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Case report

Amplifying the spectrum of SPAST gene mutations

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Abstract. Hereditary spastic paraplegias (HSPs) include a group of neurodegenerative disorders characterized by slowly progressive spasticity and weakness of the lower extremities, caused by axon degeneration of corticospinal tracts. Spastic paraplegia type 4 (SPG4) is the most common autosomal dominant form of HSP and is caused by mutations in the SPAST gene. SPAST gene encodes for the protein spastin, a member of the ATPases Associated with a variety of cellular Activity (AAA) family. We describe a newly variant in SPAST gene, within an Italian family affected by pure HSP. In particular, we found a heterozygous intragenic microdeletion of 3T in exon 13 of SPG4 gene. The 3T deletion results in a mutated protein with a unique leucine residues deletion at the protein position 508, in the AAA ATPase domain. This variant is not registered in any public database either as rare normal variant nor as mutation in SPAST gene and the importance of this aminoacid is confirmed by the absolute conservation in multiple alignments with diverse species. We conclude that the novel SPAST gene variant identified is probably pathogenic and destabilizes the precise arrangement of the nucleotide binding domain, with a consequent loss-of-function of the mutated spastin protein. (www.actabiomedica.it)

Key words: SPAST gene, hereditary spastic paraplegia, spastin

Introduction

Hereditary spastic paraplegias (HSPs) include a heterogeneous group of neurodegenerative disorders, characterized by progressive spastic paraparesis.

HSPs are classified according to the pattern of inheritance (autosomal dominant, recessive and X-linked) and phenotype (pure HSP, with isolated pyramidal signs, and complicated HSP) (1).

At the moment, more than 60 causative genes have been linked to HSP (2).

Spastic paraplegia type 4 (SPG4), due to mutation of SPAST gene (3) is the most common subtype of HSPs, accounting for 40% of autosomal dominant cases and 20% of sporadic HSP cases.

The majority of SPG4 are classified as pure type of HSP, with isolated pyramidal signs. However, more than 30% of SPG4 patients present with sphincter disturbances and/or decreased vibratory sensation at lower limbs. In addition, cognitive impairment, ataxia and seizures have been described.

Here we report an Italian family of three-generation, including three subjects affected by a pure form of autosomal dominant HSP due to a recently identified mutation in the SPAST gene. The precise identification of this mutation, which required the application of several molecular biology techniques, resulted in the deletion of a single aminoacid in the AAA domain of the spastin protein, and amplifies the pattern of SPG4 causative mutations.

Results

Participants

All patients were evaluated at the Neurological Unit of the University Hospital "Santa Maria della Misericordia" in Udine (Italy).

After the medical interview and neurological assessment, patients underwent brain and cervical Magnetic Resonance Imaging (MRI), nerve conduction velocity (NCV) and electromyography (EMG) studies and cognitive assessment by Mini-Mental State Examination (MMSE).

A genetic test has been proposed to the proband and his symptomatic and asymptomatic relatives to confirm the clinical diagnosis.

Genetic testing

The genomic DNA of the proband and his relatives has been isolated from peripheral blood leukocytes using the Qiagen Gentra Puregene Blood Kit according to the manufacture's instructions.

The MLPA (P165) autosomal dominant spastic paraplegia kit, designed to detect deletions or duplications of exons of the SPAST gene was purchased from MRC-Holland (Amsterdam, The Netherlands). MLPA reactions were carried out according to the manufacturer's instructions. Electrophoresis of polymerase chain reaction products was performed using an ABI 3500 sequencer (Applied Biosystems). MLPA data were analyzed using Coffalyser software (MRC-Holland).

All exons and exon/intron junctions of the SPAST gene were sequenced following standard procedures on an ABI 3500 Genetic Analyzer (Applied Biosystems) sequencer. PCR amplification of the SPAST gene regions was performed with oligonucleotides (SIGMA) provided with M13 tails at the 5' end to simplify the next step of the amplicons forward and reverse sequence.

The Single Base probe Extention (SBE) assay was performed with the Abi Prism SnaPshot multiplex kit (Applied Biosystems) following the manufacturer's instructions. SAP (shrimp alkaline phosphatase - USB Affymetrix) was used to clean the single base extended products and the GeneScan-120 Liz Size Standard, Hi-Di Formamide and an ABI 3500 sequencer (Applied Biosystems) were used for capillary electrophoresis.

The clinical presentation and patient investigations described in this study are summarized in Table 1. The family tree is shown in figure 1. DNA and protein mutations are represented in figure 2 and figure 3 respectively. Detailed descriptions for the clinical and molecular characterization of each case are provided below.

