A novel missense mutation in *SPAST* causes hereditary spastic paraplegia in male members of a family: A case report

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Abstract. Hereditary spastic paraplegia (HSP) comprises a group of hereditary and neurodegenerative diseases that are characterized by axonal degeneration or demyelination of bilateral corticospinal tracts in the spinal cord; affected patients exhibit progressive spasticity and weakness in the lower limbs. The most common manifestation of HSP is spastic paraplegia type 4 (SPG4), which is caused by mutations in the spastin (*SPAST*) gene. The present study reports the clinical characteristics of affected individuals and sequencing analysis of a mutation that caused SPG4 in a family. All affected family members exhibited spasticity and weakness of the lower limbs and, notably, only male members of the family were affected. Whole-exome sequencing revealed that all affected individuals had a novel c.1785C>A (p. Ser595Arg) missense mutation in *SPAST*. Bioinformatics analysis revealed changes in both secondary and tertiary structures of the mutated protein. The novel missense mutation in *SPAST* supported the diagnosis of SPG4 in this family and expands the spectrum of pathogenic mutations that cause SPG4. Analysis of *SPAST* sequences revealed that most pathogenic mutations occurred in the AAA domain of the protein, which may have a close relationship with SPG4 pathogenesis.

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Introduction

Hereditary spastic paraplegia (HSP) is a group of clinically and genetically heterogeneous neurodegenerative diseases with a global incidence of 4.3-9.8 per 100,000 individuals (1). Four inheritance patterns have been identified: i) Autosomal-dominant (AD), ii) autosomal-recessive, iii) X-linked recessive and iv) mitochondrial; additionally, *de novo* mutations have been found in a number of patients with HSP (1). Thus far, >70 related pathogenic genes have been identified in patients with HSP (1). According to the clinical phenotype, HSP can be divided into pure and complex forms. The pure form is characterized by progressive spasticity and weakness of the lower limbs, with occasional sensory impairment or bladder dysfunction; the complex form includes the pure phenotype with additional abnormalities, including mental and cognitive changes, optic atrophy, muscular atrophy, ataxia, deafness, ichthyosis and/or peripheral neuropathy (2).

Spastic paraplegia type 4 (SPG4) is the most common subtype of AD-SPG; it is present in ~40% of patients with AD-SPG. SPG4 is caused by mutations in the spastin (*SPAST*) gene and usually manifests as pure HSP (3). Mutations in *SPAST* are the most common causes of familial and sporadic HSP. In China, the frequency of *SPAST* mutations is 40% in patients with familial HSP and 33.33% in patients with sporadic HSP (2). The *SPAST* gene is located on chromosome 2p22.3 and contains 17 exons; it encodes the Spastin protein, a member of the ATPase associated with diverse cellular activities (AAA) protein family. Spastin comprises four functional domains: i) Hydrophobic domain (HD; amino acid residues 1-86); ii) microtubule interacting and trafficking domain (MIT; amino acid residues 116-194), which is involved in cytokinesis and endosomal-tubule recycling (4); iii) microtubule-binding domain (MTBD; amino acid residues 270-328), which enables Spastin to bind to microtubules; and iv) AAA domain (amino acid residues 342-599), which is required for Spastin hexamerization and microtubule-severing activity (5). There are two translation initiation codons in *SPAST*, which encode the full-length isoform M1 (expressed in the spinal cord) and the slightly shorter isoform M87 (expressed in the spinal cord and cerebral cortex) (5). HD is present only in

the M1 Spastin isoform; MIT, MTBD and AAA are present in both Spastin isoforms (4).

Spastin is an ATPase that severs microtubules. During the severing process, the six Spastin subunits assemble into a ring-shaped hexamer that is attached to the microtubule and energy from adenosine triphosphate (ATP) hydrolysis is used to sever the microtubule by pulling the negatively charged C-terminus of tubulin through the central pore of the hexamer (4). In neurons, this process can convert long microtubules into short microtubules that move rapidly and harmoniously within axons, thereby enabling efficient microtubule transport that is crucial to axonal growth and axon branch formation; microtubule transport also maintains neurite complexity (5). Mutations in *SPAST* are presumed to cause partial loss of Spastin microtubule-severing activity or the production of a neurotoxic mutant protein; these changes contribute to the onset of SPG4 (5). Additionally, SPG4 penetrance may be influenced by biological sex and age (6).

In the present study, the clinical characteristics of affected individuals and sequencing analysis of a mutation that caused SPG4 only among male members of a family are reported.

Materials and methods

Clinical characteristics. The family was recruited in November 2021 from the Affiliated Hospital of Jining Medical University (Jining, China). Familial history and clinical data of the patients were collected. All patients provided written informed consent to participate in the study.

