LETTER TO THE EDITOR

A Familial Phenotypic and Genetic Study of Mutations in *PFN1* Associated with Amyotrophic Lateral Sclerosis

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Dear Editor,

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease that prominently affects both upper and lower motor neurons. The prevalence of ALS has been estimated at 2.6–3.0 per 100,000 in Europe, 5.2 per 100,000 in the USA, and 1.9–9.9 per 100,000 in Asia [1–3]. ALS is classified as sporadic (sALS) or familial (fALS), but only 5%–10% of cases are identified as familial [4, 5]. In 1993, the first mutation associated with ALS was found in the superoxide dismutase 1 (*SOD1*) gene [6]. Since then, > 30 genes with such mutations have been reported, of which four genes *SOD1*, *FUS* (FUS RNA binding protein), *TARDBP* (TAR DNA binding protein), and *C9orf72* (C9orf72-SMCR8 complex subunit), account for 60%–70% of fALS cases and 10% of sALS cases [5]. The discovery of mutations in these genes has established a pivotal rationale for understanding the pathogenic processes and mechanisms in ALS. In particular, mitochondrial dysfunction, disruption of RNA metabolism, abnormal regulation of protein structure, and cytoskeletal defects are now considered to be the main pathways involved in the pathogenesis of ALS [5].

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In 2012, mutations in the profilin 1 (*PFN1*) gene were identified in two large families with ALS by exome sequencing, and the *PFN1* gene was further identified as a causative gene by sequencing in 272 cases of fALS, with five other cases of fALS containing variants in this gene [7]. Since then, an additional 12 reports of *PFN1* screening have identified 10 cases from among 5,385 ALS cases, including fALS and sALS (Table S1). A total of seven ALS-causing mutations in the *PFN1* gene have been reported so far (Table S1) [7, 8]. Current reports show that fALS cases caused by *PFN1* mutations all have limb symptoms at the onset, while the specific clinical manifestations have not been described [7, 8].

In this study, we report for the first time fALS patients in Asia with a *PFN1* mutation. Peripheral blood samples were obtained from the affected and unaffected family members (II-2, II-7, III-3, III-5, III-12, IV-1, and IV-2). Next-generation exome sequencing was performed using the Illumina NextSeq 500 platform (Illumina, San Diego, CA) to screen the gene variations. Custom-designed Roche NimbleGen SeqCap probe libraries were used to capture all coding regions and 10 bp of the flanking intronic regions of genes. The gene detection interval included 182 causative genes and 2,851 coding regions, which contained a total of 465,264 bases (Table S2). Sanger sequencing was used to validate the identified variant in the proband's mother and other relatives for segregation analysis.

The proband (Patient 1: III-3) of the pedigree first started to experience progressive right lower extremity weakness at the age of 56. The proband's symptoms gradually worsened, and the left lower extremity was also involved, resulting in instability while walking. As the disease progressed over the next 11 months, muscle atrophy and fasciculation appeared in both lower extremities. Findings from neurological examination of the

proband and other affected family members are summarized in Table 1. Electromyographic (EMG) examination of the proband showed neurogenic damage in both lower limbs and the thoracic paraspinal muscles. No significant abnormality was found on magnetic resonance imaging (MRI) of the brain and the whole spinal cord. Cerebrospinal fluid (CSF) testing showed no abnormalities in color of the fluid, CSF pressure, the presence of white and red blood cells, protein, or glucose levels. Anti-GD1b IgM antibody was weakly positive, and the remaining human anti-ganglioside antibodies were negative.

The mother of the proband (Patient 2: II-2) was unable to stand and was wheelchair-bound from the age of 78. By 86 years of age, she could not lift her head, and had developed dysarthria within the previous few months. Clinical information on this patient was limited. Due to her stiff muscles, muscle twitching, and muscle atrophy, a neurological examination could not be performed. Patient 3 (II-7) started to experience weakness of her right lower limb, especially the distal extremity, at the age of 64. The weakness became worse, and the left lower limb was involved at the age of 66. The patient had muscle atrophy in both lower extremities with fasciculation. Patient 4 (III-12) first started to experience progressive right lower limb weakness at the age of 50. This weakness gradually worsened with muscle atrophy, and the left lower extremity was also involved two years after the onset of symptoms. The patient had complained of numbness in both lower extremities, but no sensory abnormalities were observed on neurological examination. The EMG result suggested neurogenic damage in both lower extremities, consistent with anterior horn cell damage in the L2–S2 spinal segments. No significant abnormality was found on MRI of the lumbosacral spinal cord. CSF testing showed no abnormalities and serum human anti-ganglioside antibodies were all negative.

