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Whole-Genome Sequencing Reveals a Novel Insertional Mutation in *SPAST* in a Chinese Han Family with Hereditary Spastic Paraplegia

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Abstract

Hereditary spastic paraplegia (HSP) mainly results from the progressive degeneration of longcorticospinal tract. HSP displays a heterogeneity of clinical manifestation and genetic mutation. More than 50 genes and loci have been linked to HSP and thus its heterogeneous genetic causes render its diagnosis not so efficient. Using whole-genome sequencing, we examined two affected and one unaffected members of a Chinese Han family with HSP across four generations. We identified a novel insertional mutation in *SPAST* (c.756insA, p.M253Nfs*12) which segregated with disease in the family.We further looked up genome data to reveal other possible variants segregating with HSP in the family and found that no other variant in known HSP genes or novel genes segregated with disease. Therefore, the novel insertional mutation in *SPAST* is the sole variant revealed segregating with HSP in our HSP family.This novel mutation caused a frame shift to generate a premature terminating codon which is located by 76 nucleotides upstream of splicing site, likely leading to nonsense-mediated decay of aberrant SPAST mRNA and possibly causing HSP by haploinsufficiency. We established our diagnosis using whole-genome sequencing alone.

Keywords: hereditary spastic paraplegia, SPAST, insertion mutation, whole-genome sequencing, next-generation sequencing

1. Introduction

Hereditary spastic paraplegia (HSP)is known for its heterogeneity in clinical manifestation and genetic variation[1]. According to clinical manifestation, HSP is classified as pure and complex forms[2]. The disease is characterized by the progressive weakness and spasticity of lower extremities which results from the degeneration of long corticospinal tract[3]. Pure HSP manifests mainly as the phenotypes of corticospinal tract degeneration, occasionally with isolated sensory deficit or the disturbance of urinary bladder function. By contrast, complex HSP is involved in more non-motor phenotypes such as dementia, epilepsy, peripheral neuropathy and retinopathy. Besides its heterogeneity in clinical manifestation, HSP has diverse genetic causes[4,5,6,7]. The transmitting patterns of HSP can manifest as autosomal dominant, autosomal recessive, or X-linked forms[1,2,8]. A maternal trait of inheritance is also reported for HSP[2].More than 50 genes and loci have been linked to HSP, rendering HSP diagnosis not so efficient. As technology is advanced[9], next generation sequencing is recommended for determination of genetic mutation in HSP patients[10,11,12,13]. Using whole genome sequencing, we identified a novel insertional mutation in *SPAST* which segregated with HSP in a family across four generations.

2. Materials and Methods

2.1. Participants

A Chinese Han family with seven HSP patients across four generations was examined of genetic variants segregating with the disease. Except two deceased patients, the rest five patients were examined by two specialized neurologists and were clinically diagnosed with pure HSP according to Harding criteria [1]. Five close siblings from this family were also recruited as normal controls. Five hundred normal subjects of Han Chinese origin were recruited from the same outpatient clinic as additional controls. All the participants provide their written informed consent to participate in this study without minors/children enrolled in our study. The study was approved by the Sichuan University Ethics Committee. Neurological examination was performed at the hospital and detailed clinical data were collected meanwhile.

2.2. Genetic analysis

Two HSP patients (IV3 and IV8) and one unaffected sibling (III8) of the same family were screened for genetic variants by next generation sequencing. Genomic DNA was extracted from peripheral white blood cells using standard phenol/chloroform extraction. The whole genomes of three selected subjects were sequenced with Hiseq-2000 at Axeq Inc. Genome data was analyzed with Enlis Genomics. Selected variants were verified by PCR analysis and Sanger sequencing.

