

A novel SPAST gene mutation identified in a Chinese family with hereditary spastic paraplegia

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Research article

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Abstract

Background: Hereditary spastic paraplegia is a heterogeneous group of clinically and genetically neurodegenerative diseases characterized by progressive gait disorder. Hereditary spastic paraplegia can be inherited in various ways, and all modes of inheritance are associated with multiple genes or loci. At present, more than 76 disease-causing loci have been identified in hereditary spastic paraplegia patients. Here, we report a novel mutation in *SPAST* gene associated with hereditary spastic paraplegia in a Chinese family, further enriching the hereditary spastic paraplegia spectrum.

Methods: Whole genomic DNA was extracted from peripheral blood of the 15 subjects from a Chinese family using DNA Isolation Kit. The Whole Exome Sequencing of the proband was analyzed and the result was identified in the rest individuals. RaptorX prediction tool and Protein Variation Effect Analyzer were used to predict the effects of the mutation on protein tertiary structure and function.

Results: Spastic paraplegia has been inherited across at least four generations in this family, during which only four HSP patients were alive. The results obtained by analyzing the Whole Exome Sequencing of the proband exhibited a novel disease-associated in-frame deletion in the *SPAST* gene, and the this mutation also existed in the rest three HSP patients in this family. This in-frame deletion consists of three nucleotides deletion (c.1710_1712delGAA) within the exon 16, resulting in lysine deficiency at the position 570 of the protein (p.K570del). This novel mutation was also predicted to result in the synthesis of misfolded *SPAST* protein and have the deleterious effect on the function of *SPAST* protein.

Conclusion: In this case, we reported a novel mutation in the known *SPAST* gene that segregated with HSP disease, which can be inherited in each generation. Simultaneously, this novel discovery significantly enriches the mutation spectrum, which provides an opportunity for further investigation of genetic pathogenesis of HSP.

Background

Hereditary spastic paraplegia (HSP), also called familial spastic paraparesis or Strümpell-Lorrain disease, is a group of neurodegenerative and inherited heterogeneous neurological disorders characterized by a length-dependent distal axonal degeneration of the corticospinal tracts[1]. The progressive spasticity and pyramidal signs of the lower limbs are the prominent features of HSP, which can be well explained by the fact that the innervated function of the longest fibers toward to the lower extremity is prone to be affected[2].

According to whether accompanied by additional neurological or psychiatric symptoms such as ataxia, mental and cognitive changes, extrapyramidal signs, visual dysfunction or epilepsy, or with extra neurological signs, the diseases can be categorized into either pure HSP (pHSP) or complicated HSP (cHSP)[3, 4]. The onset age of HSP exhibits a wide range from childhood to over 70 years old depending on the underlying genetic defect, even in the family with a same mutation[5]. Therefore, it is hard to explain the interaction between the genotype and the phenotype of HSP.

Based on the distinguishably inherited trait, HSP can be classified into as autosomal dominant, autosomal recessive, X-linked, mitochondrial, or de novo[6]. Meanwhile, autosomal dominant HSP is the most common mode that accounts for approximately 70% of all HSP patients[7]. All modes of inheritance are associated with multiple genes or loci. Until now, there have been at least 76 spastic paraplegia associated with loci and more than 59 corresponding spastic paraplegia genes (SPG) have been identified[8]. The *SPG4/ SPAST* gene comprising 17 exons, identified as the 90-kb genomic region on chromosome 2 (2p22.3)[9], has been reported to be the most frequent cause of HSP and accounts for approximately 40% of pure autosomal dominant HSP and 10% of sporadic cases[10, 11]. Over 500 mutations have been identified in the *SPAST* gene. Generally, *SPAST* gene mutations have a tendency to cause pure HSP[12] and are more common in males than females[5].

Here, we report a novel *SPAST* gene mutation site (c.1710_1712delGAA) that presented in a Chinese family with HSP, significantly enriching the mutation spectrum of HSP gene. The study was conducted in accordance with the Declaration of Helsinki and the Ethics Committee of Peking University First Hospital. The related written informed consent from all members of the family participating in the study was obtained to publish the case details and any accompanying images used in medical journals.

