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Case Report

Novel homozygous *GBA2* mutation in a patient with complicated spastic paraplegia

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ABSTRACT

Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurological disorders characterized primarily by a pyramidal syndrome with lower limb spasticity, which can manifest as pure HSP or associated with a number of neurological or non-neurological signs (i.e., complicated HSPs). The clinical variability of HSPs is associated with a wide genetic heterogeneity, with more than eighty causative genes known. Recently, next generation sequencing (NGS) has allowed increasing genetic definition in such a heterogeneous group of disorders. We report on a 56- year-old man affected by sporadic complicated HSP consisting of a pyramidal syndrome, cerebellar ataxia, congenital cataract, pes cavus, axonal sensory-motor peripheral neuropathy and cognitive decline. Brain MRI showed cerebellar atrophy and thin corpus callosum. By NGS we found a novel homozygous biallelic c.452-1G > C mutation in the b-glucosidase 2 gene (GBA2), known to be causative for autosomal recessive hereditary spastic paraplegia type 46 (SPG46). The rarity of this inherited form besides reporting on a novel mutation, expands the genetic and clinical spectrum of SPG46 related HSP.

1. Introduction

Hereditary spastic paraplegias (HSPs) represent a clinically and genetically heterogeneous group of neurodegenerative diseases [1]. Clinically, HSPs are defined as "pure" or "uncomplicated" forms if neurological manifestations consist of an isolated pyramidal syndrome with lower limb spasticity and weakness with or without bladder disturbance and diminution of lower extremity vibration sensation [1]. "Complex" HSPs include other signs and symptoms as cerebellar ataxia, peripheral neuropathy, optic atrophy, congenital cataract, epilepsy, mental retardation, skeletal abnormalities, thin corpus callosum (TCC). Inheritance may be autosomal dominant (AD), autosomal recessive (AR), X-linked or maternally inherited by mitochondrial DNA. Among all recognized HSPs, 79 are classified as spastic paraplegia (SPG1 → 79) on online mendelian inheritance in man (OMIM). The majority of genes are inherited by AR traits. The prevalence of AR-HSP was recently calculated as 1.8/100.000, with SPG11 being the most frequent form, followed by SPG15 [1]. Diagnostic genetic approach to such heterogeneous diseases has been considerably improved by next generation sequencing (NGS). In this paper, we describe the first Italian patient with SPG46 harboring biallelic novel mutations in b-glucosidase 2 gene (*GBA2*).

2. Case presentation

The proband is a 56-year-old Italian man affected by a form of early-onset complicated spastic paraplegia with non-consanguineous parents. His father died for acute myocardial infarction at 50 and his mother is a 70-year-old healthy woman. The onset of disease was at the age of 6 with subtle impairment in maneuvering of the hands and associated

Abbreviations: AD, autosomal dominant; AOA1, ataxia with oculomotor apraxia type 1; AR, autosomal recessive; ARCA, autosomal recessive cerebellar ataxia; ATM, ataxia telengiectasia; FA, Friedreich ataxia; GBA2, b-glucosidase 2; HSPs, hereditary spastic paraplegias; NGS, next-generation sequencing; NPC, Niemann-Pick type C; OMIM, online mendelian inheritance in man; SCA, spinocerebellar ataxia; SPG, spastic paraplegia genes; TCC, thin corpus callosum; XL, X-linked