3. Results

3.1. Clinical features

The proband (Figure 1: IV-3) complained unsteady gait and the stiffness and weakness of lower limbs and thus he was hospitalized. The proband recalled that he had difficulty in walking and running since he was 8 years old. His symptom was progressing slowly over the past 27 years and was accompanied by the mild numbness of the right leg below knee joint in recent two years. Neurological examinations showed that hypermyotonia of lower limbs, spastic gait, a mildly superficial sensory disturbance in his right leg below the knee-joint, hyperreflexia of lower extremities. In addition, ankle clonus andBabinski sign were positive.No abnormality was detected in the proband by electromyography and intracranial magnetic resonance imaging. Except for the absence of ankle clonus, the mother of the proband (Figure 1: III-2) revealed similar symptoms of signs including unsteady gait and the weakness of lower limbs. The proband and his mother showed similar symptoms characteristic of pure HSP, implying that the disease likely is caused by genetic mutation. Therefore, the rest members of the proband's family were examined and five additional patients were revealed. Five of the seven affected family members were alive and were then diagnosed with pure HSP. The clinical data of affected members in the family were summarized in table1.No consanguineous marriage was noticed in the family. Pedigree analysis revealed that HSP was transmitted across four generations in an autosomal dominant pattern. The age of disease onset spanned from 8 to 35

years and the progression of disease was relatively slow. The unique clinical features of HSP in the family implies that novel mutation of an unknown or known gene could be the culprit of disease. (Figure1: Pedigree of a family suffering from the pure autosomal dominant hereditary spastic paraplegia; Table 1: Clinical characteristics of patients with hereditary spastic paraplegia in the family.)

3.2. Genetic findings

Considering that HSP has a heterogeneous genetic cause, we took a bold but efficient approach to sequence the whole genome for two affected (IV3 and IV8) and one unaffected (III8) members of the family. Genomic DNA was sequenced with Illumina HiSeq-2000 system which provided 30X coverage on target. Genome data in CASAVA format were analyzed with Enlis Genomics. We first generated a complete list of genes which have been linked to HSP and then checked the three genomes against HSP gene list to reveal all variants in the exons and introns of any HSP genes. A variant that was found in the two affected subjects and was not found in the unaffected subject was selected for further analysis. Using this criterion, we found an insertional mutation in *SPAST*(c.756insA, p.M253Nfs*12) which caused a frameshift to generate a premature stop codon (Figure 2: An insertional mutation in *SPAST* causes frameshift to introduce a premature stop codon). The premature stop codon is located in the exon 5 of *SPAST* and is 76 nucleotides away from exon-intron junction. As mutation of *SPAST* has been linked to HSP, we considered the insertional mutation in *SPAST* responsible for disease in the family. We verified this variation by PCR analysis followed by Sanger sequencing (Figure 2). Indeed, the insertional mutation in *SPAST* segregated with HSP in the family. To validate the pathogenic role of the insertional mutation in *SPAST*, we looked up 133 normal genomes and did not find this variation in any of the 133 normal subjects. We examined 500 normal subjects of the same origin by PCR-based sequencing and did not find the mutation in these normal subjects. Also, this unique insertional mutation was not found in 1000 Genomes and SNP and ESP databases. We examined the other HSP genes and did not find in HSP genes any variant that met our criterion.

We also considered that mutation of an unknown gene might be the true culprit of HSP in the family. Using our own genome library which contains 133 normal genomes, we examined the genome sequence of three family members to reveal novel variants which must meet all of the following criteria: 1) which were found in the two affected subjects of the family; 2) which were not found in the unaffected subject of the family; 3) which were not found in all of the 133 normal subjects; and 4) which were not found in SNP, ESP, and 1000 Genomes databases. In total, we found 13 variants which met all of these criteria. We used PCR and Sanger sequencing to determine whether the 13 selected variants segregated with HSP in the family. None of these variants was confirmed of segregation with disease in the family. Thus, the insertional mutation of *SPAST* is the sole genetic variant segregating with HSP in the family.

4. Discussion

In this study, we demonstrated that next-generation sequencing is an efficient approach for the diagnosis of heterogeneous genetic diseases such as HSP. Both clinical manifestation and genetic mutation display heterogeneity in HSP [1,2,3,4,5,6,7]. More than 50 genes and loci have been linked to HSP and varying types of mutation in the genes have been detected in patients with HSP [4,5,6,7]. Apparently, the traditional approach PCR-based sequencing is time-consuming and inefficient, particularly for a heterogeneous disease like HSP. Moreover, it is unlikely to exclusively establish a diagnosis using traditional genetic approach alone when a novel mutation is revealed in a known gene because the pathogenesis of the novel mutation cannot be validated until functional relevance is confirmed by functional assay. By contrast, next-generation sequencing provides unprecedented advantages for the diagnosis of heterogeneous diseases because it can simultaneously reveal disease-causing variation and whole genome information[10,11,12,13]. Using next-generation sequencing, we were allowed to simultaneously examine both known and unknown genes for variations segregating with disease. In our HSP family, we first examined all of known HSP genes and found a novel mutation in the HSP gene *SPAST* and confirmed the segregation of this variation with disease.