Methods

Subjects

In this study, we recruited 15 subjects (female: male ratio is about 1:1; age range: 3–63 years) in total from a Chinese family with HSP. This family was enrolled in our study on the basis of the following criteria: (1) Based on Harding's criteria[4], the proband, a 63-years-old female, was diagnosed with HSP; (2) The family of the proband had at least four affected relatives with HSP; (3) The family was potentially informative for designing a study to investigate the genetic mutations. Total of 15 individuals were performed with neurologic examination, four of which had the same clinical manifestations and the rest were all asymptomatic. The study was approved by the Peking University First Hospital Ethics Committee. Written informed consents were obtained from the participants and the legal guardian of the patient aged eleven years in our study.

Dna Extraction

Whole genomic DNA was extracted from peripheral blood of the 15 family members using DNA Isolation Kit (Biotek, AU1802) according to the manufacturer's instructions. Concentrations of each DNA sample were assessed on a Qubit fluorometer (Invitrogen, Q33216) using Qubit dsDNA HS Assay Kit (Invitrogen, Q32851). Simultaneously, 1% agarose gel electrophoresis was performed for quality control of each DNA sample.

Libraries Preparation And Amplification

DNA libraries were prepared with KAPA Library Preparation Kit (Kapa Biosystems, KR0453) following the manufacturer's instructions, which mainly comprises three major steps: end-repair of fragmented DNA, A-tailing and adapter ligation. Purifications between steps were performed using Agencourt AMPure XP beads. After the ligation reaction with beads, 50 µl ligation was thoroughly resuspended in 45 µl PEG/NaCl SPRI® Solution and incubated at 37°C for 2 min. Subsequently, the beads were captured by a magnet and incubated until the liquid is clear. The clear supernatant was removed and discarded and 200 µl 80% ethanol was used to wash the beads for three times and then the beads were dried at room temperature until all the ethanol was sufficiently evaporated. Finally, the beads were thoroughly resuspended in 25 µl water and incubated for 2 min at room temperature, which was captured on a magnet and incubated until the liquid was clear to proceed with library amplification.

Library amplification was accomplished by polymerase chain reaction (PCR) under the following 25 µl reaction system: 2 × KAPA HiFi HotStart ReadyMix 12.5 µl, 5 µM each primer 1 µl, 10 µl captured library beads suspension and 1.5 µl water. PCR amplification program was set up as following: 98°C 2 min; 98°C 30 s; 65°C 30 s; 72°C 30 s, 13 cycles; and a final step at 72°C for 4 min. Subsequently, repeat the steps of washing and resuspending the beads as described above. The amplified libraries that were prepared for array capture were assessed with Qubit dsDNA HS Assay kit (Invitrogen, Q32851).

Array Capture And Sequencing

Hybridization of the capture probes to pooled libraries and removal of non-hybridized library molecules were performed via the Agilent SureSelectXT2 Target Enrichment System. The pooled libraries were mixed with a buffer solution and oligo-blockers (including a blocker of repetitive elements, Human Cot-1 DNA) to capture array hybridization and then the hybridization mixture was incubated for 24 hours at 65 °C. The hybridized library molecules were carried out with Dynabeads® MyOne™ Streptavidin T1 (Invitrogen, #65601), which was thoroughly resuspended in 40 µl water. The captured library was amplified as following: 2 × KAPA HiFi HotStart ReadyMix 21 µL, 5 µM primer 1 µL, 20 µL captured library beads suspension. PCR amplification program was 98°C 2 min; 98°C 30 s; 65°C 30 s; 72°C 30 s, 13 cycles and a final step at 72°C for 4 min. Purifications between steps were carried out with Agencourt AMPure XP beads and the libraries were estimated with Qubit dsDNA HS Assay kit (Invitrogen, Q32851). Finally, DNA libraries of the proband were analyzed by whole exome sequencing (WES). WES was performed on the HiSeq2500 platform as paired-end 200-bp reads. Illumina Sequence Control Software (SCS) was used to evaluate the sequencing data, thus removing adapter sequences in the raw data and discarding low-quality sequencing reads. Conventional Sanger sequencing of the SPAST gene was further performed in 15 individuals from the Chinese family.

