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A novel mutation of *SPG4* gene in a Chinese family with hereditary spastic paraplegia

A novel SPG 4 variant in HSP family

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Abstract

BACKGROUND

Hereditary spastic paraplegia (HSP) is a group of neurogenetic diseases of the corticospinal tract, accompanying by distinct spasticity and weakness of the lower extremities. Mutations in the spastic paraplegia type 4 (*SPG4*) gene, encoding the spastin protein, are the major cause of the disease. This study aimed to report a Chinese family with HSP caused by a novel mutation of the *SPG4* gene.

CASE SUMMARY

A 44-year-old man was admitted to our hospital for long-term right lower limb weakness, leg stiffness, and unstable walking. His symptoms were gradually worsened, while no obvious muscle atrophy in the lower limbs was found. Neurological examinations revealed that the muscle strength of the lower limbs was normal, and knee reflex hyperreflexia and bilateral positive Babinski signs were also detected. Members of his family also had the same symptoms. Using mutation analysis, a novel heterozygous duplication mutation, c.1053dupA, p. (Gln352Thrfs*15), was identified in the *SPG4* gene in this family.

CONCLUSION

A Chinese family with HSP had a novel mutation of the *SPG4* gene, which is autosomal dominant and inherited as pure HSP. The age of onset, sex distribution, and clinical manifestations of all existing living patients in this family were analyzed. The findings may extend the current knowledge on the existing mutations in the *SPG4* gene.

Key Words: Hereditary spastic paraplegia; *SPG4* gene; Mutation; Genetic testing; Autosomal dominant HSP

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Core Tip: It is difficult to distinguish HSP from other spasticity-related genetic diseases, because the different affected genes lead to large differences in the pathogenic mechanisms, clinical features, and imaging abnormalities of HSP. Therefore, genetic testing is important for the diagnosis and typing of HSP. A Chinese HSP male patient was identified, and pedigree surveys of his relatives were performed. Furthermore, genomic DNA was extracted for the whole-exome sequencing, and pathogenic variants were screened by bioinformatics methods and verified using Sanger sequencing. A novel heterozygous duplication mutation, c.1053dupA, p. (Gln352Thrfs*15), was identified in the *SPG4* gene in this family.

INTRODUCTION

Hereditary spastic paraplegia (HSP) is a group of neurogenetic diseases caused by degeneration of the corticospinal tract, which is characterized by slow progressive spasms and weakness of the lower limbs. The disease has obvious clinical and genetic heterogeneity [1]. According to their clinical manifestations, HSPs are divided into pure and complex types. Pure type presents with slowly progressive lower extremity weakness and spasticity, corticospinal tract signs, disturbance in the vibration sense and

proprioception, and possible sphincter disturbances. Complex HSP form is characterized by leg spasticity and other complications, such as ataxia, a thin corpus callosum, extrapyramidal signs, chorioretinal dystrophy, peripheral neuropathy, and mental retardation [2, 3]. HSP can be inherited by autosomal dominant (AD), autosomal recessive (AR), X-linked (XL), and mitochondrial maternal patterns. More than 80 genes or loci involved in the inheritance of this disease have been identified. Spastic paraplegia type 4 (SPG4) is the most common cause of pure HSP, accounting for 45% of pure HSP cases. It is inherited in AD mode and is caused by the mutation of the *SPAST* gene [1, 4, 5].

CASE PRESENTATION

Chief complaints

Seven years before his admission to our hospital, the right lower limb weakness leg stiffness, and unstable walking appeared without obvious inducement in a 44-year-old man (patient V: 4) from the Han Chinese population in Shandong Province, China.

History of present illness

Initially, he felt that his symptoms were mild and he was never treated, his symptoms were gradually worsened over time. In the fourth year, after the onset, he began to experience hotness of his right knee joint; his gait became increasingly slow, his legs felt stiff , and lumbago appeared. The patient's lumbago symptoms were most severe at night during his sleep and in the morning. Meanwhile, the patient felt that the range of motion of his both knees was limited, which aggravated when he was tired. He could not run and had difficulty in climbing stairs.

History of past illness

The patient was healthy before, without any trauma or cardiovascular history, no long-term history of smoking and alcohol consumption.