Whole-exome sequencing (WES). Genetic screening was performed to determine the genetic etiology of the disease. Peripheral blood (5 ml) was collected from the proband's father (II-4) and a portion (2 ml) was sent to Beijing Kangxu Medical Laboratory Co., Ltd. for WES. A total of 2 ml of peripheral blood were collected from the proband (III-6), his uncle (II-3), third aunt (II-6), brother (III-5) and sister (III-7). The proband's other two aunts (II-1 and II-2) declined to be tested Genomic DNA was extracted from blood samples (2 ml) using a FlexiGene DNA Kit (Qiagen). The degree of DNA degradation, the presence of RNA, and protein contamination were analyzed by agarose gel electrophoresis (data not shown). The DNA concentration was accurately quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Inc.). Genomic DNA was fragmented using a Covaris bath sonicator (duty cycle, 10%; intensity, 5; cycles per burst, 200; 3 min for 25˚C) into 180-280 bp fragments to construct a DNA library. Adaptors from *Trans*NGS® Index Primers (384) Kit for Illumina® (TransGen Biotech Co., Ltd.), were ligated to both ends of each DNA fragment, and cohesive ends of the DNA fragments were trimmed. Next, the DNA library was amplified by polymerase chain reaction under the following thermocycling conditions according to the *Trans*NGS® Index Primers (384) Kit manufacturer's protocol: Initial denaturation at 98˚C for 3 min; followed by 5 cycles of 30 sec at 98˚C, 35 sec at 60˚C and 30 sec at 72˚C; with a final extension at 72˚C for 3 min. The following adaptor-specific primers were used to amplify DNA library: Forward, 5'-AATGATACGGCGACCACCGAGATCTAC ACTAGCTGCCACACTCTTTCCCTACACGACCTCTTC CGATC-s-T-3' and reverse, 5'-CAAGCAGAAGACGGC ATACGAGATTCCGCGAAGTGACTGGAGTTCAGA CGTGTGCTCTTCCGATC-s-T-3'; where -s- represents a phosphorothioate bond. The PCR products were then purified using *MagicPure*® Size Selection DNA Beads (TransGen Biotech Co., Ltd.) according to the manufacturer's protocol. The subsequent DNA fragments were hybridized in liquid phase using up to 500,000 biotin-labeled Agilent SureSelect Human All Exon V6 probes (Agilent Technologies, Inc.), which were then captured using streptomycin magnetic beads and amplified using the SureSelect Target Enrichment System (Agilent Technologies, Inc.) under the following thermocycling conditions: Initial denaturation at 98˚C for 2 min; followed by 15 cycles of 30 sec at 98˚C, 30 sec at 62˚C and 1 min at 72˚C; with a final extension at 72˚C for 10 min. The following adaptor-specific primers were used to amplify: Forward, 5'-AATGATACGGCGACCACCGA-3' and reverse primer, 5'-CAAGCAGAAGACGGCATACGA-3'. The products were subsequently purified using *MagicPure*® Size Selection DNA Beads as aforementioned, and the Qubit 2.0 fluorometer and an Agilent Technologies 2200 TapeStation qPCR (7500 Fast Dx Real-Time PCR Instrument, Thermo Fisher Scientific, Inc.) were used to accurately quantify the effective concentration (3 nM) of the library, which was then subjected to single-read sequencing using a NextSeq500 (Illumina, Inc.).

Sequencing reads were aligned to the human reference genome (version hg19) using Burrows-Wheeler Alignment (version 0.7.15). Quality control was conducted by analyzing global alignment depth, interval alignment depth, interval coverage, site alignment quality and other indicators, such as data volume and duplication rate. The alignment results were translated into BAM format; GATK UnifiedGenotyper (v3.6) (https://www.broadinstitute.org) was used to detect single-nucleotide variants and small insertion or deletion variants. Possible copy number variations were analyzed using CODEX (v1.14.1), XHMM (v1.0) and KSCNV (developed by Beijing Kangxu Medical Laboratory Co., Ltd.) (7,8). ANNOVAR (v2016-02-01) was used to annotate the locations of variants in genes and transcripts. Subsequently, gene-related annotations were conducted, involving databases such as RefSeq (version of the reference genome, GRCH37/Hg19; https://www.ncbi.nlm.nih.gov/refseq), Ensembl (April 2021 update; https://www.ensembl.org/index.html) and UCSC (version of the reference genome, GRCH37/Hg19; https://genome.ucsc.edu). The frequencies of annotated variants in the population were investigated using 1000G (2015 update; http://www.1000genomes.org), dbSNP (v150; https://www.ncbi.nlm.nih.gov/SNP), ESP6500 (2014 update) (https://evs.gs.washington.edu/EVS) and ExAC (v0.3; ExAC is now in gnomAD; (www.gnomad-sg.org) databases. SIFT (version 2; https://sift.bii.a-star.edu.sg), PolyPhen2 (version 2; http://genetics.bwh.harvard.edu/pph2) and MutationTaster (NCBI 37/Ensembl 69; http://www.mutationtaster.org) were used to investigate the impacts of mutations on Spastin protein function (9,10). Disease-related annotations were performed using Online Mendelian Inheritance in Man, The Human Gene Mutation Database and ClinVar databases. Next, annotation results were filtered according to mutation location, mutation type, mutation frequency and mutation site characteristics to retain mutations potentially associated with disease.

MutationTaster were used to assess sequence conservation across species. Variants (pathogenic, likely pathogenic, benign, likely benign and variants of uncertain significance) were classified according to the American College of Medical Genetics and Genomics (ACMG) Variation Interpretation guidelines (11). Finally, clinical analyses, such as clinical symptom matching and genetic pattern matching, were conducted for each sample according to ACMG (11), family history and protein damage prediction of gene locus according to the results of SIFT, Polyphen2 and MutationTaster and the clinical presentation.

