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BRIEF COMMUNICATION

Identification of *IFRD1* variant in a Han Chinese family with autosomal dominant hereditary spastic paraplegia associated with peripheral neuropathy and ataxia

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Abstract

Spinocerebellar ataxias (SCAs) are a group of autosomal dominant, clinically heterogeneous neurodegenerative disorders. SCA18 is a rare autosomal dominant sensory/motor neuropathy with ataxia (OMIM#607458) associated with a single missense variant c.514 A>G in the interferon related developmental regulator 1 *(IFRD1)* gene previously reported in a five-generation American family of Irish origin. However, to date, there have been no other reports of the *IFRD1* mutation to confirm its role in SCA. Here, we report a Han Chinese family with SCA18; the family members presented with a slowly progressing gait ataxia, pyramidal tract signs, and peripheral neuropathy. We identified a missense variant (c.514 A>G, p.I172V) in *IFRD1* gene in the family using targeted next-generation sequencing and Sanger direct sequencing with specific primers. Our results suggest that the *IFRD1* gene may be the causative allele for SCA18.

Introduction

Spinocerebellar ataxias (SCAs) are a group of autosomal dominant, clinically heterogeneous disorders characterized by progressive ataxia [1]. The pathological changes of SCAs are mainly caused by cerebellar atrophy as well as degeneration of the spinal cord and brainstem [2, 3]. To date, over 40 subtypes of SCAs have been identified with a wide array of neurodegenerative symptoms and more than 28 genes were reported in SCA cases [1, 4]. The genetic mechanism associated with SCA1-3, SCA6, SCA7, and SCA17 involve CAG-coding polyglutamine repeat expansions in the respective proteins [2]. Alternatively, noncoding repeat expansions and conventional mutations in the encoding genes were associated with other subtypes of SCAs. However, the causative mutations in a significant number of SCAs cases remain undetermined [5].

SCA18 is an autosomal dominant sensory/motor neuropathy with ataxia (OMIM#607458) that was associated with a single missense variant c.514 A>G in the interferon related developmental regulator 1 (*IFRD1*) gene reported in a five-generation American family of Irish origin [6, 7]. Their symptoms included features of motor and sensory neuropathy, ataxia, pyramidal tract signs, dysmetria, and muscle weakness.

Here, we report a Han Chinese family presented with SCA18 characterized by slowly progressing gait ataxia, pyramidal tract signs and peripheral neuropathy. We identified a variant in the *IFRD1* gene (c.514 A>G, p.I172V) in the family. To the best of our knowledge, this is the first report describing SCA18 in persons of Han Chinese ethnicity.

Materials and methods

Subjects

This study was approved by the Ethics Committee of the Shandong University School of Medicine and informed written consent was obtained from all participants. A proband, II-3, 16-year-old female presented with gait disturbance that commenced in her early childhood. Further, the proband's sister and brother (II-1 and II-2, respectively)

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displayed similar gait disturbances. All affected family members underwent physical examinations, and blood samples were taken. We then performed nerve conduction velocity (NCV) tests to examine nerve conduction as well as brain magnetic resonance imaging (MRI) to evaluate the brain structures.

Next generation sequencing

Genomic DNA was extracted from the blood leukocytes using a TIANamp Blood DNA Midi Kit (DP332) according to the manufacturer's protocol. First the affected individuals were excluded for mutations in SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 35, 36, Dentatorubral-pallidoluysian atrophy (DRPLA), and Friedreich ataxia (FRDA) by polymerase chain reaction (PCR) and capillary electrophoresis. Then target sequences were enriched using customized capture probes chips (Illumina, San Diego, CA) that included 214 genes associated with known ataxia and spastic paraplegia diseases. This list of the targeted genes associated with ataxia and spastic paraplegia was provided in the supplementary material. A total of 1 μg genomic DNA was fragmented into 200–300-bp length fragments using the Covaris Acoustics System. Next, the DNA fragments were processed by end-repairing, A-tailing, and adaptor ligation—a 4-cycle pre-capture PCR amplification and targeted sequences capture. Captured DNA fragments was eluted and amplified by 15-cycle post-capture PCR. The final product was sequenced with 150-bp paired-end reads on the Illumina HiSeq X Ten platform (Illumina, San Diego, CA) according to the standard manual. The clean short-reads were mapped to human genome (hg19) using BWA software. SOAPsnp software and SAM tools Pileup software were used to detect single nucleotide variants (SNPs) and small insertion and deletions. Variants were annotated by ANNOVAR software. Interpretation of the variants followed the recommended standards of the American College of Medical Genetics and Genomics [8]. Finally, we performed Sanger sequencing with specific primers to confirm the identified mutations in all family members.

