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Clinical short communication

A novel hemizygous *SACS* mutation identified by whole exome sequencing and SNP array analysis in a Chinese ARSACS patient

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ABSTRACT

The array of autosomal recessive spastic ataxia of Charlevoix–Saguenay (ARSACS) has expanded worldwide after the first description in the Charlevoix–Saguenay region of Québec. Here, we report a Chinese ARSACS patient presenting progressive peripheral neuropathy (CMTNS2 = 15) with horizontal gaze nystagmus and mild spastic gait. Genetic studies including whole exome sequencing (WES), Sanger sequencing and single nucleotide polymorphism (SNP) array analysis revealed a novel hemizygous nonsense mutation (c.11803C > T, p.Gln3935X) of *SACS* and a 1.33 Mb deletion involved in *SACS* on chromosome 13q12.12 in the patient. Our findings highlight the necessity of *SACS* mutation screening in the gene panel of inherited peripheral neuropathies, and stress the need of testing copy number variation (CNV) in *SACS* mutation screening.

1. Introduction

Autosomal recessive spastic ataxia of Charlevoix–Saguenay, more commonly known as ARSACS, was originally described in families from the Charlevoix–Saguenay region of Québec in the northeast of Canada [1]. The typical phenotype of ARSACS is characterized by progressive spinocerebellar ataxia, dysarthria, nystagmus, upper motor neuron dysfunction, distal sensorimotor peripheral neuropathy, normal mentality and retinal striation [2]. Increasing atypical ARSACS cases including non-ataxia, non-spasticity, autonomic dysfunction, hearing loss and neuropsychiatric disorders have been reported in French, Belgian, Tunisian, Italian, Spanish, Turkish, Dutch and Japanese descent, which resulted in great clinical heterogeneity [1, 3–15].

ARSACS phenotype is due to the alteration of the activity of sacsin (MIM 604490), a protein which includes a UbL domain at the N-terminus, a DnaJ domain, and a HEPN domain at the C-terminus [16]. The function of sacsin is not yet fully known, only a few evidences reveal that it protects against mutant ataxin-1 and recruit Hsp70 chaperone action [17]. Sacsin is encoded by *SACS* which is located on chromosome 13q12.12 and comprises 10 exons consisting of 13,737 base pairs, and the last coding exon is giant spanning more than 12.8 kb (this exon is the largest identified in vertebrate organisms) [18]. To date, more than 126 mutations have been reported worldwide (Human Gene Mutation database, http://www.hgmd.cf.ac.uk).

The genetic diagnosis of inherited peripheral neuropathies is still a great challenge due to the great clinical and genetic heterogeneities. The next-generation sequencing (NGS) techniques have provided a new approach to solve this problem. Here, we report an ARSACS patient presenting progressive peripheral neuropathy with horizontal gaze nystagmus and mild spastic gait caused by a novel hemizygous nonsense mutation of *SACS* and a ~1.33 Mb deletion on 13q12.12 involved in *SACS*.

2. Patient and methods

2.1. Patient and clinical evaluation

A 12 year-old Chinese female was referred to our genetic department for evaluation of inherited peripheral neuropathy in 2015. No variations were found after screening 60 genes in a gene panel of Charcot–Marie–Tooth disease once in another hospital. Four family members including her healthy father, mother and younger sister were comprehensively checked by two experienced neurologists. The family tree is shown in Fig. 1B. The routine hematological and biochemical examinations (including creatine kinase level), nerve

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electrophysiological study, brain and whole-spine MRI, echocardiography, optical coherence tomography and fundus examinations were performed on the patient.

2.2. Whole exome sequencing

Our study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University (2015-S046). All study participants provided written informed consent. Genomic DNA of all available family members was extracted from peripheral white blood cells according to standard protocols. Exonic regions were captured and enriched with the Agilent SureSelect Human All Exon 50 Mb kit (Agilent, Santa Clara, CA), and the DNA-sequencing libraries were prepared with an Illumina Paired-End DNA Sample Preparation kit. A total of 90 bp paired-end reads were obtained by sequencing on HiSeq2000 (Illumina, San Diego, CA, USA). Data were analyzed using the SOAPaligner [19]. Variants were filtered by dbSNP database, the 1000 Genomes Project, ESP6500 database and SNP database of BGI. Candidate variants were confirmed by co-segregation analysis using polymerase chain reaction and Sanger sequencing.

2.3. SNP array analysis

Single nucleotide polymorphism (SNP) array analysis of genomic DNA from the patient and her mother was performed using the Human Omni Cyto-12 Beadchip (Illumina, San Diego, CA). The labeling and hybridization of gDNA were performed following the manufacturer's protocol. The data were analyzed using the GenomeStudio2011 software (CNV Partition v3.1.6).