Sanger sequencing. Peripheral blood (2 ml) was collected from the proband (III-6), proband's father (II-4), his uncle (II-3), third aunt (II-6), brother (III-5) and sister (III-7). The proband's other two aunts (II-1 and II-2) declined to be tested. The blood samples were sent to Beijing Kangxu Medical Laboratory Co., Ltd. for Sanger sequencing. Genomic DNA was extracted from the blood samples using a FlexiGene DNA Kit (Qiagen). According to the results of WES of the proband's father, the c.1785C>A mutation in S*PAST* gene was selected for further validation. Primers were designed using the website Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast) (12) and synthesized by Tianyi Huiyuan Biotech Co., Ltd (101.43.170.175/tyhy). The primer sequences for *SPAST* were designed using the gene sequence from GenBank (accession no. NM_014946), as are as follows: Forward, 5'-TCCATCATTTCGTTAACCACCA-3'; reverse, 5'-GCCGATGACGTTCATTGAAGA-3'. The c.1785C>A mutation in *SPAST* was amplified by PCR using a EasyTaq PCR SuperMix (Beijing TransGen Biotech Co., Ltd.), under the following thermocycling conditions: Initial denaturation at 95˚C for 10 min; followed by 35 cycles of 30 sec at 95˚C, 30 sec at 60˚C and 45 sec at 72˚C; with a final extension at 72˚C for 5 min. The amplicons were then sequenced by Sanger sequencing using an ABI 3730xl DNA analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). The resulting sequences were aligned with the results of WES, and false-positive sites obtained by next-generation sequencing were excluded.

Whole-exome sequences accession numbers in ClinVar. Whole-exome sequences of the family members in this study have been deposited in ClinVar under the following accession number: SCV002761959.

Bioinformatics analysis. Bioinformatics analysis was used to predict the possible effects of gene mutations on relevant mRNAs and proteins. The RNAfold web server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) was used to predict the effects of new mutations on mRNA structure. PSIPRED (V4.0) (http://bioinf.cs.ucl.ac.uk/psipred) and RaptorX (http://raptorx.uchicago.edu) were used to predict the secondary and tertiary structures of mutated and wild-type Spastin proteins, respectively.

Summary of various pathogenic mutations. ClinVar and gnomAD databases were searched for pathogenic variant data regarding the *SPAST* gene, using the search term 'SPAST'. We did not include mutations that were considered 'benign' or 'likely pathogenic.'

Results

The proband was a 30-year-old male who was admitted in November 2021 to The Affiliated Hospital of Jining Medical University with a >10-year history of unstable gait; the patient first began to experience difficulty walking because of tremors in the lower limbs. Subsequently, weakness and tremors in the lower limbs during walking gradually worsened, and the patient could not bend his knees; however, he could stand and walk slowly unaided at the time of admission. His urinary and bowel functions were normal. Neurological examination revealed increased muscle tone in both lower limbs, muscle strength grade 3-4 (normal muscle strength is grade 5), tendon hyperreflexia and bilateral Babinski sign positive; lower limb sensation was normal. Examination of cranial nerves and upper limbs revealed no abnormalities. General physical examination, routine laboratory tests and magnetic resonance imaging of the brain, cervical spine and thoracic spine did not reveal any obvious pathognomonic alterations (Fig. 1). The patient had been born through full-term vaginal delivery, and he had exhibited good health from birth until the onset of the present symptoms.

The proband's deceased paternal grandfather had exhibited difficulty walking (Fig. 2); his age at death was not provided by the family. The proband's 66-year-old uncle (II-3) had begun to experience difficulty walking at the age of 36 and he began walking with crutches at the age of 56 (Video SI). The proband's 61-year-old father (II-4) had begun to experience difficulty walking at the age of 41 and is currently confined to a wheelchair. The proband's youngest uncle (II-5) died of diabetes at the age of 54; he could not walk before death, although his age at the onset of walking difficulty was unknown. The proband's 33-year-old brother (III-5) exhibited muscle weakness at the age of 15 and has been confined to a wheelchair since the age of 29. The urinary and bowel functions of these living relatives were normal. Moreover, neurological examinations of these relatives revealed increased muscle tone, reduced muscle strength, tendon hyperreflexia, positive bilateral Babinski sign and sensory deficits in both lower limbs. Muscle strength was normal in the upper limbs; examinations of the cranial nerves and upper limbs revealed no abnormalities. General physical examinations of these relatives also did not reveal any abnormalities. The proband's 72-year-old aunt (II-1), 70-year-old aunt (II-2), 51-year-old aunt (II-6) and 27-year-old sister (III-7) did not exhibit signs of difficulty walking or muscle weakness; general physical and neurological examinations revealed no abnormalities in these relatives. Because no equipment for MRI was available at the local hospital, magnetic resonance imaging scans of family members other than the proband could not be obtained. Patient information and clinical features of affected individuals in this family are summarized in Table I.