Index patient

The proband (subject II.1, figure 1) is a 55-years-old male patient who came to our attention for progressive gait difficulties, started at the age of 15 and characterized by lower limbs stiffness, slow running and abnormal gait, which deteriorated over time. He denied paraesthesias or sensory loss, but complained of dysuria and pollakiuria. Medical history was significant for type2 diabetes, hypertension and dyslipidemia.

The general physical examination was unremarkable. The neurological assessment showed a typical spastic gait. Deep tendon reflexes were normal at the upper extremities and increased at the lower extremities, with clonus in the ankle bilaterally. The plantar responses were flexor bilaterally. Muscle mass and strength, as well as all sensory modalities, were normal. Cranial nerves were functioning normally.

Table 1. Summary of clinical findings

Patient	Sex	Lower limb stiffness (age of onset)	Neuropathy	Cognitive impairment	Comorbidities
I-1	F	24	Mild sensory neuropathy	-	Hypertension
II-2	M	15	-	-	Diabetes, hypertension and dyslipidemia
III-1	F	18	-	-	-

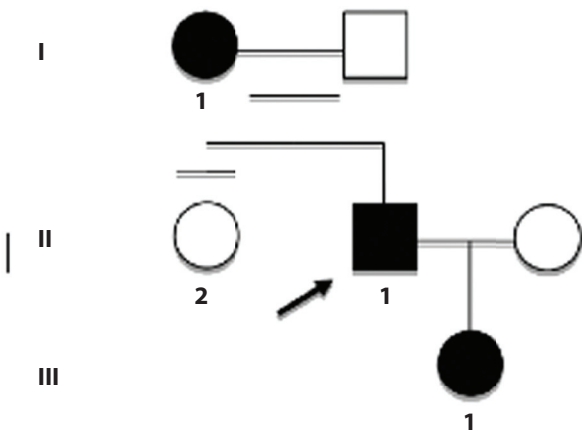

Figure 1. Family tree of the SPG4 family. Affected individuals are shown as filled symbols and unaffected individuals as unfilled symbols. The black arrow shows the proband (subject II.1)

No abnormalities were noted in laboratory tests, including blood cell count, serum electrolytes, thyroid hormone level, erythrocyte sedimentation rate, C-reactive protein, folic acid and vitamin B12. MRIs of the brain and cervical spinal cord were unremarkable. Nerve conduction studies and needle EMG were normal, particularly did not reveal signs of neuropathy. No cognitive impairment was observed at the neuropsychological evaluation.

The proband has a positive family history for spastic paraparesis (see the next paragraph).

Thus, a diagnosis of HSP as been formulated. To identify the mutated gene, the proband's genomic DNA was isolated from peripheral blood leukocytes using standard procedures (see methods).

Multiplex ligation-dependent Probe-Amplification (MLPA) analysis, SPAST gene sequence and SBE assays were performed to find the causative HSP mutation carried by the proband. The MLPA analysis detected the heterozygous deletion of exon 13 of the gene. Since exon 13 is a small exon of 43 base pair we wanted to evaluate the hypothesis of an intra-exon microdeletion. The first step was to sequence the exon 13 of the proband's SPAST gene. A deletion spanning the entire exon 13 of the mutated allele, would produce clear forward and revers sequences from the wild type/undeleted allele. On the contrary, by analyzing the proband's DNA we obtained only a partially clear sequence of the exon both in forward and reverse (Figure 2B). Indeed, the sequence of exon 13 showed a pattern of shifted banding and the quality of the forward sequence was rather poor due to the presence of a stretch of 11T, just 2 bases upstream the 5' end of exon 13. Therefore, from the analysis of the sequence, we could only derive a suggestion for a 3T microdeletion (c.1521_1523delTTT, Ref seq NM_014946) within exon 13. To verify this hypothesis, we performed the Single Base Extension (SBE) assays, as shown in figure 2C. The wild type allele would present a T in forward and an A in reverse SBE, while the mutated allele would present an A in forward and a G in revers SBE. The double fluorescent signals obtained with the SBE assays of the proband demonstrated a wild-type/mutated heterozygous condition for this subject (figure 2C, right panel). The left panel of figure 2C shows the results of SBE analysis on a negative control subject for the disease. The individual signals (T in forward and A in reverse) corresponding to a homozygous wild-type condition are evident.

Family relatives

The proband's mother (I.1), a 78 years old woman, has been complaining, since the age of 24 about the same symptoms of her son, gradually progressed. At the age of 76, she was permanently in a wheelchair. The medical history was significant for hypertension.

