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The clinical manifestations of two novel SPAST mutations

Authors: Dénes Zádori[1], Adrienn Máté[2], Krisztina Róna-Vörös[1], Gyurgyinka Gergev[3,4], Alíz Zimmermann[3], Nikoletta Nagy[5], Márta Széll[5] László Vécsei[1,6], László Sztriha[3], Péter Klivényi[1,*]

Affiliations:

[1]Department of Neurology, Faculty of Medicine, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, Hungary

[2]Department of Neurosurgery, Faculty of Medicine, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, Hungary

[3]Department of Pediatrics and Pediatric Health Care Centre, Faculty of Medicine, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, Hungary

[4]2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary

[5]Department of Medical Genetics, Faculty of Medicine, Albert Szent-Györgyi Clinical Centre, University of Szeged, Szeged, Hungary

[6]MTA-SZTE Neuroscience Research Group, Szeged, Hungary

***Corresponding author:**

Péter Klivényi, MD, PhD, DSc

Department of Neurology, Faculty of Medicine, Albert Szent-Györgyi Clinical Centre, University of Szeged,

Semmelweis u. 6, H-6725 Szeged, Hungary

Phone: +36 62 545351;

Fax: +36 62 545597

E-mail: klivenyi.peter@med.u-szeged.hu

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Running title:

Clinical manifestation of novel SPAST mutations

Research highlights:

- There are numerous mutations in the *SPAST* gene with a wide spectrum of clinical phenomenology
- We report 2 novel mutations (c.1732A>T p.R578X and c.1334G>C p.S445T) affecting the AAA catalytic domain with quite different age at onset and some difference in phenomenology
- Establishing genotype-phenotype correlations would promote the understanding of the function of spastin under normal and pathological conditions

Introduction

Hereditary spastic paraplegia (HSP) is heterogeneous group of genetically inherited disorders mainly characterized by spastic gait impairment [1]. It affects approximately 1.2 to 9.6 out of 100,000 individuals. There are more than 50 mutations which can be responsible for the development of uncomplicated or complicated (spastic paraplegia associated with additional neurologic or systemic abnormalities) forms, and there are several modes of inheritance. The most common causes of autosomal dominantly inherited cases, which account for 70-80% of all HSP forms in the Western countries, are mutations in the *SPAST* gene mapped to 2p22 [2]. All types of mutations (missense, nonsense, splicing site, deletions and insertions) have been detected in the *SPAST* gene at numerous loci with wide age range of onset. The gene product, spastin possesses 2 main structural domains: the microtubule interacting and trafficking domain (MIT) in the N-terminus, which is responsible for the association of spastin with microtubules, and the catalytic AAA domain at the C-terminus with ATPase activity [3]. The spastin-mediated severing of microtubules is particularly involved in axonal transport and axonal branching. The haploinsufficiency of spastin – mainly caused by mutations in the AAA domain – is responsible for the progressive retrograde degeneration preferentially affecting long corticospinal axons with the consequential development of characteristic symptoms [3].

The aim of this study is to present the genotype-phenotype correlation of a previously unpublished novel mutation site in the *SPAST* gene, and furthermore to report a *de novo* mutation leading to the development of characteristic symptoms in early childhood.

Case reports

The first case is a 47-year-old female patient who was first admitted to our neurology department with the aim of the diagnostic work-up of her unknown gait disorder in 2008. She

had already had some less expressed symptoms since her childhood, but without significant complaints. Her gait worsened progressively from 2007, it became more and more stiff. In addition to her motor symptoms, frequent urge to urinate and to have bowel functions both persisted since her childhood. Beyond tonsillectomy and dysmenorrhea, there were no other relevant diseases in her case history. She did not suffer traumatic injury. With regard to her family history, her father is wheelchair-bound because of a similar gait disorder. On neurological examination, our female patient presented slight spasticity in her lower limbs considerably disturbing her gait. Her tendon reflexes were brisk with bilateral ankle clonus. With regard to pathological reflexes the patient presented Babinski and Hoffman's signs. There were no seeming alterations in cognitive functions, muscle strength, movement coordination and sensation. The applied laboratory test (ions, inflammatory markers, complete blood count, thyroid functions, vitamin B_{12} and folic acid) did not show any unequivocal abnormality. The skull, cervical and thoracic MRI scans revealed no pathognomonic abnormality. The lumbar MRI demonstrated multiple discopathy and signs of Scheuermann's disease without any stenosing effect. Cerebrospinal fluid diagnostics did not show any signs of inflammation or immunological disorder. The examination of somatosensory evoked potentials demonstrated the preservation of sensory functions. The clinical picture and the positive family history raised the possibility of uncomplicated HSP (Figure 1a). The genetic testing for a disease-causing mutation in the *SPAST* gene (Centogene AG, Rostock, Germany) revealed heterozygous mutation in exon 17 (c.1732A>T p.R578X) not only in our patient, but also in her father (Department of Medical Genetics, University of Szeged, Szeged, Hungary) (Figure 1b).