3. Results

3.1. Clinical features

The patient learned to walk at the age of 18 months. She complained of frequent falling at age 6, and developed muscle atrophy and weakness of distal extremities at age 7. Neurological examination at age 12 showed symmetrical muscle atrophy and weakness in distal extremities (score 4/5 in distal upper limbs, score 3/5 in distal lower limbs). She had horizontal gaze nystagmus, however, performed correct finger–nose–finger test and heel–shin test. Deep tendon reflexes of upper limbs and knees were brisk but ankle reflexes were absent. Bilateral patellar clonus and Babinski's sign were positive. She exhibited obvious steppage and mild spastic gait, and developed pes cavus. The muscle tension and sense were normal. Electrophysiological studies revealed peripheral neuropathy with mixed axonal degeneration and demyelination findings. The motor nerve conduction velocities (MNCVs) were moderately reduced (Median: 35.8 m/s, Peroneus: 32.7 m/s), and the amplitudes of compound muscle action potentials (CMAPs) were decreased (Median: 15 mV, Peroneus: 0.7 mV). The sensory nerve conduction velocities (SNCVs) were reduced (Median: 36.6 m/s, Sural: 39.5 m/s), and the amplitudes of sensory nerve action potentials (SNAPs) were decreased (Median: 0.5 μV, Sural: 1.4 μV). The second version of Charcot–Marie–Tooth disease neuropathy score (CMTNS2) was 15. Magnetic resonance imaging (MRI) showed cerebellum atrophy particularly in the vermis superior, mild atrophy of the spinal cord and linear T2 hypointensities in the basis pontis (see Fig. 1A). The optical coherence tomography (OCT) study of retina found increased thickness of retinal nerve fiber layer (RNFL) around the optic nerve head and papilledema. The routine hematological and biochemical examinations (including creatine kinase level), echocardiography and ocular fundus appearance were normal.

3.2. Genetic findings

Two variations were found in the large exon 10 of *SACS* (NCBI reference sequence NM_014363.4) after filtered in the database of dbSNP (snp137), 1000 Genomes (phase I), ESP6500 (Exome Sequencing Project) and BGI-DB (HGVD). The c.11803C>T is a nonsense variation and c.8948A>G is a missense variation, which are predicted to a formation of premature stop at codon 3935 [p.Gln3935X] and a substitution of asparagine with serine at amino acid residue 2983 [p.Asn2983Ser] respectively. The c.8948 A>G is registered as SNP (rs200106708) in dbSNP (MAF = 0.0004, 1000 Genomes). The c.11803C>T was confirmed by Sanger sequencing in the whole family members. It was present with heterozygosis in the healthy father, but was absent in the mother and sister (Fig. 1B). The c.11803C>T was not detected in 200 healthy controls, and was predicted as damaging by SIFT software. We performed SNP array analysis to shed light on the mechanism underlying the observed homozygosity. SNP array analysis in the patient and her mother detected a novel 1.33 Mb deletion of 13q12.12 spanning from 23,539,563 to 24,874,926 (Fig. 1B).The deleted region contains six genes, namely *SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *C1QTNF9* and a part of *SPATA13* (Fig. 1C).

4. Discussion

The patient is diagnosed as ARSACS based on the clinical manifestations, neuroimaging features and genetic results. It is noteworthy that cerebellar ataxia and leg spasticity, which is the core clinical features of ARSACS, were not obvious in the patient. The main presentation of the patient is progressive peripheral neuropathy. Increasing atypical cases including non-ataxia, non-spasticity, autonomic dysfunction, hearing loss and neuropsychiatric disorders result in the clinical heterogeneity of ARSACS [1,13–15,20]. Inherited peripheral neuropathies with mild ataxia and pyramidal tract signs also exist in Roussy–Lévy syndrome and Refsum disease [21,22]. The overlap of clinical spectrum between ARSACS and inherited peripheral neuropathies should be recognized, and the neuroimaging test is necessary for differential diagnosis in these untypical cases. Moreover, increasing *SACS* mutations were detected by screening the gene panel of inherited peripheral neuropathies in the patient cohort [23]. We proposed that the *SACS* should be added in the gene panel of inherited peripheral neuropathies.

Most of the *SACS* mutations identified worldwide located in the gigantic exon 10, only a few mutations located the upstream from this exon [24]. We found a novel nonsense mutation (c.11803C>T) located in the gigantic exon 10 of *SACS* of this patient. The c.11803C>T mutation leads to the formation of a premature stop at codon 3935 [p.Gln3935X] and results in the loss of DNA J domain and higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domain of sacsin protein, which are critically involved in protein translation, folding, translocation and degradation [18,25]. The nonsense mutations of *SACS* (p.R4325X, p.E4350X, p.R4378X and p.R1877X) have been reported in a Japan, a UK and two Italy families [16,26,27]. We speculated the *SACS* mutation c.11803C>T (p.Gln3935X) was the pathogenetic mutation of the patient. Two deletions (1.54/1.5 Mb) including six genes (*SGCG*, *SACS*, *TNFRSF19*, *MIPEP*, *C1QTNF9* and *SPATA13*) were reported in a Belgian and two Italian ARSACS patients with hearing loss [15,28]. Another 0.7 Mb deletion contained five genes (*SGCG*, *SACS*, *TNFRSF19*, *MIPEP* and *C1QTNF9*) in an ARSACS family with no hearing loss [26]. We identified a novel 1.33 Mb deletion including six genes (*SACS*, *SGCG*, *TNFRSF19*, *MIPEP*, *C1QTNF9* and a part of *SPATA13*) in a Chinese ARSACS patient without hearing loss. The information of these five ARSACS cases with deletion on 13q12.12 was summarized in Table 1. Taken together, the chromosome 13q12.12 region including *SACS* might be prone to deletion and ARSACS can be added to the list of genomic disorders, which stress the need of testing CNV in *SACS* mutation screening.