In-silico Predictions

Effects of the novel mutation on SPAST tertiary structure were predicted by RaptorX prediction tool[13]. Additionally, PROVEAN (Protein Variation Effect Analyzer)[14], a new algorithm, is also adopted to predict

whether the mutation has an functional impact on the SPAST protein sequence variations.

Results

Clinical characteristics

The proband (II:2) (Fig. 1A), a 63-years-old female, presented to the outpatient clinic in our hospital due to her progressive difficulty walking caused by moderate spasticity of the lower limbs for 24 years. She has felt unknown gait disorder as early as in 1992. For the last 11 years, this gait disorder has gotten worse, especially in cold weather. And she was confined to a wheelchair at the age of 52. In the last year, the proband suffered from the frequently urged to urinating and bowel functions. Physical examination showed that she had brisk deep tendon reflexes in all four limbs, simultaneously accompanied with obvious corticospinal tract signs (Babinski's signs was positive), and decreased sense of pain, light touch and vibration in the lower limbs characterized with stocking pattern-distributed sensory loss. Muscle strength of the upper limbs was normal, while both the extensors and flexors in the lower limbs were 3/5. The results obtained from routine laboratory tests, electromyography, cranial and cervical MRIs did not reveal any obvious pathognomonic alteration. For her previous history, the proband had ever received some treatment on rheumatoid arthritis because of the pain in both hips and knees 26 years ago, but the uncomfortable symptom was not getting better. 9 years ago, a traumatic injury on her back further aggravated her discomfort though the cranial and cervical MRIs were both normal at that time.

With regard to the proband's family history (Fig. 1A), her parents were deceased, but her father had similar symptoms. In addition, she had three brothers and two sisters. One of her brothers who had similar symptoms has passed away and the rest were all asymptomatic. Details about her symptomatic father and brother are not clear. Physical examination gave the below results. ♂:9 aged 32 years had an abnormal spine physiological curvature. The shoulders of ♂:9 aged 11 years were not equal. The (♂:1) aged 46 years, ♂:9 and ♂:9 showed brisk deep tendon reflexes in all four limbs and positive Babinski signs. All the three symptomatic patients all could not run and squat since young age. Despite their motor symptoms, the proband's nephew(♂:1) and the third daughter (♀:9) have frequent urge to urinate and to have bowel functions at the age of 40 years and 27 years.

The proband's the third daughter (♀:9) and grandson(♂:9) suffer from other disease except HSP. The ♀:9 patient who was diagnosed with pulmonary hypertension has been suffering from chest tightness and shortness of breath for three years, losing the ability to work. Similarly, the echocardiography of ♂:9 aged eleven years showed mild reflux at mitral valve and tricuspid valve.

Clinical features of the four affected individuals in the family have been summarized in Table 1, and their clinical commonalities and personalities are exhibited respectively. The four symptomatic patients have the different degrees of disability. The disability score was evaluated according to a four-point scale (1: normal, 2: able to walk but not run, 3: need the help of a walking aid or support, 4: walk on wheelchair)

[15]. In addition, the onset age ranges from 3 to 30 years old, although they have the same mutation in the exon 16.

Table 1: Clinical features of the affected individuals within the family.