WES identified the missense mutation in the *SPAST* gene: c.1785C>A as a likely pathogenic variant according to ACMG guidelines (11). Sanger sequencing analysis of the proband, his father (II-4), uncle (II-3), third aunt (II-6), brother (III-5) and sister (III-7) revealed that all tested male family members carried the same heterozygous missense mutation in the *SPAST* gene: c.1785C>A in exon 17; genomic coordinates, Chr2:32379499 (Fig. 3). This mutation resulted in a serine to arginine substitution at position 595 (Ser595Arg) in the AAA

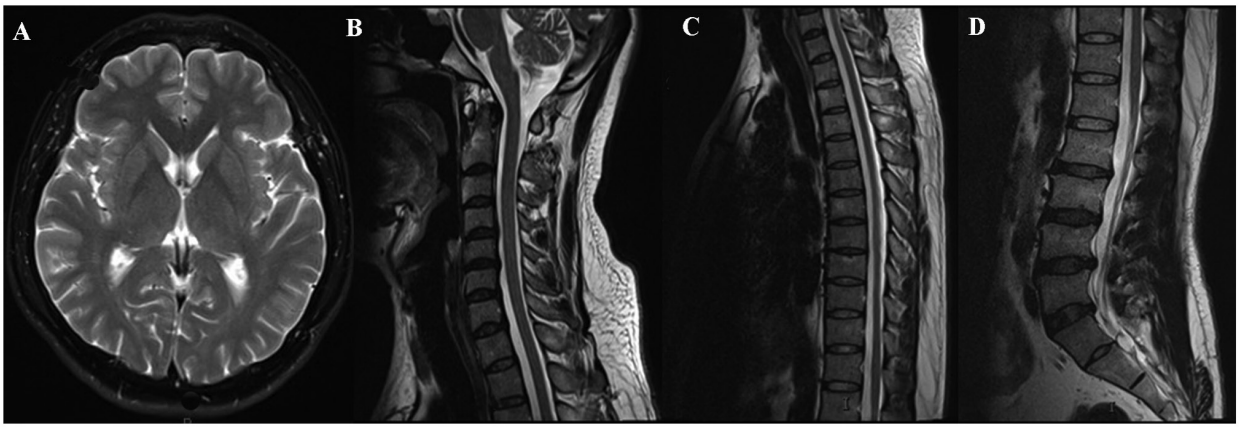

Figure 1. Magnetic resonance imaging of the proband's (A) brain, (B) cervical spine, (C) thoracic spine and (D) lumbar spine did not reveal any obvious pathognomonic alterations.

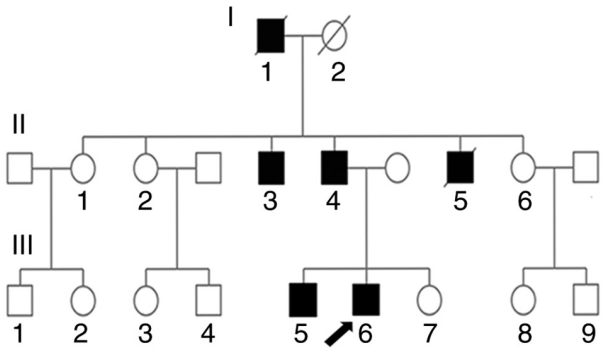

Figure 2. SPG4 family pedigree of 17 members. I-1, grandfather, deceased, exhibited difficulty walking. I-2, grandmother, deceased, who did not exhibit muscle weakness. II-1, oldest aunt who has no muscle weakness. II-2, second aunt who has no muscle weakness. II-3, oldest uncle, 66 years old, who has SPG4. II-4, father, 61 years old, who has SPG4. II-5, youngest uncle, deceased, who had SPG4. II-6, third aunt who has no muscle weakness. III-1 and III-2, children of older aunt who have no muscle weakness. III-3 and III-4, children of second aunt who have no muscle weakness. III-5, older brother, 33 years old, who has SPG4. III-6, proband (arrow), 30 years old, who has SPG4. III-7, sister who has no muscle weakness. III-8 and III-9, third aunt's children who have no muscle weakness. /, deceased family member; SPG4, spastic paraplegia type 4.

ATPase cassette. However, the missense mutation was not present in the proband's aunt and sister.

According to American College of Medical Genetics and Genomics criteria, this variant was considered likely pathogenic: PM1 (located in mutation hotspots and/or in critical functional areas where no benign variation is known), PM2 (no variation or very low frequency in recessive mode was found in normal control populations), PP3 (variants that would have a deleterious effect on the function of the gene or gene product) and PP4 (the clinical phenotype or family history of the mutation carrier is highly consistent with the characteristics of a certain monogenic genetic disease). SIFT and PolyPhen2 analyses indicated that the Ser595Arg mutation is likely to be 'deleterious' and 'probably damaging,' respectively. MutationTaster software identified the Ser595Arg mutation as a 'disease-causing' mutation.

The RNAfold web server was used to predict the effects of the *SPAST* mutation on mRNA structure; the results revealed that it could lead to changes in mRNA structure, as shown by the increase of hairpin structure at 1564-1904 ribonucleic acid site (Fig. 4A and B). PSIPRED (V4.0) was used to predict and compare the mutant and wild-type Spastin secondary protein structure; the c.1785C>A missense mutation was predicted to cause changes in Spastin amino acid polarity, from neutral to positively charged (Fig. 4C and D), and in the secondary structure, which was manifested as the strand at amino acids 252-253 changed to coil, the helix shortened at amino acids 331-337, the helix shortened at amino acids 436-441, and the helix lengthened at amino acids 443-447 (Fig. 4E and F). RaptorX was used to predict the mutated Spastin tertiary structure, which revealed the spatial structure of the HD, MIT and MTBD domains was reversed compared with wild-type Spastin (Fig. 4G and H). Protein sequence alignment revealed that the Spastin amino acid sequence is highly conserved across species at amino acid 595 (red frame in Fig. 4I). Therefore, we hypothesized that the mutation causes a change in tertiary structure that affects protein function.