Personal and family history

His family history revealed that he was born to non-consanguineous parents, but similar symptoms were found in several of his relatives. After obtaining written informed consent from the proband and his relatives, it was attempted to conduct a detailed investigation of this Han Chinese family from Shandong Province, China. This family was traced back to the sixth generation, with a total number of 11 people suffering from the disease, with an autosomal dominant genetic pattern (AD, Figure 1A). Five deceased individuals had spastic paraplegia in their lifetime, including four men (patients I: 1, II: 1, II: 2, and IV: 6) and one woman (patient III: 2). Of the six living patients, five were male (patients IV: 2, IV: 4, V: 4, VI: 1, and VI: 3), and one was female (patient V: 3).

Physical examination

Neurological examination showed that the cranial nerves of the proband were not abnormal, the proximal and distal muscle strength and muscle tone of the upper extremities were normal, biceps reflexes, tricepral triceps, radial reflexes were normal, and bilateral Hoffman signs were negative. The proximal and distal muscle strength of both lower limbs was normal, Besides, lower limb hypermyotonia, Besides, bilateral knee reflex was enhanced, and bilateral Chaddock's sign, Oppenheim sign, and Gordon sign were negative, while Babinski sign was positive. There was no muscle atrophy, or abnormal involuntary movement.

Other living and symptomatic patients initially showed weak legs or unstable walking, and they developed different clinical symptoms as they grew older and the course of the disease progressed. The proband's father (patient IV: 4) and uncle (patient IV: 2) affected by illness in middle age, with a long course of disease, severe symptoms, and unable to lift the leg, with a scissor gait. Neurological examination revealed the increased lower limb hypermyotonia and tendon hyperreflexia, and bilateral Babinski signs were positive. They started using crutches for assisting walking approximately 20 and 10 years after onset, respectively. His (The proband's) nephew (VI: 1), now 20

years, and developed a gait disturbance at the age of 10 years old. Although there was an obvious difficulty in walking, he did not need the assistance of appliances. His cousin (V: 3) was a female patient with an onset age of 46 years old and a disease course of 2 years who presented only with mild weakness of both lower limbs. His son (VI: 3), who aged 19 years old, was an asymptomatic mutation carrier. The mean age of onset of symptoms was 36.6 ± 14.1 years old. The disease course was 14.8 ± 10.9 years. All the survived patients were intellectually normal; no cognitive impairment, peripheral neuropathy, bladder dysfunction, claw-feet, or scoliosis were detected. Their age, age of onset, course of disease, gender, SPRS[6], and disability stage were recorded and evaluated. Additional data are presented in Table 1.

Laboratory examinations

The proband was initially suspected of having myelopathy. Further laboratory examinations, such as cerebrospinal fluid sample analysis and determination of the serum vitamin B12 Level, were conducted. No inflammatory or immune lesions were detected. Neuro-electrophysiological examination suggested normal motor nerve conduction, sensory nerve conduction, and needle electromyography.

Imaging examinations

The magnetic resonance imaging (MRI) of the cervical spinal cord, thoracic spinal cord, and lumbar spine cord revealed only inter vertebral disc herniation of the cervical and lumbar spine. No obvious swelling, atrophy, or compression of the spinal cord was found.

MUTATION ANALYSIS

The aforementioned manifestations and examination results could not explain the clinical symptoms of the patient. After obtaining written informed consent from participants, DNA was extracted from seven peripheral blood samples, including the proband (V: 4), his father (IV: 4), his mother (IV: 5), his wife (V: 5), his sister (V: 6), his

son (VI: 3), and his daughter (VI: 4). Whole-exome sequencing was first performed, pathogenic variants were analyzed by bioinformatics methods, and were then verified using Sanger sequencing. It was found that the proband (V: 4) and the patient (IV: 4, VI: 3) carried a pathogenic heterozygous variant of the SPG4 gene, namely c.1053dupA, p.(Gln352Thrfs*15), located at the shear site of the SPG4 exon 7 (Figures 1B, C). This mutation has not previously been reported. It was also not registered in the Clinvar, dbSNP, and HGMD databases.

MULTIDISCIPLINARY EXPERT CONSULTATION

Not applicable

FINAL DIAGNOSIS

Referring to the HARDING diagnostic criteria[7], based on genetic testing, the proband and other related relatives were finally diagnosed with SPG4 HSP.

TREATMENT

The proband was given an intravenous injection of methylcobalamin (500 μg/day) for two weeks; then, the route was changed to oral administration (1.5 mg/day) for three months. He also took baclofen orally (15 mg/day at the initial stage, which then increased to 30 mg/day), for six weeks.

OUTCOME AND FOLLOW-UP

Although his low back pain was resolved somewhat, his slow walking and stiffness in both lower limbs were not significantly improved, he still could not run, and he had difficulty in climbing the stairs.

