Detailed Phenotype Characterization of a Patient with a Novel Mutation in the SPAST Gene

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Abstract

Introduction: Spastin mutations are the most frequent cause of Hereditary Spastic Paraparesis (HSPs). To date, almost 160 SPAST variations have been reported as responsible of both pure and complicated phenotypes. We described a novel SPAST pathogenic variant in a 5-generation pedigree from Southern Italy, along with a detailed phenotypic characterization of the proband.

Methods: A comprehensive clinical and paraclinical assessment of the proband have been done. The NGS analysis was performed using the SeqCap EZ Choice Enrichment Kits (Hoffmann-La Roche, Basel) on an Illumina MiSeq (San Diego, CA). GenomeUp software (https://platform.genomeup.com) was used for data analysis.

Results: We described a 73-years old Caucasian male with walking impairment started around his fifties and gradually progressed over the following 23 years. The predominant phenotypic manifestation was a gradually progressive spastic paraparesis, associated to upper-limb hyperreflexia. Impaired vibratory sensation, and autonomic dysfunction appeared after more than 15 years from symptom onset. Although cognition was normal, a high-field MRI scan of the brain showed moderate subcortical and cortical atrophy, especially of the right frontal and parietal lobes. Genetic analysis revealed a new heterozygous deletion NM_014946:c.[1005-1delG] in the SPAST gene. This variant was classified as pathogenic based on the American College of Medical Genetics for variants’ classification.

Conclusion: We reported a novel mutation of the SPAST gene, and a detailed phenotypic description of the proband. The very late age of onset and the presence of cortical and subcortical atrophy at the brain high-field MRI scan, expand the phenotypic manifestation of SPAST-mutation carriers.

Keywords: SPAST mutation; HSP; Phenotype

Introduction

The term Hereditary Spastic Paraparesis (HSP) identifies a group of clinically and genetically heterogeneous neurodegenerative diseases mainly characterized by a slowly progressive spasticity and weakness of lower limbs [1]. To date, more than 80 genes have been identified as causative of autosomal dominant, autosomal recessive, and X-linked forms of HSP [2], and the scenario is still incomplete. HSPs can be clinically grouped into uncomplicated (or pure) when spasticity is the only clinical finding, and complicated (or complex) when other clinical features (intellectual disability, dementia, cerebellar ataxia, epilepsy, peripheral neuropathy, extrapyramidal signs, amyotrophy, deafness, or optic neuropathy) are also present [3]. Mutations in SPAST/SPG4 are the single commonest cause of autosomal dominant HSPs, accounting for up to 40% of familiar cases and up to 6% to 15% of apparently sporadic forms [4-6]. SPAST gene encodes Spastin, a member of the AAA (ATPase associated with various cellular activities) family of proteins [7]. Spastin interact with microtubules, and contributes to membrane modeling, and intracellular and axonal transport of vesicles [8,9]. To date, 159 SPAST variations have been reported (https://databases.lovd.nl/shared/variants/SPAST/unique; date last updated May 10th, 2021). Spastin mutations are...
usually associated with a pure or uncomplicated phenotype, even though complex forms of HSP have been described for specific SPG4 mutations. We report a detailed phenotype characterization of a new SPAST mutation identified in the index case of a 5-generation family showing an autosomal dominant pattern of inheritance.

Materials and Methods

Clinical and paraclinical assessment

Patient was assessed and followed-up at the Center for Neurodegenerative Diseases and the Aging Brain, University of Bari “Aldo Moro” – A.O. Pia Fondazione Cardinale G. Panico, Tricase (LE), Italy. He underwent a detailed clinical assessment, including personal medical history, standard neurological examination, and cognitive evaluation, including the Clinical Dementia Rating Scale (CDR), the Mini Mental State Examination (MMSE) [10], and a complete neuropsychosocial assessment, using a battery of cognitive tests, described already [11]. As per diagnostic protocol, patients underwent routine blood biochemical analyses, a high-field (3-Tesla) brain Magnetic Resonance Imaging (MRI) scan, motor evoked potentials, and somatosensory evoked potentials. After a written informed consent, a blood sample was also collected for genetic analyses and shipped to the Centre of Molecular Genetics, ICRCS Neuromed, Pozzilli (IS), Italy. Family pedigree was reconstructed and drawn based on the information collected from the examined patient and his caregiver (wife). No other family members were available for genetic analysis. The study was approved by the Institutional Review Board of the “Azienda Sanitaria Locale, Lecce”. All methods were carried out in accordance with relevant guidelines and biosystems.