A summary of all mutations occurring in the four domains of spastin and the various pathogenic mutations (according to the clinical significance of mutations shown in the ClinVar and gnomAD databases) affecting the four domains of Spastin is provided in Table II. Among these pathogenic mutations, frameshift mutations were most common, followed by missense mutations, nonsense mutations were least common. Approximately 84% (78/93) of the frameshift mutations were pathogenic; most occurred in the AAA domain (47/78), followed by the MIT domain (13/78) and MTBD (13/78), and then the HD (5/78). Among all missense mutations, ~30% (65/208) were considered pathogenic; most missense mutations occurred in the AAA domain (63/65), and two occurred in the MTBD, whereas no pathogenic missense mutations occurred in the MIT domain or the HD. Similar to frameshift mutations, ~65% (32/49) nonsense mutations were pathogenic, and most occurred in the AAA domain.

Pathogenic mutations in exons that encode AAA functional structure and splicing mutations in introns that may affect AAA were identified as shown in Table III. Further analysis revealed that point mutations (specifically missense mutations) were most common overall (Table III). Base deletions were also common. Analysis of exons 7-17 encoding the AAA

Table I. Clinicopathological features of affected individuals within the family.

Individual ID	I-1	II-3	II-4	II-5	III-5	III-6
Sex	M	M	M	M	M	M
Age at onset, years	---	36	41	---	15	20
Age at examination, years	---	66	61	---	33	30
Disease duration, years	---	30	20	---	18	>10
Lower limb hyperreflexia	---	+	+	---	+	+
Lower limb spasticity	---	+	+	---	+	+
Lower limb muscle strength[a]	---	2-3	1-2	---	1-2	3-4
Reflex	---	+	+	---	+	+
Babinski sign	---	+	+	---	+	+
Upper limb hyperreflexia	---	–	–	---	–	–
Upper limb spasticity	---	–	–	---	–	–
SPATAX-EUROSPA[b] disability score	6	5	6	---	6	3
Neurogenic bladder dysfunction	–	–	–	–	–	–
Sensory deficits	---	+	+	---	+	–
Mental retardation	---	–	–	---	–	–
Concomitant diseases	---	–	–	Diabetes	–	–

[a]Muscle strength score: 0, no contraction of the muscle; 1, muscle contraction, but no joint movement; 2, the limb can move on a flat surface, but cannot complete the movement against gravity; 3, the limb can complete the movement against the gravity, but cannot overcome the external resistance; 4, able to complete exercise and overcome resistance, but not as strong as normal limbs; 5, normal muscle strength. [b]SPATAX-EUROSPA disability score: 0, no disability; 1, no abnormal gait, but positive signs; 2, mild abnormal gait, can still run, walking is not limited; 3, moderate abnormal gait, hindering running, can walk independently; 4, severe gait abnormalities, walking requires a single walker; 5, severe gait abnormality requiring a bilateral walker for walking; 6, unable to walk and wheelchair-bound; 7, severe disability/confined to bed. +, presence of a feature; –, absence of a feature; ---, unknown.