Her neurological assessment revealed spastic paraparesis, ambulation possible only with support and for few steps, diffuse brisk tendon reflexes, with bilateral clonus and Babinski's sign, loss of vibratory sensation at the ankles, with preservation of other sensory modalities.

Brain and cervical spinal cord MRIs were unremarkable. Nerve conduction and needle EMG studies were consistent with mild sensory neuropathy at lower limbs. Neuropsychological tests were normal.

The proband has a 20-year-old daughter (III.1), who complains of lower limb stiffness and difficulty in running. Her neurological examination reveals only an increase in deep tendon reflexes at the lower

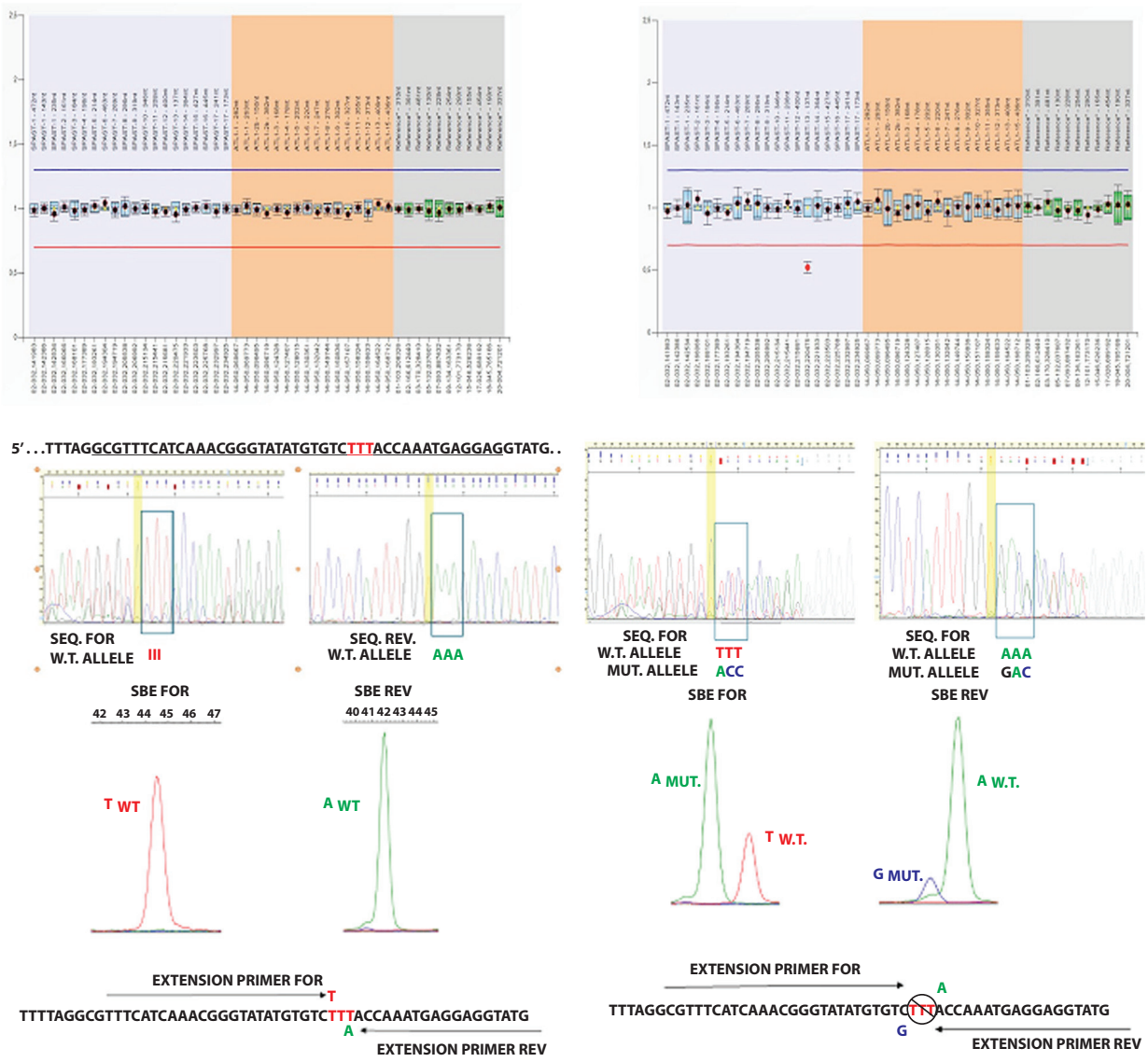

Figure 2. The results obtained with the Multiplex Ligation-dependent Probe Amplification, DNA sequence analysis and Single Base probe Extension tests for healthy control and affected proband are shown. A left) MLPA analysis of the SPAST gene of a healthy control showed no deletion/duplication of SPAST gene exons. A right) MLPA analysis of the SPAST gene of the proband showed heterozygous deletion of exon 13 (yellow arrow). B left and right) The coding sequence of exon 13 is shown (top left, bold letters underlined) as well as the 3T (red) deleted in the family. The partial electropherograms (forward and revers) of exon 13 of a healthy control (left) and the SPG4 affected proband (right) are shown. The rectangle shows the deleted 3T in the family. Seq. For = Forward DNA sequence; Seq. Rev = Reverse DNA sequence; W.T. allele = wild type allele; MUT. Allele = mutant allele. C left and right) The results of the SBE assay of a healthy control (left) and the affected proband (right) are shown. At the bottom of the 2C panel on the left and right respectively are represented schematically the expected single base elongated in forward and revers SBE assays for the wild type and the mutated exon 13.

limbs. She underwent MRI scans of her brain and cervical spinal cord which were unremarkable and neurophysiological examinations, including nerve conduction and needle EMG study, with normal results.

Furthermore, the proband has a 50-year-old sister (II.2), without neurological symptoms and with normal neurological examination.

The proband's relatives (mother, subject I.1; daughter, subject III.1; sister subject II.2, figure 1)