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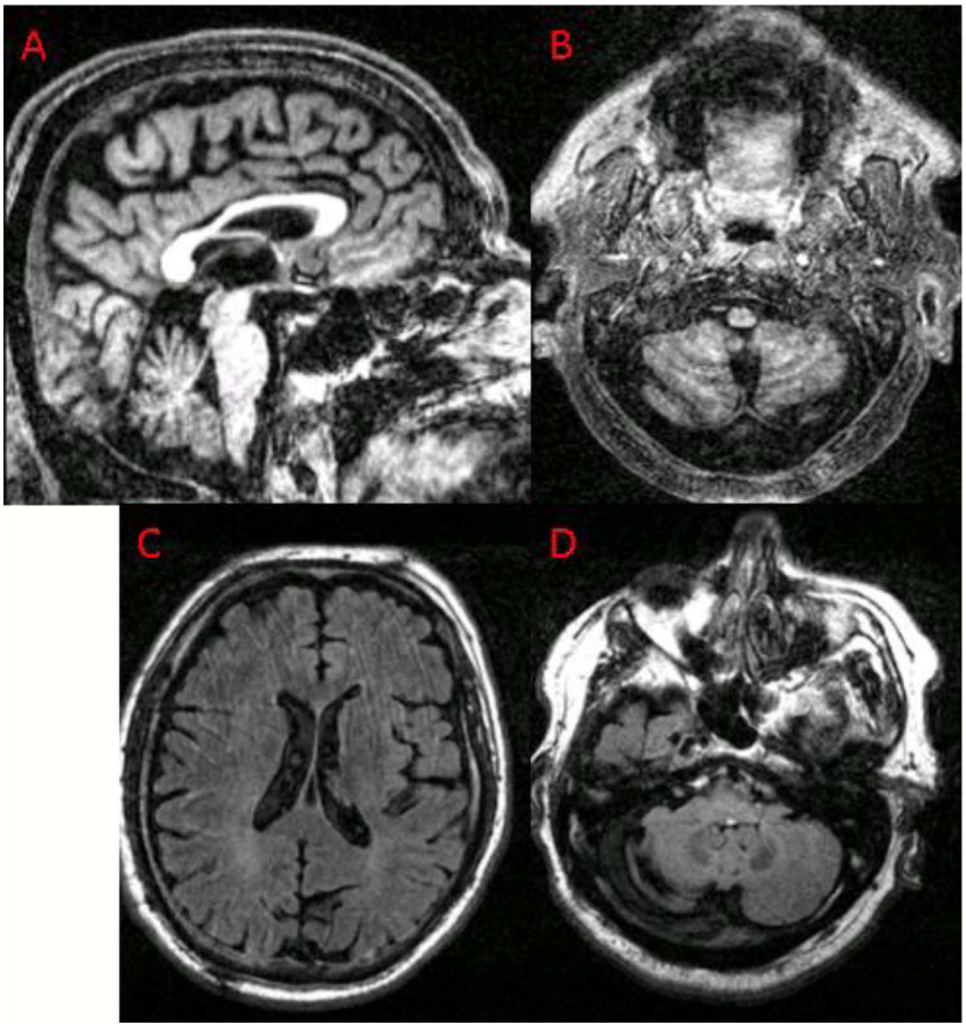

Fig. 1. Brain MRI of SPG46 patient (homozygous mutation c.452-1G > C). A. Sagittal T1 weighted image showing a moderate cerebellar atrophy associated with thinning of the corpus callosum body. B and D. Axial T1 weighted image and Axial T2-FLAIR weighted image demonstrating mild atrophy of the cerebellar hemispheres. C. Axial T2-FLAIR weighted image showing mild and diffuse cortical atrophy without evident white matter changes (MRI quality reduced by motion artifacts).

postural-action tremor. Over the years, upper limbs dysmetria and dysarthria were associated to progressive spastic ataxia. Patient complained weakness, stiffness of the lower limbs and balance disorder, requiring a crutch at 18 years. Bilateral cataract was diagnosed at age 28. The first brain MRI (at age 47) showed cerebellar atrophy, while spinal cord MRI was normal. Genetic tests ruled out Spinocerebellar ataxia (SCA) type 1 (SCA1, *ATXN1*), type 2 (SCA2, *ATXN2*), type 3 (SCA3, *ATXN3*), type 6 (SCA6, *CACNA1A*), type 7 (SCA7, *ATXN7*), Friedreich Ataxia (FA, *FXN*), Ataxia talangiectasia (*ATM*), and Ataxia with oculomotor apraxia type 1 (AOA1, *APTX*), as well as Niemann-Pick type C (*NPC1* and *NPC2*) by a cutaneous biopsy. Vitamin deficiencies, endocrine and coeliac diseases and other metabolic abnormalities were excluded as well. Intellectual development was normal in early childhood, without scholar or professional impact. Mild cognitive decline was noticed at about age 49 and neuropsychological examination at age 52 showed executive functions and declarative memory impairment and ideomotor apraxia.