Individual ID	II:6	III:1	III:9	IV:9
Sex	F	M	F	M
Age at onset(years) ^a	early 30s	early 10s	early 10s	3
Age at examination(years)	63	46	32	10
Disease duration(years)	>33	>36	>22	7
Disability score ^b	4	2	2	2
Lower limb hyperreflexia	+	+	+	+
Lower limb spasticity	+	+	+	+
Lower limb pyramidal weakness	-	-	-	-
Babinski sign	+	+	+	+
Upper limb hyperreflexia	+	+	+	+
Upper limb spasticity	-	-	-	-
Sphincter disturbances	+	+	+	-
Scoliosis	-	-	+	-
Pes cavus	+	-	-	+
Sensory deficits	+	-	-	-
Mental retardation	-	-	-	-
concomitant diseases	-	-	Pulmonary hypertension	Reflux at mitral and tricuspid valve

+ indicates the presence of a feature, – indicates the absence of a feature, respectively.

a: Age at onset was calculated approximately when appeared to have difficulty in walking first.

b: Disability stages: 1: normal, 2: able to walk alone but not run, 3 : need the help of a walking aid or support, 4 : wheelchair user

Genetic findings and prediction results of protein structure and function by different methods

The Exome Sequencing analysis of the proband exhibited a novel disease-associated mutation in exon 16 of the already known disease-associated *SPAST* gene, and the in-frame deletion was identified in the three affected family members (Fig. 1B II:2, III:1, III:9, IV:9). It is an in-frame deletion mutation in the heterozygous state: the GAA nucleotides deletion at codon 1710–1712 position and the circled nucleotide represents the codon 1710 position, where the mutation starts. (Fig. 1B II:2, III:1, III:9, IV:9). According to Human Gene Mutation Database (HGMDpro), the pathogenic mutation site c.1710_1712delGAA has not been reported until now. Therefore, it is a novel mutation. And there are no mutations were identified when analysis of other genes associated with HSP was performed : *PLP1*, *L1CAM*, *SPG11*, *SPG7*, *ATL1* and so on. While the rest asymptomatic family members had no mutations at this site (Fig. 1B III:5).

As highlighted in Fig. 1B, the results of RaptorX prediction showed that this new-found mutation we reported resulted in the synthesis of misfolded protein(Fig. 2B) (http://raptorx.uchicago.edu/StructPredV2/myjobs/37184781_551773) in comparison to native one

(Fig. 2A). (http://raptorx.uchicago.edu/StructPredV2/myjobs/12115358_551772). We performed a protein sequence alignment across species showing the area of this in-frame amino acid deletion and the surrounding residues (<https://www.uniprot.org/uniprot/?query=SPAST&sort=score>). The result revealed that the spastin protein sequence across species is highly conserved at the position 570 of the protein (red frame) (Fig. 2C). Therefore, the lysine deficiency at the position 570 of the protein has a significant impact on the function. Additionally, the result of PROVEAN demonstrated that the mutation site c.1710_1712delGAA has an functional impact on the SPAST protein sequence variations. Given a list of genomic coordinates and variants (2,32372308,AGAA,A), the amino acid change (p.K570del) can be quickly determined and PROVEAN score is computed to be -11.55, which is significantly lower than the score threshold (cutoff=-2.5) (http://provean.jcvi.org/genome_view_table_2.php?jobid=2280831231964070&outfmt=one). The deletion variant was predicted as deleterious (Table 2). According to American College of Medical Genetics and Genomics (ACMG) criteria[16], we score this variant as likely pathogenic PM1, PM2, PM4, PP3

Table 2: The genome variants results are represented as PROVEAN scores and predictions.

INPUT	PROTEIN_ID	LEN GTH	CODON_CHANGE	POS	RESIDUE _REF	TYPE	SCORE	PREDICTION (cutoff=-2.5)
2,32372308,AGAA,A	ENSP00000320885	616	GTG A[AGAA/A]T ATG	570	K	Deletion	-11.55	Deleterious

Discussion And Conclusion

According to the family history, accurate description of the clinical phenotype as well as cerebral and spinal MRI, we diagnosed the proband with HSP. Analyses of the Exome Sequencing revealed a novel disease-associated in-frame deletion in the *SPAST* gene. This mutation consists of three nucleotides deletions (c.1710_1712delGAA) within the exon 16. The onset age of patients in this family is highly variable, which is accordance with the previous study[17]. However, the onset age of affected individuals in our study is obviously earlier than previous studies[18], which promotes us to consider the existence of the new mutation site c.1710_1712delGAA. Additionally, affected individuals in our study presented clinical features of the pHSP, which is consistent with Orlacchio A' s study[19].