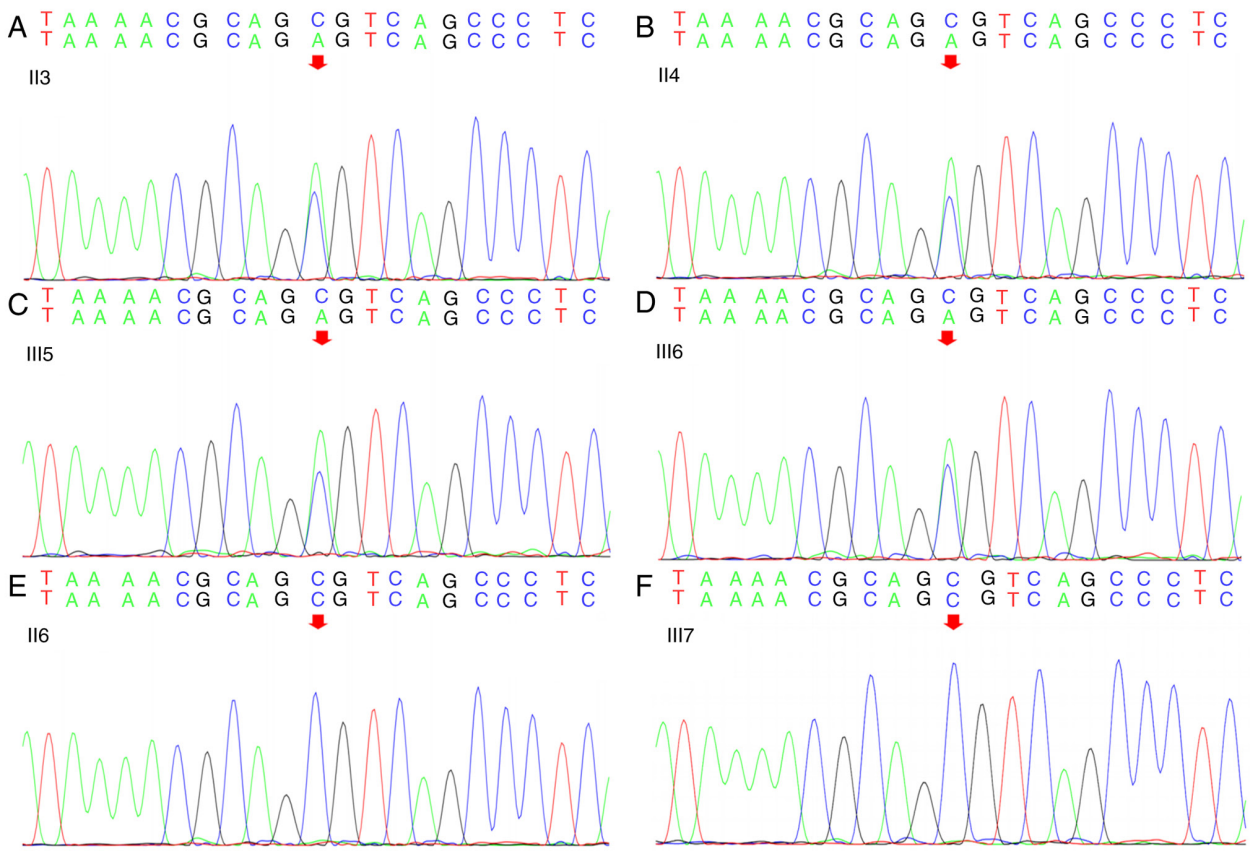

Figure 3. Sanger sequencing results. The (A) oldest uncle (II-3), (B) father (II-4), (C) brother (III-5) and (D) proband (III-6) all carried the c.1785C>A missense mutations in the *SPAST* gene. The (E) third aunt (II-6) and (F) and sister (III-7) carried a wild-type *SPAST*. *SPAST*, spastin.

Table II. Summary of the numbers of mutations affecting the Spastin domains.

		Pathogenic[a]				
Gene mutation	Total	HD	MIT	MTBD	AAA	Total
Frameshift	93	5	13	13	47	78
Missense	208	0	0	2	63	65
Nonsense	49	7	11	6	18	32

[a]According to ClinVar and gnomAD databases. AAA, ATPase associated with diverse cellular activities; HD, hydrophobic domain; MIT, microtubule interacting and trafficking domain; MTBD, microtubule-binding domain.