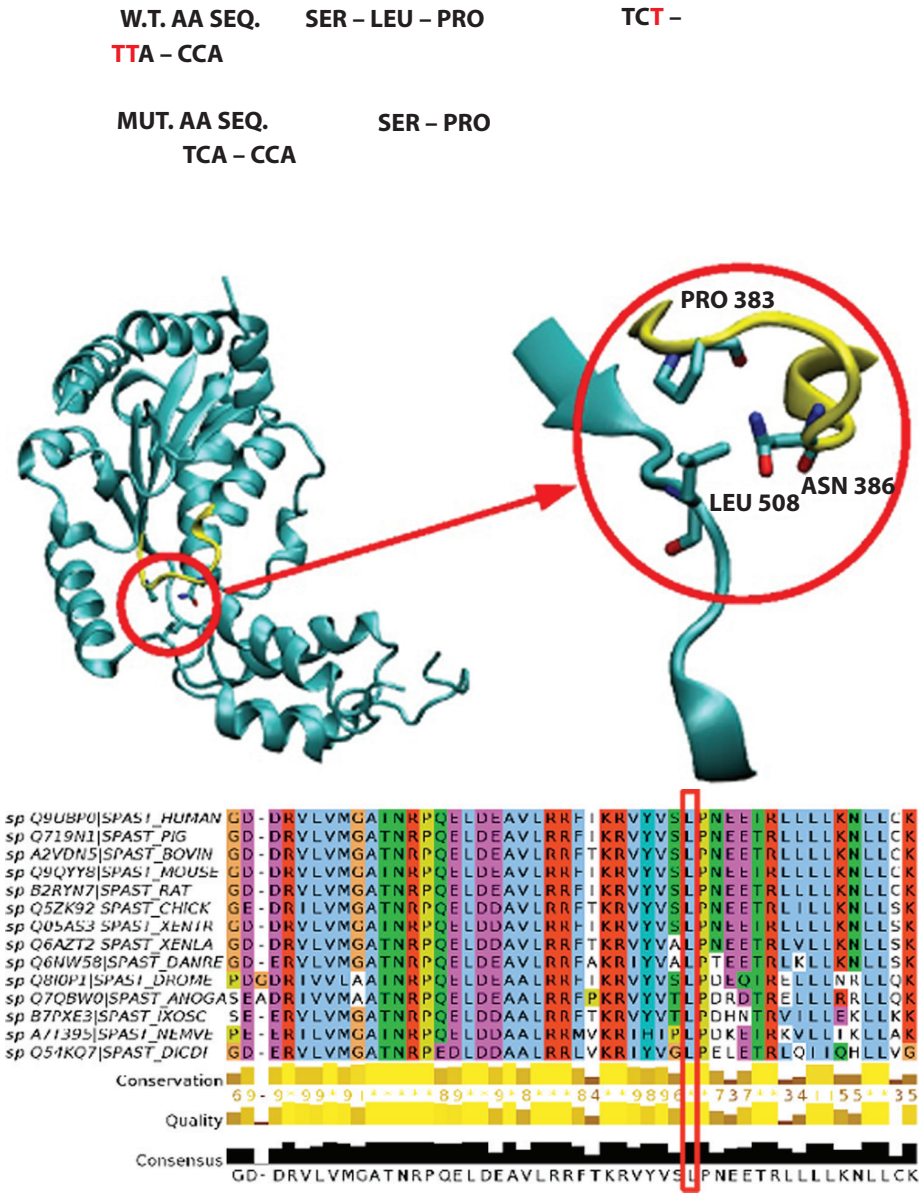

Figure 3. Figure 3: Structure of the AAA domain of the human spastin and alignment of different species. A) The Amino acid sequence of protein spastin resulting from the 3T deletion in exon 13 is represented (top of the panel). Cartoon representation of the AAA domain structure of the human spastin (pdb id.: 3vfd) (bottom of the panel). The nucleotide binding domain is highlighted in yellow. L508 = Leu 508, P383 =Pro 383 and N386 = Asn386 are shown in sticks. Details of the spatial arrangement of the three aminoacids are shown in the right panel. B) Alignment of the spastins from different species. The Swiss-Prot codes indicate the following: Human: homo sapiens; Pig: Sus scrofa; Bovin: Bos taurus; Mouse: Mus musculus; Rat: rattus norvegicus; Chick: Gallus gallus; Xentr: Xenopus Tropicalis; Xenla: Xenopus Laevis; Danre: Danio rerio; Drome: Drosophila melanogaster; Anoga: Anopheles gambiae; Ixosc: Ixodes scapularis; Nemve: Nematostella vectensis; Dicdi: Dictyostelium discoideum. The red rectangle evidences the position of the conserved Leu508 in the ATPase domain.

were evaluated for the inheritance of the identified mutation. For this purpose, the genomic DNA of the relatives, obtained from a peripheral blood sample, was analysed by MLPA and SBE to determine the presence or absence of the TTT deletion. The mutation identified in the proband segregated in all the affected relatives (mother and daughter), while the asymptomatic sister did not arbor the mutation (data not shown).

Discussion

SPG4 is the most common autosomal dominant form of HSP and is caused by mutations in the SPAST gene. The SPAST gene is located on the 2p22.3 chromosome and encodes for the spastin protein, a member of the ATPase Associated with a variety of cellular Activity (AAA) family, with a role in microtubule dynamics and membrane trafficking.

The Spastin has a modular organization in domains, therefore the C-terminal of the protein contains the domains involved in the microtubule severing and the N-terminal half of the protein is involved in targeting the spastin to specific sites of action. In particular, the protein C-terminal includes the AAA ATPase domain and the Microtubule Binding Domain (MTBD), while the N-terminal includes a Hydrophobic Domain (HD) and a Microtubule Interacting and Trafficking (MIT) domain (4).