The *SPAST* gene has 17 coding exons and encodes the protein spastin, a member of the AAA ATPase protein family. The protein spastin contains two main structural domains: the microtubule interacting and trafficking (MIT) domain in the N- terminus and the catalytic AAA domain at the C-terminus, in which the former mainly regulates microtubule organization and the later focus on ATPase activity associated with various cellular activities[20]. The two main domains are both essential to accomplish the main known function of spastin: microtubule (MT) severing[21]. More than 200 different mutations located in sites within the AAA region have been identified in patients with HSP-*SPG4*[22]. Therefore, it is believed that some mutated spastins may result in insufficient microtubule-severing activity by dominant-negative fashion. Additionally, another prevalent hypothesis is neurotoxicity of mutant spastin proteins. The *SPAST* gene presents two translation initiation codons, which allows to synthesis two spastin isoforms: a full-length isoform called M1(616 amino acid) and a slightly shorter isoform called M87(530 amino acid) that lacks the first 87 amino acid[23]. Studies on rodents show that M87 is more abundant in various

tissues, whereas M1 is only appreciably detected in brain and spinal cord[24]. Besides that, axonal transport and neurite growth are not affected by the mutated M87[25]. However, Mutant spastin proteins can form defective heterohexamers with wild-type (WT) spastin, and simultaneously produce toxic effect when presented as the tissue-specific M1 isoform[26, 27]. Even though M87 likely harbors the same AAA mutations as the M1 isoform, it is somehow degraded more effectively than mutated M1 in a dominant-negative scenario[28], thus possessing a lower toxicity.

In Our study, a novel mutation in *SPAST* gene was found in a Chinese family with multiple affected family members, which significantly enrich the mutation spectrum of HSP. This novel mutation has been inherited at least four generations according to the related investigation, further emphasizing the closed interaction between the phenotypic and genetic heterogeneity of HSP. However, some shortcomings are still existed. For example, we haven't performed functional laboratory studies about the novel mutation due to the unavailable patient cell lines and the related verification on which one molecular mechanisms above of *SPAST* gene mutants in our study cannot be realized. Haploinsufficiency is the prevalent mechanism at present. Using the haploinsufficiency model of HSP-SPG4 with a 50% decrease in active spastin levels has shown a lower MT severing[29, 30]. Another hypothesis is mainly that mutated spastin can form defective heterohexamers with wild- type (WT) spastin, and exert a dominant-negative effect. However, there is still considerable debate about the latter one hypothesis. It is unclear whether the mutant M87 can effectively impair the enzymatic activity of WT spastin[31]. Thus, further study should ascertain the role of causative genes to help better understand the relationship between genotypes and phenotypes.

Abbreviations

hereditary spastic paraplegia(HSP); spastic paraplegia genes (SPG); computed tomography(CT); magnetic resonance imaging(MRI); Sequence Control Software (SCS); Next Generation Sequence (NGS); Human Gene Mutation Database (HGMDpro); microtubule interacting and trafficking (MIT); microtubule (MT); wild- type (WT); reticulum membrane(ER); casein kinase 2(CK2); fast axonal transport(FAT);Protein Variation Effect Analyzer (PROVEAN); American College of Medical Genetics and Genomics (ACMG); polymerase chain reaction (PCR).

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and the Ethics Committee of Peking University First Hospital. Written informed consents were obtained from all of the participants of this family in the study. The written informed consent of the boy aged eleven years in our study was obtained from his parents.

Consent to publish

Written informed consent was obtained from all of the participants in the study to publish the clinical details and any accompanying clinical images in medical journals. The written informed consent to publish of the boy aged eleven years in our study was obtained from his parents.

Availability of data and material

All datasets generated or analyzed during this current study are available in this published article. Details are available from the corresponding author on reasonable request.