The proband's grandfather (I-1) had died in his 40s due to an accident, before which he was asymptomatic. The proband's grandmother and great-grandparents were asymptomatic and died at 80–90 years of age. Patient 5 (II-5) had symptoms of lower limb weakness since the age of 63; these started on the right and then involved the left side. He was diagnosed with ALS and died in 2014 at the age of 70. Limb weakness, muscle atrophy, and stiffness were not present in family member III-5.

Next-generation sequencing analysis indicated a heterozygous c.353G > T missense mutation (p.G118V) of the *PFN1* gene in the proband (III-3) (Fig. 1B); this had been identified as a clinically significant pathogenic mutation. Sanger sequencing showed that the other symptomatic family members (II-2, II-7, III-3, and III-12) and an asymptomatic family member (IV-1) also had the p.G118V mutation of *PFN1* (Fig. S1). All the affected

Table 1 Clinical features of patients from the family with a p.G118V mutation in the *PFN1* gene

Patient	Onset site	Age	Age at onset	Muscle strength				Muscle tension of LL	Deep tendon reflexes		Babinski's sign	Cognitive impairment	Sensory abnormalities
				RPLL	RDUL	LPLL	LDLL	UL	LL	UL			
Patient 1 (III-3)	RLL	58	56	2	1–2	3	1–2	5	Weakness	Weakness	–	–	–
Patient 2 (II-2)	Not sure	88	Not sure	NA	NA	NA	NA	NA	Weakness	Weakness	–	NA	–
Patient 3 (II-7)	RLL	68	64	4	3	5	4	5	Weakness	Weakness	–	–	–
Patient 4 (III-12)	RLL	53	50	4	3	5	4	5	Weakness	Briskness	±	–	–
Patient 5 (II-5)	RLL	Died at 70	63	NA	NA	NA	NA	NA	Weakness	Weakness	–	NA	NA

RLL right lower limb, RPLL right proximal lower limb, RDUL right distal upper limb, LPLL left proximal lower limb, LDLL left distal lower limb, UL upper limb, LL lower limb, NA not applicable (unknown), ±, suspicious positive; –, negative.

Fig. 1 The family pedigree and results of gene sequencing. **A** The family pedigree. Patient III-3 is the proband. The proband's grandfather (I-1) died in his 40s due to an accident, before which he was asymptomatic, and the question mark (?) means that it is unclear whether he was a patient or not. Family member IV-1 was

found to have the p.G118V mutation in the *PFN1* gene, but relevant clinical symptoms had not yet occurred (*genetic testing performed). **B–D** Genetic testing results for the proband (III-3), the proband's mother (II-2), and the proband's brother (III-5).

family members were older than 50 years, and the asymptomatic family member was less than 40 years old. Genetic testing of the other two asymptomatic family members, III-5 and IV-2, showed that both had negative results for the gene mutation (Fig. S1). According to the genetic results and the clinical phenotypic manifestations in the affected family members (Fig. 1A), an autosomal dominant inheritance pattern was confirmed.

In this study, we describe a missense mutation (c.353G > T, p.G118V) in the *PFN1* gene in a family with a phenotype proposed to be ALS. In 2013, a case of sALS associated with the p.R136W mutation in the *PFN1* gene was found in China [9]. However, our study is the first report of fALS in the Asian population with a mutation in the *PFN1* gene. This gene encodes the 140-amino-acid protein profilin 1, which is essential for the regulation of actin polymerization in response to extracellular signals. Mutations in the *PFN1* gene have been previously identified as a cause of ALS. Cells expressing a *PFN1* gene mutation have shown cytoskeletal defects [7] and altered stress granule dynamics [10]. Higher expression levels of TAR DNA-binding protein 43 (TDP-43), which is encoded by the *TARDBP* gene, have been reported in cells expressing ALS-associated *PFN1* mutations than in the wild-type *PFN1* [11]. The p.G118V mutation was discussed in 2012 when the *PFN1* gene was first identified to be associated with fALS [7], and was further confirmed as a pathological site rather than a benign polymorphism by examining 7,560 control samples [7]. And this variant has

not been reported in population databases, including the 1000 Genomes Project, the Database of Single Nucleotide Polymorphisms (dbSNP), and the Genome Aggregation Database (GnomAD). Cells expressing the p.G118V mutation in the *PFN1* gene have been found to contain a pathological aggregation of TDP-43 and show cytoskeletal defects that are known to be associated with motor neuron diseases [7]. Transgenic mice expressing *PFN1* with the p.G118V mutation have been shown to develop ALS-associated clinical and pathological features, including loss of upper and lower motor neurons, muscular atrophy, and reduced survival [12]. Based on *in vivo* and *in vitro* studies, there is evidence to support the hypothesis that the p.G118V missense mutation in the *PFN1* gene leads to the development of ALS.

