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ORIGINAL COMMUNICATION

Complex phenotype in an Italian family with a novel mutation in *SPG3A*

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Abstract Mutations in the *SPG3A* gene represent a significant cause of autosomal dominant hereditary spastic paraplegia with early onset and pure phenotype. We describe an Italian family manifesting a complex phenotype, characterized by cerebellar involvement in the proband and amyotrophic lateral sclerosis-like syndrome in her father, in association with a new mutation in *SPG3A*. Our findings further widen the notion of clinical heterogeneity in *SPG3A* mutations.

Keywords Hereditary spastic paraplegias · SPG3A · Amyotrophic lateral sclerosis

Introduction

Hereditary spastic paraplegias (HSP) are a group of neurodegenerative diseases characterized by progressive spasticity in the lower limbs, presenting either in isolation ("pure") or associated with a wide array of additional features ("complicated" forms), and great genetic heterogeneity sustained by the identification of 39 loci, 19 of which are described in autosomal dominant (AD) kindreds (HUGO database, www.genenames.org).

The *SPG3A* form of ADHSP typically presents with a pure phenotype, early onset and benign prognosis. The *SPG3A* gene is mutated in approximately 10% of ADHSP families, and it is considered the most common cause of early-onset ADHSP [1].

We report peculiar phenotypic features of two patients from an Italian family carrying a novel *SPG3A* mutation. Written informed consent was obtained for both patients.

Patients and methods

Case 1

A 39-year-old woman had experienced progressive walking disturbance since 5 years of age. Her neurological examination showed spastic gait, lower limb spasticity and proximal weakness, four-limb hyperreflexia, positive jaw jerk reflex, bilateral Troemner, ankle clonus and Babinski signs. Slight bilateral dysmetric finger-to-nose testing and dysdiadochokinesia were also present. There were no sphincter or cognitive disturbances. The neurophysiologic study showed normal motor and sensitive nerve conduction velocities (M- and S-NCV), normal brainstem auditory and visual evoked potentials; absent somatosensory and motor evoked potentials (SSEP and MEP) from the lower limbs. Brain MRI revealed mild vermis atrophy and wide frontal subarachnoid spaces (Fig. 1a). Cerebral [18F]-fluorodeoxiglucose PET showed hypometabolism in the cerebellum and the dorsolateral frontal cortex (Fig. 1b).

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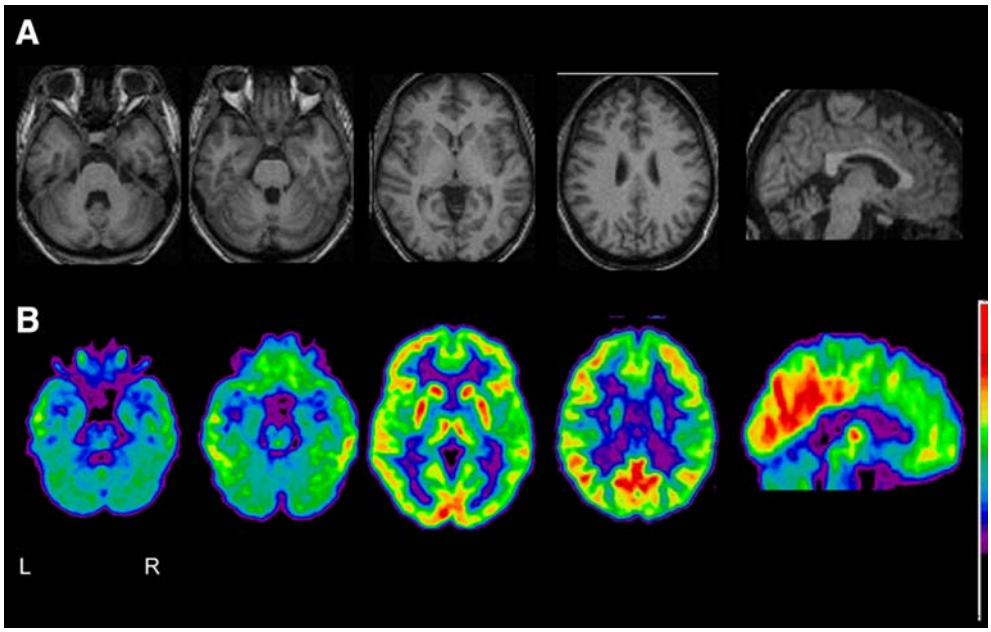