The second case is an 8-year-old boy who was born at term from the third, twin pregnancy of his mother. The other twin died in utero in the first trimester of the pregnancy, but otherwise no complications were reported. His birth weight, length and head circumference were in the

normal range. He was first examined by a pediatric neurologist at the age of 18 months for delayed motor development, i.e., being unable to walk. No neurological abnormalities were detected at this first examination, but physiotherapy was recommended to catch up to his age. He began to walk, but at the age of 22 months minor gait disturbances and mild signs of weakness in the lower limbs appeared: he had a tottering gait and could not walk more than a few steps. Speech development was also delayed: his speech was restricted to only a few words. The first signs of spastic paraparesis appeared at the age of 26 months: spasticity was detected in both legs with brisk tendon reflexes and persisting Babinski sign. He was walking on his tiptoes. No ataxia could be detected. Evaluations of serum lactate and creatine kinase levels were normal. Spine and skull magnetic resonance imaging were performed at the age of 4 years, but no abnormalities were detected. The lack of morphological abnormalities in the central nervous system and the progressive spasticity in the lower limbs turned our attention to the possibility of hereditary spastic paraplegia. Genetic testing was also performed at Centogene AG (Rostock, Germany) and the results showed a de novo mutation in exon 11 of the *SPAST* gene (c.1334G>C p.S445T), as no mutation was found in the parents (Figure 2a and 2b).

Discussion

Despite the intensive research activity, the pathomechanism of HSPs is not yet fully understood. With regard to the most commonly affected *SPAST* gene, there are numerous reported mutation sites with a relatively wide spectrum of age at onset (from the very early childhood to the sixties) and clinical manifestation [4]. The delineation of novel mutations in the *SPAST* gene seems to be very important since the detailed description of clinical phenomenology would promote the understanding of structure-function relationship of the gene product, spastin. This case study reports 2 novel mutations, located within highly

conserved regions of the protein (Figure 1c and 2c). According to the HSP mutation database (https://reseq.biosciencedbc.jp/resequence/GeneDetail.do?targetId=9&geneId=EG6683), None of the mutations (p.R578X and p.S445T) have been previously demonstrated. A mutation-causing p.S445R amino acid change has already been described [4], but the p.S445T change has not been previously reported. Although both genetic mutations affect the AAA domain of spastin – similarly to most of SPAST mutations – the clinical phenomenology is quite different.

With regard to our first patient, her symptoms, which mainly affect her gait and vegetative functions, persisted from her late childhood, but they became expressed only in her middle-age. These alterations are characteristic for uncomplicated cases. The closest known affected site by a nonsense mutation (c.1741C>T p.R581X) causes similar symptoms (gait disorder with lower limb spasticity and brisk tendon reflexes, sphincter impairment), but slightly later disease onset (the mean age is 28.5 years) [5].

The symptoms of the other patient developed in very early childhood and mainly characterized by lower limb weakness accompanied by gait disturbance. There are some available reports of *SPAST* mutations with an onset at the early childhood [4, 6], mainly affecting the 400 to 500 amino acids region of spastin, similar to our case. At the age of 8 years, he attended a regular school and he was able to walk with ankle-foot orthosis and a walking stick. His cognitive skills are in the normal range, he performs well in the elementary school. Although the age at onset is quite different from the previously reported case of p.S445R amino acid change in spastin – with a considerable disease worsening at late teenage years – the clinical course shows some similarities: the case is described as a complicated HSP form with walking difficulties with marked lower limb stiffness, and furthermore with bladder, swallowing, breathing and speech dysfunctions [4].

Conclusion

In conclusion, the clinical description of novel mutations in the *SPAST* gene would accompany to the understanding of the functioning of the gene product, spastin, both under normal and pathological conditions.

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Conflict of interest

None to declare.

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Figure Legends

Figure 1. (a) Pedigree of the investigated family demonstrates that the investigated proband (II/1) and her father (I/1) are the only affected individuals within the family. **(b)** Direct sequencing revealed a newly identified nonsense mutation. The affected patients carried the mutation in a heterozygous form, while the investigated healthy family members carried wild type sequences only. **(c)** The identified nonsense mutation is located within a highly conserved region of the protein.

Figure 2. (a) Pedigree of the investigated family demonstrates that the investigated proband (II/1) is the only affected individual within the family. **(b)** Direct sequencing revealed a novel missense mutation, which is carried in a heterozygous form by the patient. **(c)** The identified missense mutation is located within a highly conserved region of the protein.