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Spastin mutation screening in Chinese patients with pure hereditary spastic paraplegia

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ABSTRACT

Background: Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative diseases. Mutations in the spastin (*SPAST*) gene are the most common cause of pure HSP. However, few data are available regarding the clinical and genetic spectrum of HSP among Chinese patients.

Methods: Clinical data were collected at diagnosis and follow-up of 42 Chinese patients with pure HSP. All seventeen exons of the *SPAST* gene were directly sequenced. Additionally, we used a multiplex ligation dependent probe amplification (MLPA) assay targeting the *SPAST* gene to evaluate large exon deletion or insertion mutations in patients without *SPAST* point mutations.

Results: The age of disease onset of our patients was 19.6 ± 14.4 years. Six novel variations were found, including three missense mutations (p. L363P, p. D441V, and p. S595R), one insertion (c.1511dupT (p. Y505Ifs*7)), and two larger deletions (exons 5–17 and exons 10–17). Four previously reported mutations, including p. S399L, c.1215_c.1219delTATAA (p. N405Kfs*36), exon 1 deletion, and exon 16 deletion, were detected. The *SPAST* mutation rate was 40% (4/10) in Chinese familial patients and 33.33% (7/21) in Chinese sporadic pure HSP patients. The frequency of large deletions was high in both AD-HSP (20%, 2/10) and sporadic HSP (14.28%, 3/21).

Conclusion: *SPAST* mutations are common in Chinese patients with pure HSP. Large exon deletions are an important cause of AD-HSP and sporadic pure HSP in Chinese patients. Large fragment tests should be performed to explore large *SPAST* mutations in familial and sporadic HSP patients without *SPAST* point mutations.

1. Introduction

Hereditary spastic paraplegia (HSP) is a group of clinically and genetically heterogeneous neurodegenerative disorders characterized by slow progressive spasticity and weakness of the lower limbs. According to its clinical presentation, HSP can be classified into a pure form with isolated progressive spasticity and weakness of the lower limbs and a complicated form with other abnormalities, such as mental and cognitive changes, optic atrophy, amyotrophy, ataxia, deafness, ichthyosis, and/or peripheral neuropathy [1]. The inherited forms include the autosomal dominant (AD-HSP) form, which is observed most frequently (70–80%), as well as the autosomal recessive (AR-HSP) and X-linked forms [2]. To date, at least fifty-two chromosomal loci have been identified for HSPs, including seventeen autosomal dominant, thirty autosomal recessive and five X-linked inherited loci [3–5]. The spastin (*SPAST*) gene, which encodes a member of the AAA ATPase protein family and is located on chromosome 2p24-p21, has been reported to be the most common cause of HSP, accounting for 40–45% of pure AD-HSP cases and approximately 10% of sporadic cases. More than 440 different mutations have been identified in the *SPAST* gene, including 179 missense/nonsense mutations (http://www.hgmd.cf.ac.uk/ac/all.php). Cases of HSP that are caused by *SPAST* mutations

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tend to present as the pure form of the disease, which is often associated with a decreased sensation of vibration in the lower limbs and urinary problems [6]. In addition, HSP caused by mutation of the *SPAST* gene is reported more often in males than in females [7]. However, the age of onset, severity of symptoms, and progression of symptoms are highly variable, even within families [7]. Therefore, it is difficult to describe the correlation between the genotype and the phenotype of HSP.

To date, most of the data regarding clinical and molecular correlations related to HSP have been generated using patients from European countries [8–10], although *SPAST* mutations have been reported in some Han Chinese patients [11,12]. However, the prevalence and types of *SPAST* mutations among Chinese patients are still largely unknown. One small study estimated that the frequency of *SPAST* mutations in Chinese patients is 45% (5/11) in AD-HSP and 9% (1/11) in sporadic HSP [13]. Considering the limited sample size of previous studies, in the current study, we present clinical and genetic data related to the *SPAST* gene in a cohort of 42 patients with pure HSP from Southwest China.

2. Materials and methods

2.1. Subjects

Forty-two patients, including 21 sporadic and 21 familial patients from 10 families, who presented to the Department of Neurology, West China Hospital, Sichuan University, were clinically diagnosed with pure HSP according to the Harding criteria [1]. All patients presented with a pure form of the HSP phenotype, although mild associated symptoms, such as sensory disturbances or bladder dysfunction, were allowed. Patients with the complicated form of HSP, which is associated with mental and cognitive impairment, dementia, epilepsy, aphasia, dystonia, extra-pyramidal disturbances, cerebellar abnormalities, a thin corpus callosum, optic atrophy, ataxia, deafness, and ichthyosis, were excluded [1]. The disability score of patients was evaluated on a four-point scale (1 = normal, 2 = able to walk but not run, 3 = requires the use of a walking aid or support, 4 = wheelchair bound) [14]. The patient history was obtained, and neurological examinations were performed by neurologists.

2.2. Mutation screening of the SPAST gene

A total of 200 unrelated Chinese healthy controls (HC), matched with regard to gender, age, and area of residence to the HSP patients, were recruited for the study as the control group. Written informed consent was obtained from each subject, and the study was approved by the Sichuan University Ethics Committee. Genomic DNA was extracted from a 5 ml peripheral blood sample with an established method using saturated phenol-chloroform.