Fig. 1 Axial and sagittal slices of MRI T1 weighted images (**a**) and [18F] FDG PET scans (**b**) in case 1. Images were normalised in the Montreal Neurological Institute space using SPM2 (Wellcome Department of Imaging Neuroscience, London, UK). MRI shows enlargement of the IV ventricle and mild cerebellar vermis atrophy. The [18F] FDG cerebral distribution appears reduced in the cerebellum, mainly in the vermis. Slight hypometabolism is evident in the prefrontal cortex. *L* left side; *R* right side

Case 2

The 70-year-old father of the patient described in Case 1 had 3-year progressive weakness of the right leg, leading to the need of a cane. His neurological examination, at the age of 68 years, revealed weakness, wasting, and sporadic fasciculations in the lower limbs, particularly on the right side where he had foot drop. Tendon reflexes were brisk with left side prevalence and left ankle clonus, while right ankle reflex was absent; the plantar responses were flexor. Mental status, cranial nerves, sensory system, coordination, muscle tonus, and upper limb strength were normal. The neurophysiologic evaluation showed a severe decrease of right external popliteal sciatic nerve muscle action potential amplitude on MNCV; chronic denervation on EMG of different muscles (right biceps brachii, right and left first dorsal interosseous, left vastus medialis, right and left anterior tibialis, right internal gastrocnemius), signs of active denervation at the lower limbs, normal genioglossus EMG; prolonged central conduction time on SSEP and normal MEP. Brain MRI was unremarkable; spine MRI showed several disc protrusions, more evident at L4-S1 levels. At that time he received the diagnosis of clinically probable-laboratory supported amyotrophic lateral sclerosis (ALS).

Recently he developed progressive dyspnea with the need for a respiratory assistive device and severe impairment of fine hand movements. A recent EMG confirmed signs of active denervation even in the upper limbs (right and left first dorsal interosseous, left deltoid).

Molecular analyses

Sequencing the *SPG3A* gene coding exons and flanking intronic regions disclosed a novel heterozygous missense mutation in exon 12 at nucleotide c.1247 G>A (p.Arg416His) (Fig. 2a). Arginine 416 is highly conserved among species (Fig. 2b) and Polyphen modelling analysis (http://genetics.bwh.harvard.edu/pph/) predicts the amino acid substitution to be possibly damaging, with a position-specific independent counts (PSIC) score difference of 1.756.

The mutation was found also in case 2 but not in 25 ADHSP patients, in 50 sporadic cases nor in 250 Italian healthy controls. Multiplex ligation-dependent probe amplification [probe mixtures P165-HSP-B1 and P211-B2 HSP [MRC-Holland, The Netherlands)] [2], denaturing high performance liquid chromatography and direct sequencing of *SPG4* [3] in case 1 did not detect any pathogenic changes. Also, mutations in *NIPA1/SPG6, KIF5A/SPG10, REEP1/SPG31*, and *BSCL2/SPG17* had been ruled out [4]. We have also excluded Krabbe disease in the proband by β-galactocerebrosidase activity estimation (5.4 nmol/h/mgP; normal value 2.0–6.0). The two siblings of the proband refused both clinical and molecular assessment.