To establish diagnosis, we further looked up variations in novel genes and found a small number of variants which were not found in our own normal genome library and the public databases including SNP, ESP, and 1000 Genomes. We examined whether any of the few variants segregated with HSP in the family and we revealed that none of the variants segregated with disease. Our genetic analysis confirmed that the novel insertional mutation in *SPAST* is the sole genetic variant segregating with HSP in our family. Therefore, we established our diagnosis solely by next-generation sequencing.

SPAST is located on chromosome 2p21-p22 and consists of 17 exons[14]. The insertional mutation found in our HSP family was in the exon 5 of *SPAST* and the single nucleotide insertion caused frame shifting, leading to the occurrence of a premature terminating codon which is 76 nucleotides away from downstream splicing site. The insertional mutation found in our HSP family is predicted to produces the same effect on SPAST mRNA fate as nonsense mutation, which has frequently been detected in *SPAST* in HSP patients[3].The nascent SPAST mRNA produced from the mutant allele contains a premature terminating codon which likely induces the nonsense-mediated decay of aberrant SPAST mRNA[15,16,17], resulting in the reduced production of the protein spastin. If this is the case, the insertional mutation in our HSP family may cause the disease due to haploinsufficiency[18]. On the other hand, the aberrant SPAST mRNA may be translated to produce abnormal short peptides which could cause disease by a dominant-negative or gain-of-functional effect[19]. Spastin encoded by*SPAST* belongs to ATPase family and is involved in a number of important cellular functions. A follow-up mechanistic study is warranted to determine how insertional mutation in our HSP family causes neurodegeneration.

Compliance with Ethical Standards

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

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study. We also thank all the subjects for their participation in this study.

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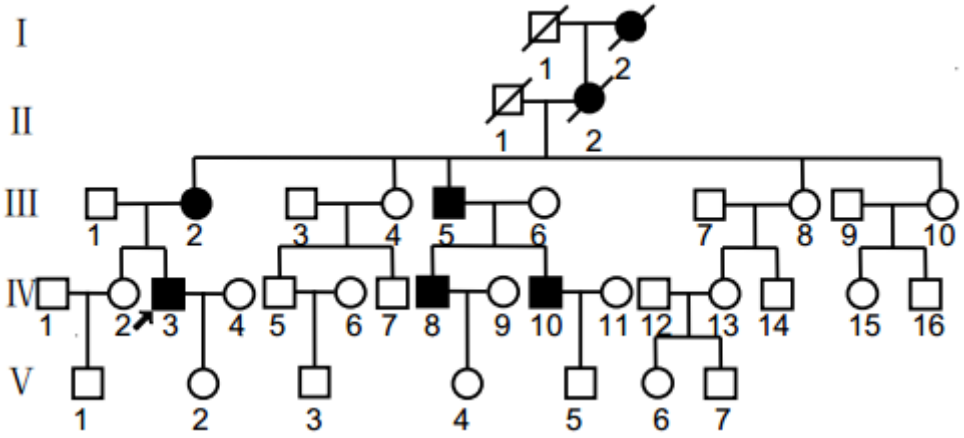
Table1

Table 1 Clinical characteristics of patients with hereditary spastic paraplegia in the family

Patient number	I-2	II-2	III2	III5	IV3	IV8	IV10
Phenotype	pure	pure	pure	pure	pure	pure	pure
Sex	F	F	F	M	M	M	M
Age at examination（age of death）	—(80)	—(70)	60	50	36	25	24
Age at onset	—	35	20	30	8	20	20
Disease duration	—	35	40	20	28	5	4
Disability score	—	—	3	4	3	1	1
LL/UL spasticity	—	—	Y/N	Y/N	Y/N	Y/N	Y/N
LL/UL weakness	—	—	YN	Y/N	Y/N	Y/N	Y/N
Spastic gait	Y	Y	Y	Y	Y	Y	Y