“The datasets generated and/or analysed during the current study are available in the repository,

Competing Interests

The authors have no conflict of interests.

Funding

No grant was provided for this analysis and this study.

Authors' contributions

YN H and WW Y designed the work and analyzed the clinical data. HQ J and JW D performed the neurologic examination and interpreted the clinical data related to the HSP. D N collected the material of these family members and analyzed the genetic data. WW Y was a major contributor to writing the manuscript. All authors contributed toward drafting and critically revising the paper, gave final approval of the version to be published.

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Figures

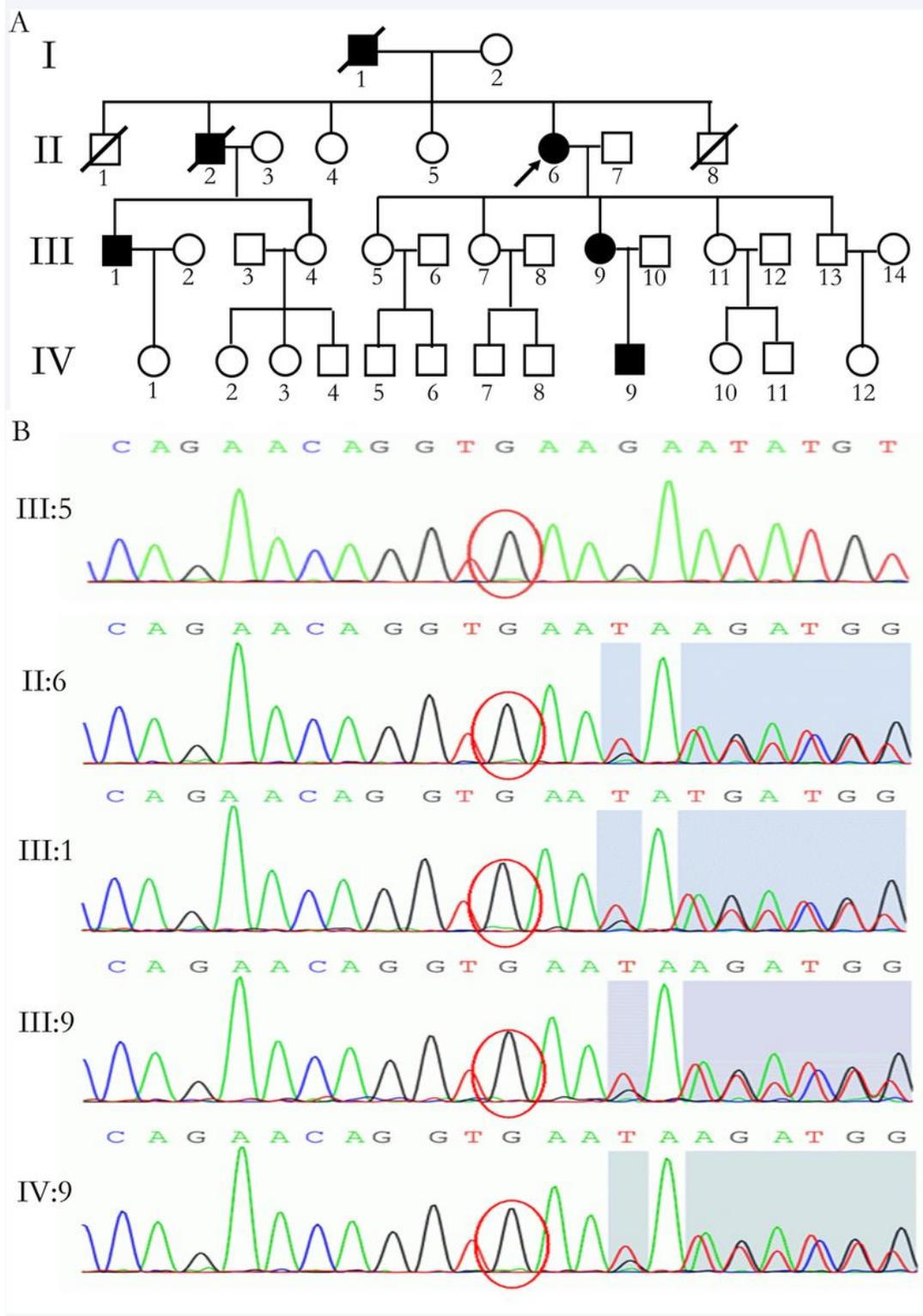


Figure 1

Pedigree of the investigated HSP family harbouring a novel SPAST gene mutation. A: The black arrow indicates the proband II:6. Squares indicate male, circles indicate females. Individuals