Results

Clinical features

Four family members presented with lower limb ataxia along with elevated deep tendon reflexes in lower limbs and positive Babinski signs (Fig. 1a and Table 1). The proband's father presented with similar symptoms in his 20s but later died in an accident. The family pedigree demonstrates an autosomal dominant pattern of inheritance. Physical examination revealed bilateral ataxia, pyramidal tract signs, and

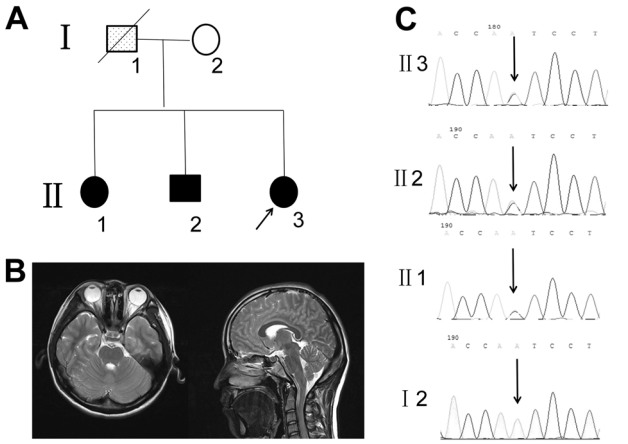

Fig. 1 **a** Pedigree of a Han Chinese family with autosomal dominant hereditary spastic paraplegia associated with peripheral neuropathy and ataxia. Filled squares and circles indicate affected family members (II-1, II-2, and II-3); the hatched square indicates the likely affected father (I-1). The arrow indicates the 16-year-old proband female (II-3). **b** T_2-weighted brain MRI of the 16-year-old proband female (II-3); there were no abnormal findings. **c** Partial sequence demonstrating the *IFRD1* c.514 A>G variant in a Han Chinese family with autosomal dominant hereditary spastic paraplegia associated with peripheral neuropathy and ataxia. The arrows mark the position of the c.514 A>G variant. All three siblings (II-1, II-2, II-3) carried the c.514 A>G variant while the mother did not have similar variant (I-2)

mild weakness in the arms and legs. The proband's symptoms were slowly progressive. NCV revealed decreased motor NCV in the legs (data not shown). Brain MRI of patients II-3 and II-2 were normal and did not reveal cerebellar atrophy (Fig. 1b and data of II-2 not shown). All patients were followed up for 1 year but no significant improvements were seen. The clinical features of all affected subjects are summarized in Table 1.

Next generation sequencing

We analyzed the genomic DNA for mutations associated with SCAs and hereditary spastic paraplegia. Next-generation sequencing was carried out on the proband's DNA, and 22 SNPs were discovered by the Illumina chip without filtration (data not shown); 18 variants with minor allele frequency > 0.01 obtained from our in-house controls (800 unrelated volunteers from China) or the Exome Variant Server (ESP6500) were excluded. Additionally, three variants (c.333_335del variant in the *ARX* gene; c.11062 G>A variant in the *SYNE1* gene; c.1702-1 G>T variant in the *COL18A1* gene) had no noticeable correlation with the phenotype of ataxia and neuropathy; therefore, they were also excluded from the analysis. Interestingly, we observed the *IFRD1* gene variant (c.514 A>G, p.I172V) in the proband's DNA (data not shown). Sanger sequencing with specific primers was conducted to confirm the identified *IFRD1* variant in all family members (I-2, II-1, II-2, and

Table 1 Clinical features in a Han Chinese family with autosomal dominant hereditary spastic paraplegia associated with peripheral neuropathy and ataxia

Variables	Patient II-1	Patient II-2	Patient II-3
Sex	Female	Male	Female
Age at examination (years)	22	16	15
Age at onset (years)	10	4	4
Ataxia	Gait ataxia; lower limb ataxia	Gait ataxia; lower limb ataxia	Gait ataxia; lower limb ataxia
Pyramidal tract signs	Elevated deep tendon reflexes in lower limbs and positive Babinski signs	Elevated deep tendon reflexes in lower limbs and positive Babinski signs	Elevated deep tendon reflexes in lower limbs and positive Babinski signs
Sensory loss	−	−	−
Weakness in LL	Mild	Mild	Mild
Wasting in LL	Mild	Mild	Mild
Pes cavus	+	+	+
EMG/NCV	Decreased motor nerve conduction velocity of the lower limbs	Decreased motor nerve conduction velocity of the lower limbs	Decreased motor nerve conduction velocity of the lower limbs
Brain MRI	N/A	Normal	Normal