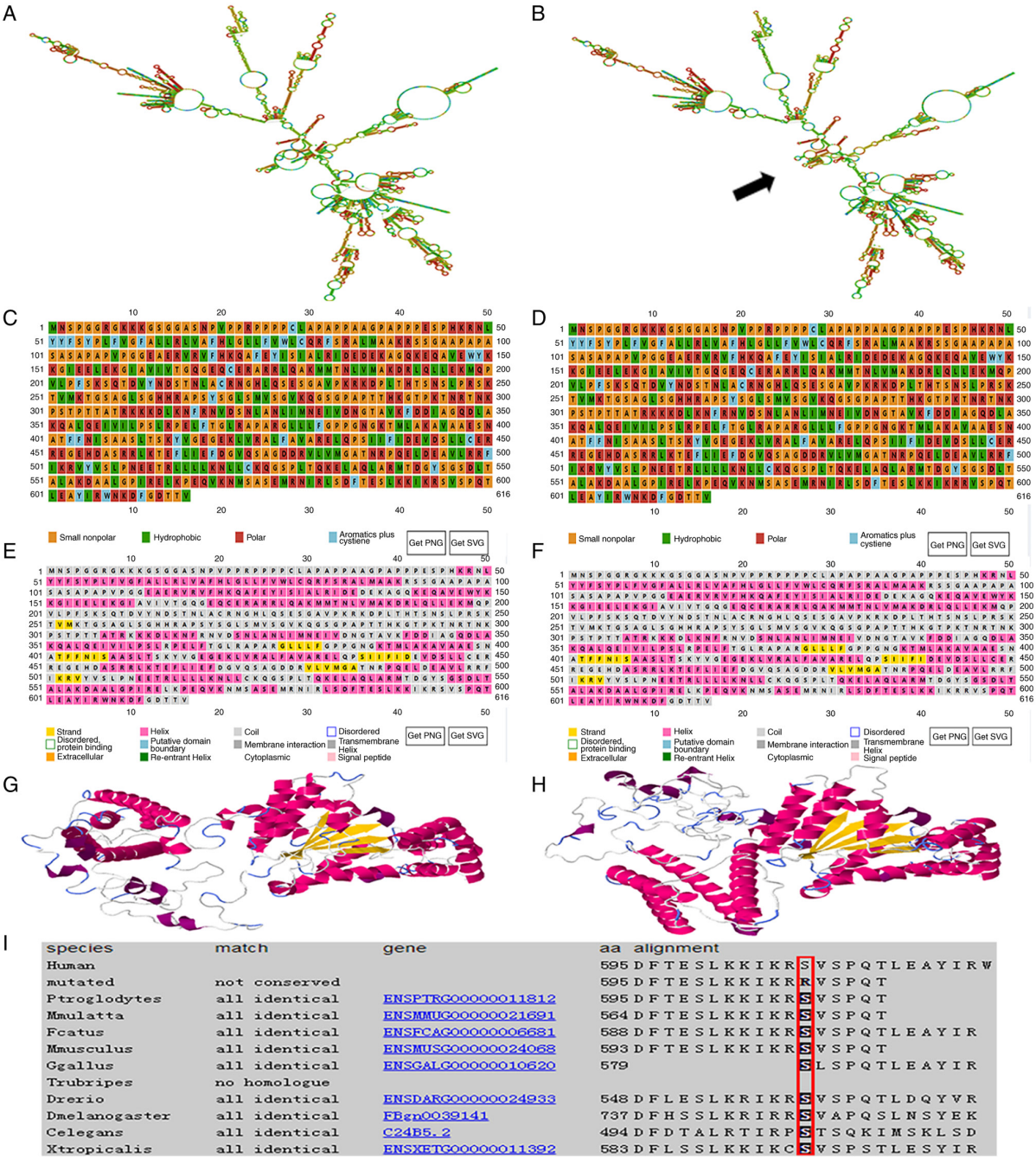

Figure 4. mRNA and protein structural predictions of the Spastin mutation. Structural predictions of (A) wild-type and (B) c.1785C>A missense mutant mRNA. Polarity predictions of (C) wild-type and (D) p.Ser595Arg Spastin amino acids. Secondary protein structure predictions of (E) wild-type and (F) p.Ser595Arg proteins. Tertiary protein structure predictions of (G) wild-type and (H) p.Ser595Arg proteins. (I) Evolutionary conservation of Ser595 among species (red box).

Table III. Summary of all pathogenic mutations affecting the AAA domain.

Region and type of mutation			Affected exon region
Intron (splice mutations)			Exon8:c.1098+1G>A;c.1098+2T>A;c.1099-4371_1245+1010del;c.1099-1062_1246-1342del;c.1099-956_1246-1672del and c.1099-1G>A Exon9: c.1173+1G>A; c.1173+185_1245del; c.1174-270_1246-1724dup; c.1174-2A>T; c.1174-1G>C and c.1174-1G>A Exon10: c.1245+1G>A; c.1246-2897_1493+523dup and c.1246-2896_1493+523dup Exon11: c.1321+1G>A; c.1321+2T>G; c.1322-30_1322-2del; c.1322-2A>C; c.1322-2A>G; c.1322-1G>T and c.1322-1G>A Exon12: c.1413+1_1413+2del; c.1413+3_1413+6del; c.1413+2dup; c.1413+1G>T; c.1414-2A>T and c.1414-1G>C Exon13: c.1493+2_1493+5del; c.1493+1G>T; c.1493+1G>A; c.1493+2T>A; c.1493+2T>C; c.1494-1393_1688-466dup; c.1494-2A>G; c.1494-2A>C and c.1494-1G>A Exon14: c.1536+1G>T and c.1537-1G>A Exon15: c.1617-15_1624dup; c.1617-2A>G and c.1617-1G>A Exon16: c.1688-378_1728+1541del; c.1688-2A>G; c.1688-1G>C and c.1688-1G>A Exon17: c.1728+1G>T; c.1728+1G>A; c.1728+1G>C; c.1728+2T>G; c.1729-3331_*1641del; c.1729-884_*1715del; c.1729-1G>C and c.1729-1G>A
Exon	Single nucleotide	Missense	Exon7: c.1031T>A; c.1047G>C; c.1066G>A; c.1067A>G; c.1082C>T; c.1085C>T and c.1085C>G c.1103T>C; c.1104C>G; c.1105A>C; c.1112T>G; c.1121C>T and c.1133T>A Exon8: c.1148C>G; c.1151C>T; c.1157A>G; c.1164G>T; c.1165A>G; c.1168A>G and c.1169T>A Exon9: c.1181C>A; c.1196C>T; c.1216A>G; c.1219A>C; c.1225G>A; c.1226C>T and c.1238C>T Exon10: c.1250G>T; c.1250G>A; c.1253A>G; c.1266G>C; c.1271G>C; c.1276C>G; c.1306T>C; c.1307C>A and c.1307C>T Exon11: c.1322A>G; c.1331A>G; c.1335C>A; c.1343G>A; c.1360G>A; c.1375A>G; c.1378C>T; c.1379G>A; c.1382T>C; c.1384A>C; c.1396C>G; c.1405T>G and c.1409A Exon12: c.1462A>G; c.1472A>G and c.1484C>T Exon13: c.1507C>T Exon14: c.1540A>G; c.1550T>C and c.1601T>C Exon15: c.1637G>A; c.1667C>T; c.1684C>G and c.1685G>A Exon16: c.1715T>C Exon17: c.1742G>C; c.1775T>A and c.1785C>A
		Nonsense	Exon7: c.1039C>T; c.1054C>T and c.1063C>T Exon9: c.1238C>A Exon10: c.1252G>T and c.1291C>T Exon11: c.1344T>A and c.1390G>T Exon12: c.1417C>T and c.1468C>T Exon14: c.1573C>T; c.1591C>T and c.1606C>T Exon15: c.1634C>G and c.1684C>T Exon17: c.1741C>T; c.1774del and c.1245del

Table III. Continued.

Region and type of mutation	Affected exon region
Deletion	Exon7: c.1053del; c.1056del; c.1069del and c.1091_1092del
	Exon8: c.1118del and c.1167_1168del
	Exon9: c.1180del; c.1204del; c.1209_1212del and c.1215_1219del
	Exon10: c.1245del; c.1253_1255del; c.1262_1263del and c.1263del
	Exon11: c.1339_1340del; c.1350_1351del; c.1375del; c.1392del; c.1395del and c.1407del
	Exon12: c.1437_1438del; c.1454_1463del and c.1469_1470del
	Exon13: c.1496del; c.1506del and c.1514_1515del
	Exon14: c.1539_1540del; c.1561_1564del; c.1577_1580del; c.1583del and c.1601del
	Exon16: c.1702_1712del
	Exon17: c.1740del; c.1767del; c.1774del and c.1781del
Duplication	Exon7: c.1027_1031dup
	Exon9: c.1224dup
	Exon11: c.1359_1360dup; c.1361dup and c.1368dup
	Exon12: c.1458_1459dup
	Exon14: c.1560_1561insTT and c.1562dup
	Exon17: c.1774dup

Del, deletion; dup, duplication.

domain did not reveal obvious differences in the distribution of pathogenic mutation types among exons or differences in the locations of splice site mutations.