Neurological examination on admission to our hospital (at age 52) showed typical signs of spastic paraplegia, such as stiffness and weakness of the lower limbs, brisk reflexes and bilateral extensor plantar responses, spastic gait requiring a medical walker. There was association with a cerebellar syndrome, characterized by ataxia, moderate dysarthria and upper limbs intentional tremor. Oculomotor dysfunction was present with saccadic pursuit, slow saccades and bilateral horizontal nystagmus. Hypopallesthesia of ankles and bilateral pes cavus were present. A motor axonal peripheral neuropathy was confirmed by nerve conduction studies (decreased amplitude of compound muscle action potential, mild slowing of conduction velocity and chronic denervation, except for thoracic and brainstem regions), while a muscle biopsy was normal. Serial brain and spinal MRIs showed stable cerebellar atrophy associated with TCC, without clear white matter or brainstem involvement [Fig. 1].

Genomic DNA was extracted from peripheral blood samples according to standard procedures, after receiving patient informed consent. We tested for a panel of 89 known and exploratory spastic paraplegia genes, by a Nextera Custom Enrichment panel (Illumina, San Diego, Ca). We identified a novel homozygous mutation c.452-1G > C in the b-glucosidase 2 gene (*GBA2*), not reported in available databases (dbSNP146, 1000 Genomes, Exome Aggregation Consortium (ExAC), NHLBI Exome Sequencing Project Exome Variant Server and gnomAD). The same mutation, in heterozygosity, was detected in a peripheral blood sample from the proband's mother [Fig. 2]. No other variants possibly associated to HSP were identified. Since the mutation is novel and the parents are not consanguineous, we excluded the possibility of a deletion of GBA2 on the paternal allele or maternal UPD of chromosome 9 by the mean of high-density SNP array. cDNA QRT-PCR showed a reduction of GBA2 mRNA expression levels of about 50% compared with those of normal control in the proband's mother and the absence of GBA2 mRNA in the proband, demonstrating a complete degradation of GBA2 mRNA transcribed from the mutated allele