NCV nerve conduction velocity test, "—" indicates absent, "+" indicates presence, *LL*, lower limbs, *N/A* not applicable

II-3). DNA sequencing analysis showed that all three siblings—the proband, her sister, and brother (II-3, II-1, and II-2, respectively)—carried the same c.514 A>G (p.I172V) variant in the *IFRD1* gene and this variant was not detected in the unaffected mother I-2 (Fig. 1c). Additionally, the c.514 A>G variant was not observed in 200 unrelated healthy control subjects by Sanger sequencing (data not shown). Furthermore, the frequency of this mutation was null in the control group comprising 800 unrelated in-house healthy individuals.

Discussion

Brkanac et al. (2002 and 2009) reported that SCA18 was associated with a single missense variant c.514 A>G (p.I172V) in the *IFRD1* gene in a five-generation American family of Irish origin [6, 7]. Clinical symptoms included neuropathy, ataxia, pyramidal tract signs, and muscle weakness, which were similar to the symptoms observed in our patients. Similarly, nerve conduction studies were consistent with neuropathy in all patients. Brkanac et al. suggested that the *IFRD1* gene mutation was the likely causative allele for SCA18 and they recommended mutation analysis of *IFRD1* in additional patients with similar phenotypes to confirm its role as a disease-causing candidate. In this study, we report the same variant in the *IFRD1* gene (c.514 A>G, p.I172V) in the affected members of a Han Chinese family with similar symptoms.

IFRD1 is ubiquitously expressed in the human brain and the spinal cord [9, 10]. In mice, *IFRD1* is expressed in the brain, spinal cord, and spinal ganglia during early gestation. Disruption of *IFRD1* in mice (IFRD1$^{-/-}$) resulted in muscle atrophy and an increase in the number of central nuclei, a small fiber diameter, and a lower total number of fibers, which resembled the phenotype of neurogenic muscle atrophy in humans [11]. Further, Vietor et al. previously demonstrated that IFRD1 interacts with several proteins of the SIN3 complex, including Sin3b, Hdac1, Ncor, and Sap30 by yeast two-hybrid and coimmunoprecipitation analyses [12]. The coprecipitated complex was enzymatically active and repressed the transcription of a reporter gene. Additionally, IFRD1 could inhibit cellular retinoic acid binding protein II (CRABP II) expression during axonal regeneration, thereby modulating retinoic acid signaling and regulating the neurite initiation and branching [13]. The above mentioned results suggest a critical role for *IFRD1* in axonal growth and the pathogenesis of SCA. However, to date, the exact physiological role of IFRD1 remains unclear.

SCA18 is a rare subtype of SCA. To the best of our knowledge, SCA18 was only previously reported in a cohort of 27 patients from an American family of Irish origin. In that study, Brkanac et al. suggested that a c.514 A>G, p.I172V variant in exon 5 of the *IFRD1* gene was the disease-causing candidate. Indeed, in our study, we provide another evidence that the heterozygous variant c.514 A>G in *IFRD1* may cause SCA18.

Brkanac et al. showed that the patients in the American family displayed sensory loss and axonal sensory/motor neuropathy symptoms. In one affected member, mild cerebellar atrophy was shown on brain MRI. However, the affected members in the Han Chinese family showed predominantly demyelinating motor neuropathy in lower limbs but otherwise normal brain MRI findings. It is worth mentioning that clinical symptoms varied slightly between patient groups in these two studies. This can be attributed to the different ages at disease-onset and/or geographical or

environmental factors. Interestingly, another *IFRD1* gene variant—c.4 C>G(p.Pro2Ala)—was recently reported in an isolated 62-year-old patient with palatal tremor, hypertrophic olivary degeneration, and cerebellar atrophy [14]. Clinical manifestations such as ataxia, muscle weakness, pyramidal tract signs, sensory axonal neuropathy, and mild cerebellar atrophy were absent in this case. More detection of mutations in the *IFRD1* gene in patients with similar phenotypes is instrumental in understanding gene function.

Finally a functional study is necessary to confirm the pathogenicity of the *IFRD1* gene mutations, and more studies are required to elucidate the molecular mechanism by which IFRD1 interact with other proteins implicated in SCAs.

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Compliance with ethical standard

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

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