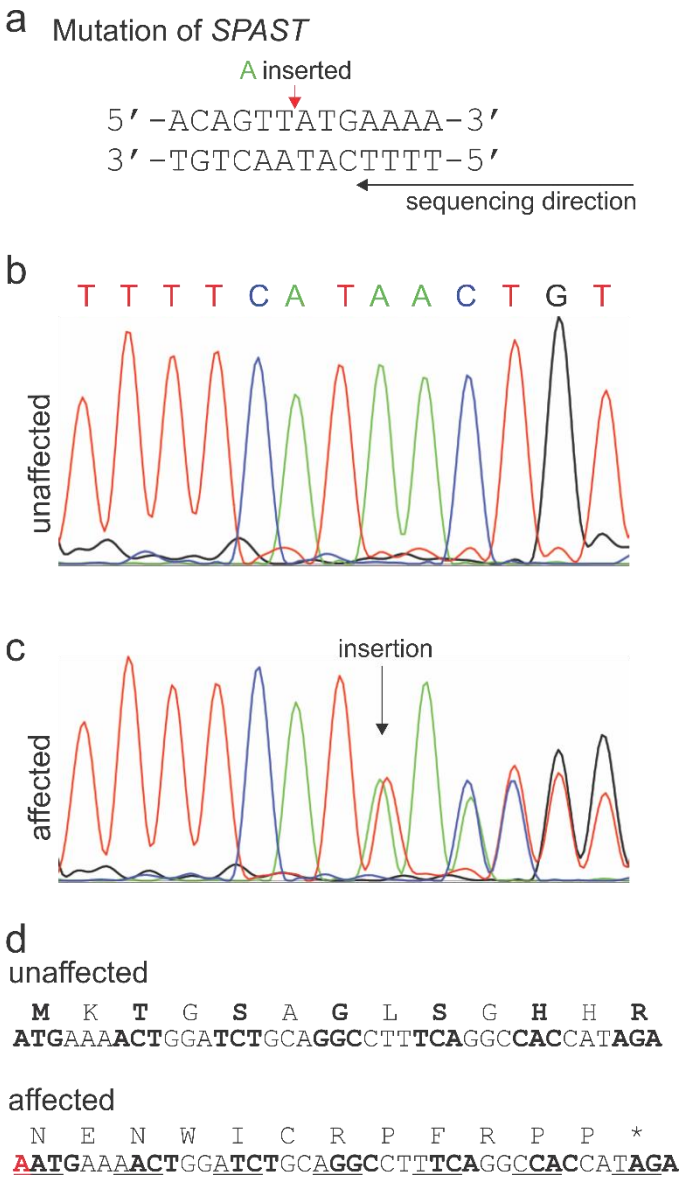
Abbreviations: F: female; M: male; LL: lower limb; UL: upper limb; Y: yes; N: no; "—"：Not available. Disability score (a five-point scale):1=normal gait or very slight stiffness of the legs, 2=moderate gait stiffness, 3=unable to run but able to walk alone, 4=able to walk with help, 5=wheelchair-bound.

Figure1 Pedigree of a family suffering from the pure autosomal dominant hereditary spastic paraplegia



Abbreviations: Symbols are used as follows: squares for males; circles for females; oblique lines for deceased people; and filled squares and circles for affected individuals. An arrow indicates the proband

Figure 2 An insertional mutation in *SPAST* causes frameshift to introduce a premature stop codon



Abbreviations: a. A schematic diagram showing the site of insertional mutation in *SPAST* gene. Note that PCR product was sequenced with a reverse primer. **b, c**. Sanger sequencing revealed that a single nucleotide "A" was inserted in the exon 5 of *SPAST* in an affected subject, but not in unaffected subject. **d**. Sequencing verified that a single nucleotide insertion caused open reading frame shift to introduce premature stop codon in *SPAST* in affected subjects