M337V mutation, an unaffected individual had the same TARDBP mutation as other affected family members [9]. Although we could not confirm the presence of the mutation in the other family members, we concluded that the possibility of the p.G376D mutation displays autosomal dominant inheritance with incomplete penetrance. Furthermore, there could be a gene modifier that regulates the expression of ALS phenotypes in people with TARDBP mutation genotypes.

We examined the mislocalization of TDP-43 with p.G376D mutation using the overexpression cellular model, for the first time with side by side analysis of previously reported disease-causing TARDBP mutants. TDP-43 cytoplasmic mislocalization has been observed in the case of these mutations in glycine-rich domains, which is known to regulate protein-protein interaction [10]. Misfolding of TDP-43 affected by TARDBP mutations was considered to be the cause of the mislocalization. In the Fig. 1B, endogenous TDP-43 was not detected in the nucleus of mutant TDP-43 overexpression models. Overexpressed mutant TDP-43 may also affect the localization of endogenous TDP-43.

The study has two limitations. At first, the genetic analysis of Patient 1’s father was not available because we could not obtain his consent. TARDBP p.G376D segregation was not confirmed in the cases. Secondly, we could not show the relation between TDP-43 mislocalization and cell death. In a past report, mutant TDP-43 overexpressed cells in which TDP-43 mislocalized cells significantly increased were likely to be stained with cleaved caspase3 antibody in comparison to WT [8]. We also performed cell death assay using immunocytochemistry of cleaved caspase3 in fixed cells or staining of ethidium homodimer-1 (EthD-1) in live cells. In these assays, there was no significant difference between WT and mutations. One of the reasons that we could not reproduce the past cell death assays might be the difference in the cell lines used. TARDBP p.G376D has the same TDP-43 mislocalization as p.M337V and p.A315T that have been proven their pathogenicity in a large number of cell and animal models. That suggests pathogenicity of p.G376D, even though we could not observe the difference in cell death between WT and mutations in our experimental setting.

4. Conclusion

We have reported rapid progressive early limb onset ALS with incomplete penetrance in a patient with p.G376D TARDBP mutation. TDP-43 mislocalization indicates that p.G376D mutation causes the pathogenicity in familial ALS. The analysis of TDP-43 mislocalization in a cellular model may help in determining the pathogenicity of VUS in side-by-side analysis with previously confirmed pathological mutations.

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Declaration of interest

The authors report no conflicts of interest.

Author contributions

S. Mitsuzawa performed cell analysis and wrote the manuscript. T. Akiyama performed cell analysis and revised the manuscript. A. Nishiyama and R. Izumi carried out genetic analysis. N. Suzuki participated in the outpatient clinic, the Sanger sequencing, cell analysis, and revised the manuscript. H. Warita and M. Kato participated in revising the manuscript. S. Koyama, T. Kato and Y. Suzuki treated the proband. S. Osana participated in the mutation analysis. M. Aoki participated in the study coordination and revised the manuscript. All authors made a substantial contribution to the manuscript.

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