affected with HSP are represented by black filled, while Healthy members are indicated by empty symbols. slashes indicate already dead. B: Detection of the mutation of SPAST gene in a Chinese family. Sequence analysis revealed a newly identified in-frame deletion mutation in a heterozygous form in four affected

individuals (II:2, I:1, I:9 and I:9) within the family. The exon16 consists three nucleotides deletion (c.1710_1712delGAA). I:5 is the representative wild type sequences of the investigated healthy family members

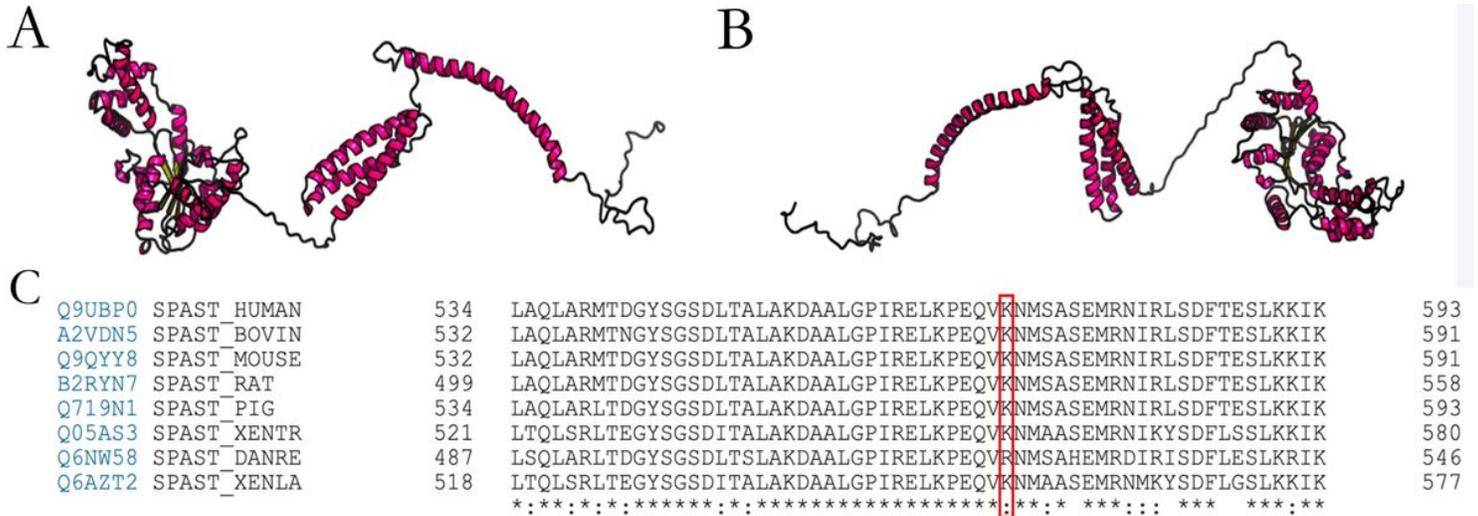


Figure 2

Tertiary structure alteration prediction of SPAST by RaptorX tool. A. The tertiary structure of native protein. B. Tertiary structure of p.K570del affected protein. The novel mutation we reported resulted in the synthesis of misfolded protein. C. The spastin protein sequence alignment across species showing the area of this in-frame amino acid deletion (red frame) and the surrounding residues