Discussion

All affected family members in this report were <40 years old at the time of onset and presented with clinical manifestations of spastic paraplegia, including lower limb spasticity and weakness, increased muscle tone in both lower limbs, varying degrees of reduction in muscle strength, tendon hyperreflexia and bilateral Babinski sign positive and had a family history suggesting autosomal dominant inheritance; furthermore, all affected family members carried the c.1785C>A missense mutation in the *SPAST* gene, met the diagnostic criteria of HSP-SPG4 (13). Although all affected family members showed progressive spasticity and weakness of the lower limbs, they did not exhibit mental, cognitive or other abnormalities that have been associated with complex HSP (2). The clinical manifestations of the patients in the present belong to simple HSP. However, this diagnosis is limited by the lack of MRI results for other family members. There is no cure for the disease, and only symptomatic treatment is available, such as intrathecal baclofen administration, botulinum toxin injections and functional electrical stimulation (13). Medication and surgical treatment were not provided to the proband or his affected family members.

The c.1785C>A missense mutation occurs in the AAA domain, which is involved in Spastin hexamerization, microtubule-severing activity and ATP hydrolysis (4). This mutation leads to changes in mRNA structure, causes a p.Ser595Arg amino acid change in the AAA ATPase cassette that may alter amino acid polarity and modify both the secondary and tertiary structures of the Spastin protein. The mutation is suspected to influence normal protein function, resulting in reduced microtubule-severing activity, which affects axon growth and development, ultimately leading to disease. Additionally, some missense mutations in the M1 isoform can reduce the activity of wild-type Spastin in a dominant-negative manner, thereby exacerbating the disease (4). The results of recent studies have suggested that the neurotoxic effects of mutant Spastin (mainly the M1 isoform) contribute to disease onset (14-16). Therefore, the neurotoxicity of this mutant protein is presumed to serve a role in the pathogenesis experienced by the proband's affected family members.

SPG4 is an autosomal dominant disease (1); in the present study, it was noted that all male members of this family carried the pathogenic gene and exhibited varying degrees of disease presentation. Among the female family members who provided samples for sequencing, none carried the *SPAST* mutation. There is a need to investigate whether the high heritability of the pathogenic gene among men in this family is a chance event. Moreover, the family members differed in terms of age at onset, disease progression and symptom severity, indicating substantial intrafamilial variability.

The present study determined that the proportion of pathogenic mutations was greatest in the AAA domain, probably because the long sequence of nucleotides encoding the AAA domain carries a greater risk of mutation. The high rates of pathogenicity for frameshift and nonsense mutations may be related to the early termination of translation caused by frameshift and nonsense mutations, which seriously affect protein structure and function, thus leading to the onset of disease. Although missense mutations occur most frequently, pathogenic missense mutations comprise ~30% of all missense mutations. These findings suggested that the reduced microtubule-severing activity or loss of AAA domain function is a critical component of SPG4 pathogenesis. No significant difference was identified in the distribution of pathogenic mutation types among exons or differences in the locations of splice site mutations. However, few pathogenic mutations occurred in exon 16, although this finding requires confirmation in future studies.

Approximately 75% of HSP-SPAST cases are inherited, and the remaining 25% of cases involve *de novo* mutations; patients with SPG4 mainly receive symptomatic treatment because no cure is available (17). Therefore, genetic counseling is essential for affected families. When progressive walking difficulties, lower limb spasticity and other symptoms, such as tendon hyperreflexia, occur in patients without a family history, HSP should also be suspected and genetic screening should be conducted. In the present report, the clinical characteristics and sequencing analysis results of a family with SPG4 were reported. The identification of a novel *SPAST* mutation expands the spectrum of pathogenic mutations that cause SPG4; it also provides information for use in genetic counseling. Furthermore, specific pathogenic mutations in the *SPAST* gene were analyzed and the findings suggested that the loss of AAA domain function is a crucial component of SPG4 pathogenesis.

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Availability of data and materials

The datasets generated and analyzed in this study are available in the ClinVar (accession no. SCV002761959; https://www.ncbi.nlm.nih.gov/clinvar). Other datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

Authors' contributions

XCW, RHL and QXK designed the study. YLW, DDC, YJ, XYW and TSH collected the data. XCW, RHL and TW contributed to data analysis and interpretation. XCW and RHL drafted the manuscript; QXK and RHL contributed to

the revision. XCW and RHL confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by The Ethics Committee of The Affiliated Hospital of Jining Medical University (Jining, China). The proband, his father (II-4), his uncle (II-3), third aunt (II-6), brother (III-5) and sister (III-7) provided written informed consent to participate.

Patient consent for publication

The proband, his father (II-4), his uncle (II-3), third aunt (II-6), brother (III-5) and sister (III-7) provided written informed consent for the publication of any associated data, as well as accompanying images and videos. The two medical staff in the Supplementary data video also provided consent for publication.

Competing interests

The authors declare that they have no competing interests.

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