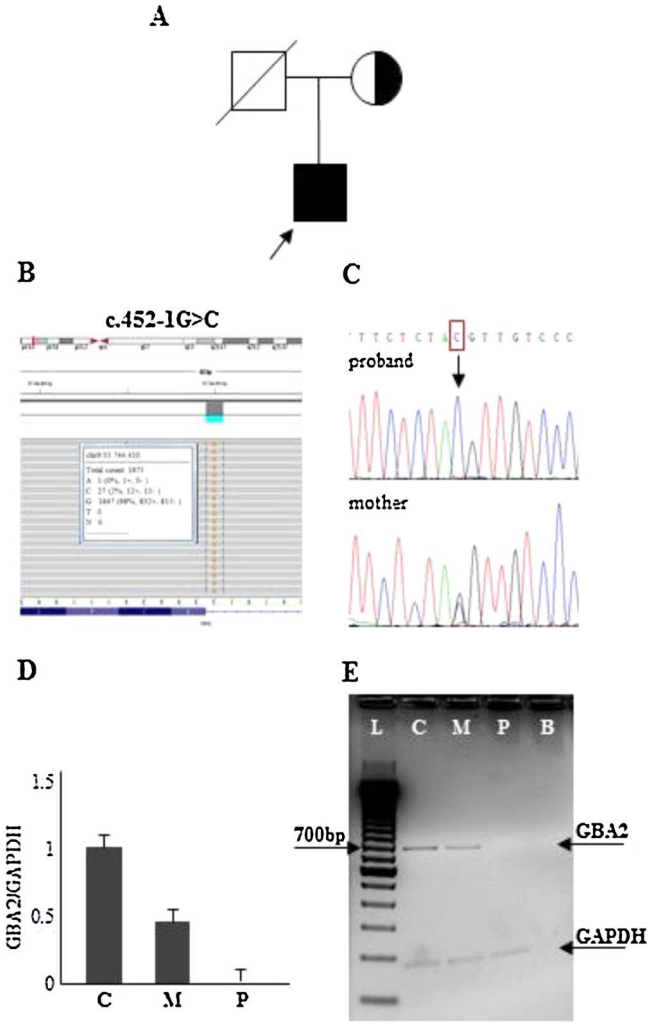

Fig. 2. Mutation analysis. A. Family pedigree. B. The output bam files are visualized on the Integrate Genome Viewer (IGV) software tool. Vertical dotter lines show the position of the pathogenic GBA2 mutation (c.452-1G > C). C. Sanger sequencing of the pathogenic mutation in proband and his mother. The arrows indicate the position of the mutation. D. cDNA QRT-PCR shows the absence of GBA2 mRNA in the proband and a reduction of GBA2 mRNA expression levels of about 50% in his mother compared with those of normal control. E. 2% agarose gel electrophoresis shows the presence of a single fragment of about 700 bp in both control sample and heterozygous proband's mother. L: 100 bp ladder; C: control; M: mother; P: proband; B: blank.

[Fig. 2D].

RT-PCR with primers located in GBA2 exons 1 and 5 revealed the presence of the expected wild-type fragment (700 bp) in the normal control and heterozygous mother, while no amplification was observed in the proband [Fig. 2E].

3. Discussion

We report on the identification of a novel biallelic mutation in *GBA2*, c.452-1G > C, located in the splice acceptor site of exon 3. The identified variant produces a complete loss of *GBA2* mRNA expression caused by an altered splicing process generating an unstable transcript that is rapidly degraded. This mutation should be considered causative for this phenotype. Clinical presentation of our patient matches the SPG46 phenotype [2,3]. In fact, an early-onset, slowly progressive spastic paraplegia complicated by cerebellar ataxia with cerebellar atrophy, bilateral cataract, intellectual deterioration, axonal neuropathy and skeletal abnormalities is consistent with clinical manifestations so far reported in SPG46 [3]. *GBA2* mutations are found also in patients with progressive ataxia as the main symptom at onset in their first two decades, associated successively with other features (*GBA2*-related autosomal recessive cerebellar ataxia (ARCA) with spasticity) [4]. So, sometimes, the different classification between cerebellar ataxia and HSP can appear artificial, revealing instead a continuous spectrum of phenotypes linked to some genes (first of all *SPG7*).

Characteristic brain MRI abnormalities (e.g., cerebellar atrophy and TCC) were compatible with SPG46 [1]. Hypogonadism, that has also been reported in SPG46 [1], was not present in our patient, excluded by hormone blood test.

GBA2 is a non-lysosomal glucosylceramidase, localized on plasma membrane and endoplasmic reticulum. This enzyme degrades glucosylceramide to ceramide and glucose. While the lysosomal form of the enzyme, GBA, is implicated in Gaucher disease, the relevance of GBA2 activity is less understood. GBA2-deficient mice present impaired liver regeneration and male infertility, but it is not clear why they do not develop spastic paraparesis or ataxia. However, alterations in lipid metabolism are known as pathogenic mechanisms underlying several HSPs, such as *CYP7B1* (SPG5), *B4GALTN1* (SPG26), *DDHD1* (SPG28), *FA2H* (SPG35), *PNPLA6* (SPG44), *CYP2U1* (SPG49/56), *DDHD2* (SPG54). Lipid metabolism is involved in membrane composition and its alteration may compromise the fluidity of the cell membrane. In addition, organelle structure modification and intracellular trafficking dysregulation are also linked to impairment of lipid metabolism. All these features are known as potential factors of HSPs pathophysiology [5].

4. Conclusion

Since few families affected by mutations in *GBA2* gene have been previously described [2,3,4], additional observations are interesting, particularly if related to new mutations, in order to better delineate the phenotype and the clinical spectrum. Moreover, our patient confirms the importance of searching for genetic causes in apparently sporadic individuals with spastic paraparesis, after a careful workup to exclude acquired causes, and to disclose key neurological and non-neurological signs in order to better address genomic analysis. However, the availability of a panel able to investigate in parallel an informative number of genes seems greatly effective to find rare mutations especially in AR or sporadic cases.

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Conflict of interest

The authors reports no disclosures relevant to the manuscript.

Statement of human rights

The study was in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. An informed written consent was obtained from the patient.

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