Genetic analysis

Next generation sequencing (NGS) panel: The NGS analysis was performed using the Seq Cap EZ Choice Enrichment Kits (Hoffmann-La Roche, Basel) on an Illumina MiSeq (San Diego, CA). A full list of genes sequenced is provided in Supplementary Table 1. All coding exons of the RefSeq transcripts of the genes and 15 base pairs of the flanking introns were targeted. 99% of the coding exons were sequenced with a minimal read depth of 30X. GenomeUp software (https://platform.genomeup.com) was used for data analysis. It provides automated annotation (Best Practices workflows of GATK v4.1 for germline variant calling), alignment of sequence reads to the reference genome GRCh37/hg19, and selection of potentially pathogenic variants. Direct evaluation of data sequence was performed by the Integrative Genomics Viewer v2.2.3. Mutation re-sequencing and segregation analysis were performed by Sanger sequencing ABI 3130xl Genetic Analyzer (Applied Biosystems).

Data analysis and variant interpretation: Variants were classified as Pathogenic (class 5), Likely Pathogenic (class 4), and VoUS (class 3) according to ACMG guidelines for germline variant classification [12]. Richards S; Aziz N; Bale S; Bick D; Das S; Gastier-Foster J; Grody WW; Hedde M; Lyon E; Spector E; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–424, doi:10.1038/gim.2015.30.). To this aim, public databases were used (Varsome https://varsome.com; Gnomad https://gnomad.broadinstitute.org). In silico analyses of variants were performed using SIFT (http://sift.jcvi.org), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2), PROVEAN (http://provean.jcvi.org/index.php), and Mutation Assessor (http://mutationassessor.org).

Results

Case report

A 73-years old Caucasian male came to our attention because of a long history of gait abnormalities started in his fifties. The walking difficulties progressed slowly over the last 20 years and lead to loss of autonomy for the last 2 years, when he started to use a walking aid. For one year he had been also manifesting autonomic involvement with urinary and fecal incontinence. His past medical history was remarkable for a chronic HBV-related hepatitis, followed by hepatic cirrhosis and hepatic carcinoma, treated with radiotherapy; b-colorectal cancer, diagnosed 2 months before his first appointment with us, and subsequently treated with a bowel surgical resection and chemotherapy. Neurological examination showed moderate/severe spastic paraparesis, with moderate proximal weakness of the lower limbs and mild proximal weakness of the upper limbs; asymmetrical mild atrophy of the quadriceps and gluteal muscles (dx > sx); mild spasticity of the legs (hamstrings, quadriceps, adductors, gastrocnemius, and soleus); deep tendon reflexes were all brisk both on upper and lower limbs, especially on the right lower limb; Babinski sign was present bilaterally; pinprick sensation was normal, while he had moderate hypopallesthesia, more pronounced in the lower limbs; he also had urinary and fecal incontinence. Neuro-cognitive tests were all in the normal range for his age and education level. Laboratory tests showed low levels of vitamin B12 (154 pg/ml; normal range: 187 pg/ml to 883 pg/ml), which resulted in a mild macrocytic anemia. Conventional spinal cord MRI showed several posterior disc protrusions at the cervical, thoracic and lumbar levels, with no signs of radiculopathy or spinal cord compression. High field (3-Tesla) brain MRI scan revealed small diffuse hyperintensities of the white matter, suggestive of small vessels disease; moderate subcortical and cortical atrophy, especially of the right frontal and parietal lobes (Global Cortical Atrophy scale: 2; Koedam score for parietal atrophy: 2) (Figure 1), mild left and moderate right hippocampal atrophy (Medial Temporal Atrophy score: 1 – right, 2 – left). Corticospinal tract appeared bilaterally normal. Arterial Spin Labeling identified lower perfusion of the right frontal lobe, than the left one. Nerve conduction study was normal. Motor Evoked Potentials (MEPs) were normal for the upper limbs, while revealed prolonged central conduction time when registered from both legs. Somatosensory Evoked Potentials (SEPs) were normal from both arms and legs. Genetic analysis identified a new heterozygous deletion NM_014946:c.[1005-1delG] in the SPAST gene. The disease was thus confirmed by genetic analysis. 