Fifteen pairs of primers designed with the Primer Permier 5 software (Premier Biosoft International, Palo Alto, CA, USA) were used to amplify the coding region of *SPAST*, including seventeen exons and exon/intron boundaries (GenBank Accession No. NM_014946). The PCR products were directly sequenced with an ABI3100 automated DNA sequencing system (BGI, Shenzhen, China). The primer sequences and PCR conditions are shown in Supplementary Table 1. Moreover, for patients in whom no *SPAST* point mutations were found by direct sequencing, we performed a multiplex ligation-dependent probe amplification (MLPA) assay targeting the *SPAST* gene with the Salsa kit P165-C1 HSP (MRC-Holland, Amsterdam) according to the manufacturer's instructions. The kit contains probes for all 17 exons in the *SPAST* gene. MLPA amplification products were separated by capillary electrophoresis using an ABI 3100-Avant Genetic Analyzer. MLPA data were analyzed using Coffalyser v9.4 software. Deletions were detected in exons in which the relative peak area was reduced by 35–50% of the normal control value.

2.3. Statistical analysis

Statistical analysis was performed using SPSS software (v 18.0). The results of the data analysis are expressed as the mean ± standard deviation. Student's *t* test was used to compare groups when the data were normally distributed. Categorical variables were compared using aChi-square test. Significance was set at $P < 0.05$.

3. Results

3.1. Clinical data

All patients (***Patient 1*** to ***Patient 42***) presented with gait disorder due to weakness or spasticity of the lower limbs. The spasticity gradually became worse, although none of the patients were wheelchair bound. The age of disease onset was 19.6 ± 14.4 years (ranging from 1 to 47 years), and the disease duration was 14.3 ± 10.9 years (ranging from 1 to 40 years). The babinski sign was present in 33 patients (78.6%). Eight patients (19.05%) had bladder disturbance and presented with urinary incontinence. Seven patients presented with pes cavus. The age of onset of patients with pes cavus did not differ from that of patients without pes cavus. In addition, an electromyography test was performed on these seven patients with pes cavus. Of these, only two patients showed mild peripheral neuropathy of the lower limbs and exhibited fibrillation waves and slowing of the sensory nerve conduction velocity. Six patients (17.7%, 6/35) showed thoracic spinal cord atrophy.

3.2. Genetic findings

After direct sequencing and MLPA assay screening of all seventeen exons in each patient, 10 different mutations were found in 13 patients, including 6 familial patients (from 4 families) and 7 sporadic patients. The clinical features of the patients with these mutations are presented in Table 1.

Among the ten mutations, six mutations were novel, including three missense mutations (p. L363P, p. D441V, and p. S595R), one insertion mutation (c.1511dupT (p. Y505Ifs*7)), and two large deletions (exons 5–17 and exons 10–17), whereas four known mutations (p. S399L, c.1215_c.1219delTATAA (p. N405Kfs*36), exon 1 deletion, and exon 16 deletion) were reported (Table 1) [8,10,14,15]. Direct sequencing showed that all novel mutations were absent in the 200 healthy control subjects. In addition, these mutations were not found in the single nucleotide polymorphism (SNP) database or the 1000 Genomes Project database.

The p. L363P mutation in exon 7 was identified in a sporadic male patient with disease onset at 15 years of age (***Patient 20***, Fig. 1A). The p. D441V mutation in exon 11 was identified in a familial patient from the family 10 pedigree with disease onset at 41 years of age (***Patient 17***, Fig. 1B). The p. S595R mutation in exon 17 was found in a familial male patient from the family 6 pedigree with disease onset at 25 years of age (***Patient 29***, Fig. 1C). The same mutation was found in his son (***Patient 30***), who exhibited symptom onset at 15 years of age. The mutated amino acid residues of the three novel missense mutations, p. L363P, p. D441V and p. S595R, are highly conserved among species. Furthermore, these novel missense mutations are predicted to be damaging substitutions by the Sorting Intolerant from Tolerant (SIFT) (http://sift.jcvi.org/www/SIFT_enst_submit.html) and PolyPhen-2 prediction tools (http://genetics.bwh.harvard.edu/pph2/).

The novel insertion mutation in exon 13, c.1511dupT (p. Y505Ifs*7), was found in a male sporadic patient with disease onset at 12 years of age (***Patient 9***, Fig. 1D).

The exons 10–17 deletion was found in an AD-HSP family (family 4) (***Patients 27 and 28***, Fig. 2A and B), and the novel exons 5–17 deletion was found in a sporadic male patient with disease onset at 44 years of age (***Patient 5***, Fig. 2C).

The previously reported mutation p. S399L was detected in ***Patient 8***. The previously reported deletion mutation in exon 9, c.1215_c.1219delTATAA (p. N405Kfs*36), was identified in a sporadic male patient with disease onset at 8 years of age (***Patient 35***). The previously reported exon 1 deletions were found in a familial male patient from family 7 (***Patient 23***) and a sporadic female patient (***Patient 15***). The previously reported exon 16 deletion was found in a sporadic female patient (***Patient 7***).

The mutation rate was 40% (4/10) in familial patients and 33.33% (7/21) in sporadic patients, and a higher mutation rate was found in male patients (39.29%, 11/28) compared to female patients (14.29%, 2/14). There were no significant differences in gender, mode of inheritance, or the age of disease onset between patients with and

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