DISCUSSION

HSP is a nervous system disease, accompanying by diverse clinical manifestations and complex genetic etiology and pathogenesis. The onset age ranges from infancy to

senility, and the functional impairment is highly variable [4]. The onset age span of patients in the present study was large, in which the largest age span between two relatives was 32 years. The majority of patients developed the disease in middle age.

According to the clinical symptoms, it was revealed that the pure HSP type was dominated in the proband's family, including progressive weakness of the lower extremities and spasticity [1-4]. Notably, in this family, male patients were more affected by the disease than female patients, with a male to female ratio of 9:2, which is consistent with the findings of another study [8]. Earlier reports have also confirmed that patients with *SPG4* gene mutation-related diseases were mostly man, suggesting that in some cases, gender is a stronger contributing factor to the time of onset of disease symptoms than age [9], which may be associated with the higher levels of estrogen and progesterone in female patients [10, 11]. Due to the genetic and clinical heterogeneity of HSP, its phenotype is complex and the diagnosis is difficult, which may be attributed to the effects of genetic factors, environmental modifiers, penetrance, and gender [8, 12, 13].

In the present study, a novel mutation, c.1053dupA, p. (Gln352Thrfs*15), was detected in the *SPG4* gene in the family with HSP. This variant is a frameshift mutation that results in an Gln 352-to-Thr substitution and a new reading frame, and is terminated at codon 15 downstream of the amino acid at position 352, causing early translation termination, compared with the wild-type protein. This may lead to truncation of the coding protein synthesis, thereby losing its normal function.

This novel variant was predicted to be deleterious by Mutation Taster analysis (www.mutationtaster.org/MT69/MutationTaster69.cgi).

1 The DNA sequence variants were named following the guidelines of the Human Genome Variation Society.

(www.varnomen.hgvs.org/recommendations/general/).

In 2009 and 2010, two mutations of the *SPG4* gene were reported in two families, including c.1055A > C, p.(Gln352P) and c.1054C > T, p.(Gln352X), respectively [14, 15]. Both are mutations caused by a single-base substitution, one being a missense mutation

and the other being a nonsense mutation. These two previously detected mutations and our newly identified mutation are located within the conserved AAA cassette, resulting in changes in activity of spastin protein, loss of function. The findings of the present study suggest that mutations in this region are not uncommon. To date, at least 80 genes and several variants have been found to be associated with HSP, of which those of *SPG4* cause the most common type of HSP [9, 16, 17]. Most of the mutations of the *SPG4* gene are missense (33%), frameshift (24%), splice-site (16%), nonsense variants, and deletions (12%) [4].

The *SPG4* gene encodes a microtubule-severing protein, namely spastin, which is a microtubule (MT)-severing enzyme containing MT-binding domain (MTBD) and adenosine triphosphatases associated with diverse cellular activities (AAA) domains with adequate severing activity, playing an important role in axon development, synaptic formation, and spinal cord maturation [18-20]. A number of factors lead to mutations in *SPG4* gene and the onset of related disease symptoms, of which the main factor is a decrease in the level of the functional spastin protein, resulting in insufficient MT cutting [18-21]. Another study found that aggregation of mutant spastin protein caused toxicity, while it could not explain the underlying mechanisms and possible consequences [21]. Qiang *et al* [22] demonstrated that the function acquisition mechanism of *SPG4* gene is more meaningful than its function loss mechanism.

Only symptomatic treatment of HSP is currently available. In the present study, the proband was treated with methylcobalamin and baclofen, which only relieved his low back pain, while the other symptoms were not improved significantly. Therefore, it is critically important to find an effective treatment for this disease. A recent study [23] proposed a new direction of a targeted therapeutic application. The results of this investigation showed that the mutation of the *SPG4* gene was not only associated with haploinsufficiency causing decreased spastin function, but also could be one of the important pathogenic factors of spastin function dysregulation. The phosphorylation of S268 mediated by HIPK2 may contribute to the stability of spastin protein and rescue HSP neurite defects.

CONCLUSION

In conclusion, a newly pathogenic mutation was proposed, expanding the existing knowledge of the spectrum of mutations of *SPG4* gene. The findings may provide a reliable basis for further research on the genetic etiology and pathogenesis of HSP. The diagnosis and typing of HSP through genetic analysis can not only control and treat the disease, but also avoid the transmission of pathogenic genes in the family.

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