Two domains are critical: MTBD and AAA ATPase. The MTBD, which extends from the residue 270 to 328 of the human spastin, is necessary to bind to microtubules in an ATP-independent manner. The AAA ATPase domain, located between residues 342 and 599, catalyzes severing of microtubules and requires hydrolysis of the ATP.

The mutational spectrum of the SPAST gene is broad and includes missense or nonsense mutations, splicing site mutations, deletions and insertions (5). In particular, most of the missense mutations fall within the AAA domain, reinforcing the functional significance of this domain, while the other mutations are scattered along the coding region of the gene and lead to the appearance of premature termination codons and mRNA instability. Literature data report that exon 13 of the SPAST gene is often involved in point mutations/single base insertion (6) or deletions spanning multiple exons (7). Rarely, not-in-frame microdeletions have been identified in this exon, as reported in two families by Depienne and coworkers.

Mechanisms of loss-of-function and haploinsufficiency (8) and of gain-of-function (9) have been described as the basis of the molecular pathogenesis of SPG4.

Now, we report a novel variant of the SPAST gene, a heterozygous in-frame deletion of three nucleotide bases (c.1521_1523delTTT), identified within an Italian family affected by pure HSP.

This deletion leads to the loss of the amino acid Leucine in position 508 of the protein Spastin (p.508del Leu), in the AAA ATPase domain. This variant is not registered in any public database either as a rare normal variant or as a mutation of the SPAST gene.

Leu508 is part of an extended stretch in the AAA domain and is in contact with the residues Pro383 and Asn386, which are part of the nucleotide binding region 382-389 (figure 3A). The importance of this aminoacid and the flanking Pro509 is confirmed by the absolute conservation in multiple alignments in several species, including, in addition to humans, Drosophila melanogaster and Xenopus laevis (figure 3B).

Based on clinical evidences and ACMG criteria(10), we conclude that the novel variant of the SPAST gene identified within family we studied is probably pathogenic.

In fact, it is possible that the deletion of Leu508 destabilizes the nucleotide binding domain structure, with a consequent loss-of-function of the mutated spastin protein. Our report broadens the spectrum of SPAST/SPG4 gene mutations that can cause disease.

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Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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