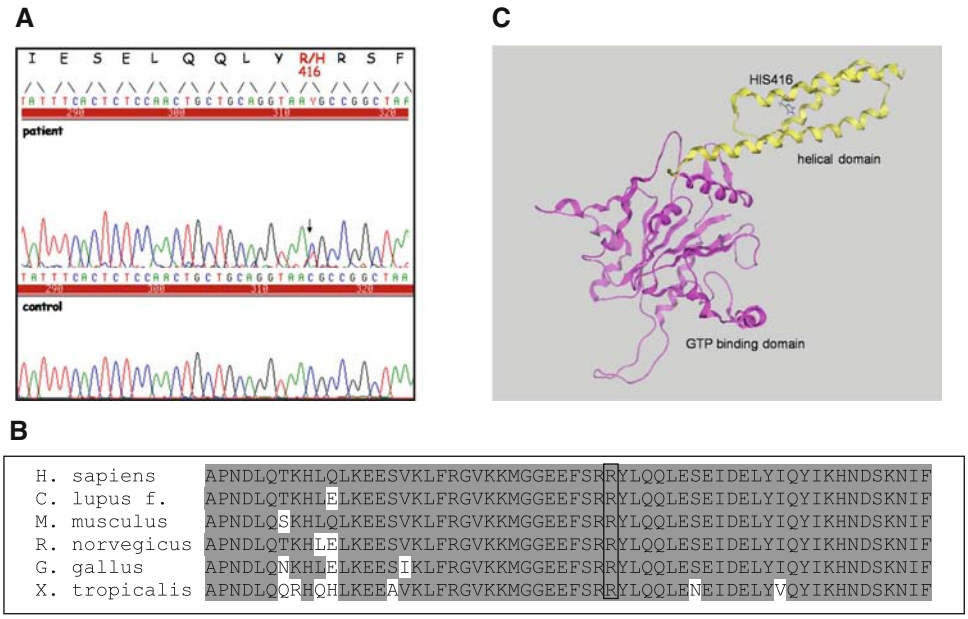

Fig. 2 **a** Electropherogram of the sequences flanking residue R416 in the patient (*upper panel*) and a normal control (*bottom panel*). An *arrow* indicates the p.Arg416His mutation. The sequence reported is in the inverse direction. **b** Species alignments of 60 human atlastin amino acids showing the conservation of residue R416 (*boxed*) (GeneBank accession num.: NP 056999.2, XP 533252.2, NP 848743.1, NP 056999.2, XP 863862.1, NP 001072222). **c** Mutated homology model of the V67-N436 sequence of human atlastin isoform "a" was built up using the MOE package starting from the crystal structures of human guanylate-binding protein 1 (hGBP1 PDB 1DG3 [15]). His416 is located in the helical domain

A homology model of region encompassing residues 67–436 in atlastin-1 was also built up using the MOE (molecular operating environment 2006, www.chemcomp.com) software package (Fig. 2c).

Discussion

We detected a novel mutation (c.1247 G>A) in exon 12 of the *SPG3A* gene in an Italian patient with HSP. This mutation was also found in her father who presented with a peculiar ALS-like phenotype. *SPG3A* encodes atlastin-1, a dynamin/guanylate-binding protein with transmembrane domains, likely involved in membrane trafficking and axonal development [5]. Of the *SPG3A* mutations described so far, about 35% are located in exon 12 [1]. The mutation here detected causes an arginine to histidine substitution in a conserved motif for protein kinase C phosphorylation according to the Prosite search [6], in the middle of an alpha helix near the C-terminus, just before the transmembrane domain (Fig. 2c). It lies far from the catalytic GTPase domain, suggesting a possible role in membrane association or oligomerization.

To date there is convincing evidence that the "apparently" pure phenotype of patients harboring mutations in *SPG4* might also be complicated by additional neurological features, including cerebellar involvement and thin corpus callosum [7] and dysarthria and ataxia with reduced regional cerebellar blood flow at PET scan [8]. Case 1 showed clinical and neuroimaging features that suggest a cerebellar involvement also in *SPG3A*, possibly related to this novel mutation.

There are some reports of an ALS-like phenotype in patients with mutations in *SPG4*. These include a case of rapidly progressive spinal and bulbar upper motor neuron syndrome [9] and that of a patient with early onset ALS and long term survival [10]. Also, in a large screen of HSP patients, neurophysiologic evidence of lower motor dysfunction was recorded in a subgroup of individuals harboring mutations in *SPG4*, one of whom showed progressive bulbar dysfunction and respiratory insufficiency [11]. Even more recently, a new *SPG4* mutation has been associated with the clinical features of the Silver syndrome (MIM #270685) which includes distal amyotrophies [12]. There are only two reports of axonal sensorymotor neuropathy in *SPG3A* families [13, 14]. Case 2 showed clinical and neurophysiologic evidence of upper and lower motor syndrome, fulfilling the El Escorial diagnostic criteria for clinically probable-laboratory supported ALS (www.wfnals.org). The relationship between the novel *SPG3A* mutation and ALS-like phenotype in this patient remains unclear. It seems possible to hypothesize either a double-trouble phenotype in a carrier of a mutation in *SPG3A* or that an ALS-like phenotype is an additional,

though rare, clinical manifestation of the novel p.Arg416His variant. Less likely, *SPG3A* mutations might increase susceptibility for ALS.

Although further studies are required to clarify these issues, it seems useful to alert neurologists to this possible comorbid association and, more importantly, to call for a complete neuroimaging assessment even in "pure" ADHSP patients.

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