Clinically, the proband presented with predominant lower motor neuron damage but no abnormalities in upper motor neurons in the current course of the disease. This does not meet the traditional ALS diagnostic criteria that require upper and lower motor neuron involvement and spread within a region or from one region to another (bulbar, cervical, thoracic, or lumbar). However, the 2015 revised El Escorial diagnostic criteria describe limited presenting phenotypes of ALS, including progressive bulbar palsy, flail arm syndrome and flail leg syndrome, progressive muscular atrophy, and primary lateral sclerosis [13]. These restricted phenotypes may develop into disseminated ALS or may be confirmed to carry pathogenic ALS mutations or pathological ALS changes [13].

Therefore, considering the manifestation of upper motor neuron signs in the proband's mother at a relatively late stage, based on the progressive nature of their symptoms and signs as well as the EMG results, the diagnosis of ALS was also established. It is worth noting that genetic testing and family history contribute to a diagnosis when early clinical symptoms are atypical.

In this family study, the same mutation in the *PFN1* gene resulted in similar clinical presentations and clinical features of ALS in the affected family members. First, all patients had a spinal onset that involved the lower limbs, characterized by initial asymmetric (mainly right lower extremity) and primarily distal symptoms and signs. The symptoms gradually progressed, and the involvement of both lower extremities occurred over a period of about two years. Previous studies have shown that cases of fALS with a *PFN1* gene mutation present with initial spinal rather than bulbar symptoms and signs [7, 8]. Second, at the onset, the involvement of lower motor neurons predominates, with subtle upper motor neuron signs usually emerging at a later stage. It is particularly important to emphasize the need to distinguish ALS from Charcot-Marie-Tooth disease, a hereditary peripheral motor and sensory neuropathy, at an early stage, so genetic testing plays a vital role in diagnosis. Third, dysphagia and respiratory failure are the major causes of death in ALS. In this report, bulbar symptoms were observed in the proband's mother more than eight years after onset, indicating that patients with ALS caused by *PFN1* mutations survive longer.

Finally, cognitive impairment was not observed; this is consistent with previous research in patients with ALS with a *PFN1* gene mutation [8]. It is worth noting that the anti-GD1b IgM antibody was weakly positive in the proband, highlighting the importance of awareness of the presence of related diseases. However, clinical symptoms such as sensory ataxia and ophthalmoplegia were not observed, and anti-GD1b IgM antibody was not detected in family member III-12. The early clinical symptoms and signs in the patients in this family might have included a differential diagnosis of flail leg syndrome, which is a rare restricted phenotype of ALS. However, as flail leg syndrome progresses, patients usually develop stiffness and muscle atrophy of almost the entire body, resulting in inactivity, and bulbar symptoms also inevitably occur, which is inconsistent with classic ALS.

This study had several limitations. First, the duration of follow-up was limited, and the pedigree in this type of study should be followed for an extended period to record the changing symptoms and signs of ALS associated with the *PFN1* gene mutation. Also, only one family pedigree has been identified so far. Therefore, it might be advisable to screen for *PFN1* gene mutations in patients with flail leg

syndrome or progressive muscular atrophy, and this deserves further study. More cases of fALS can be collected to explore further the pathogenic mechanisms of fALS and provide a theoretical basis for the development of improved diagnostic and therapeutic methods.

In this study, we describe the first ALS family in Asia with a *PFN1* mutant and a detailed phenotypic analysis was performed due to the specific clinical manifestations. In patients with progressive asymmetrical lower extremity weakness, muscle atrophy, and gradual involvement of other regions that occur at an older age, with or without upper motor neuron involvement, a diagnosis of ALS should be considered, and genetic testing would help to make an accurate diagnosis.

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Conflict of interest The authors declare that they have no conflict of interest.

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