Figure 1: T1-weighted images from the 3T brain MRI scan of the patient, showing diffuse moderate subcortical and cortical atrophy, especially of the right frontal and parietal lobes (Global Cortical Atrophy scale: 2; Koedam score for parietal atrophy: 2). Panel A: axial section. Panel B: coronal section.
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This variant is yet to be registered in the following public genetic databases: LOVD (https://databases.lovd.nl/shared/variants/SPAST/unique). Based on the guidelines of the American College of Medical Genetics for variants' classification [12], this variant was classified as pathogenic (Class 4: PVS1-PM2-PP3). Family pedigree (Figure 2) showed an autosomal dominant pattern of inheritance, spreading on 5 generations. No other family members were available for neurological examination or genetic analysis.

Discussion

We reported a novel SPAST variant with a high probability of being pathogenic, considering the type of mutation, its predicted effect using in-silico studies, and its absence in genetic databases of healthy individuals. We also reported a detailed phenotypic characterization of the proband, who presented a classical course of the disease, with insidious onset of walking difficulties, a slow disease progression, and a pure phenotype for a long time period. Impaired vibratory sensations, as well as upper-limb hyperreflexia, have also been described already as typical for SPAST mutations [13]. The Age of Onset (AO) was around 50. This late AO is not typical for autosomal dominant HSPs. A recent meta-analysis by Erfanian Omidvar et al., which included 13,570 patients with HSP, reported a mean AO of 24.79 (95% CI 21.00–28.58) for SPAST mutations, and even earlier for AT1 (4.53, 95% CI 3.09–5.98) and REEP1 (17.00, 95% CI 11.18–22.82) mutations [14]. However, clinical presentation of HSPs can be extremely heterogeneous, also regarding AO, and a “very late” AO (>35 years of age) has already been described for SPAST mutations [15,16], but is considered rare. In our family pedigree, AO, although late for all the affected members, is really variable, spanning from 30 to 50 years. It is reasonable to consider that other factors (genetic modifiers) may have influenced this phenotypic trait in our family. The proband’s phenotype was also characterized by autonomic impairment, with urinary and fecal incontinence, but only after more than 15 years from the onset of symptoms. Autonomic involvement in patients with SPAST mutation has been reported already, especially urinary and sudomotor dysfunctions, probably related to a damage of the peripheral nervous system (small post-ganglionic cholinergic fibers) [17]. Moreover, bladder dysfunction is present in SPAST-related HSPs more frequently, compared to other autosomal dominant or recessive HSPs [18]. Although high-field brain MRI showed moderate subcortical and cortical atrophy, both global and parietal, the patient did not manifest cognitive impairment. This is in line with previous studies, which showed that cognitive impairment is rarely associated with SPAST mutation [18], although mild cognitive impairment, especially in the executive and visuo-spatial domains has been reported already [19]. SPAST mutations have been previously associated to brain MRI abnormalities, with a predominant involvement of basal ganglia, thalamus, and posterior white matter [20]. Global reduction of both gray- and white-matter volumes in the brain, as well as corpus callosum volume, cortical thickness, and subcortical gray-matter volume have been also described [19]. However, a frank diffuse cortical and subcortical atrophy, detectable at the visual assessment, like in our case, was never been described. This can be partly related to the age of the proband, but it is further behind an age-related pattern of atrophy. Unfortunately, no other family members were available for genetic analysis and clinical/paraclinical examination, so we were not able to perform a proper

Figure 2: Family pedigree. Symbols: roman numbers to the left of the pedigrees denote generations. The arrow denotes the proband of the pedigree. The circles indicate females and squares indicate males. Fully black and fully gray symbols denote the affected individuals evaluated and the subjects affected by history, respectively.
segregation analysis and a detailed phenotypic characterization of other mutation carriers. However, the reported mutation, based on the guidelines of the American College of Medical Genetics for variants’ classification, is considered pathogenic. To sum up, we reported a new mutation of the SPAST gene, along with a detailed phenotypic characterization of the proband. The very late age of onset and the associated cortical and subcortical atrophy at the brain high-field MRI scan, expand the phenotypic manifestation of SPAST-mutation carriers.

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**References**