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Short communication

Identification of two novel *KIF5A* mutations in hereditary spastic paraplegia associated with mild peripheral neuropathy

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ABSTRACT

Spastic paraplegia type 10 (SPG10) is a rare form of autosomal dominant hereditary spastic paraplegia (AD-HSP) due to mutations in *KIF5A*, a gene encoding the neuronal kinesin heavy-chain involved in axonal transport. *KIF5A* mutations have been associated with a wide clinical spectrum, ranging from pure HSP to isolated peripheral nerve involvement or complicated HSP phenotypes. Most KIF5A mutations are clustered in the motor domain of the protein that is necessary for microtubule interaction. Here we describe two Spanish families with an adult onset complicated AD-HSP in which neurological studies revealed a mild sensory neuropathy. Intention tremor was also present in both families. Molecular genetic analysis identified two novel mutations c.773 C>T and c.833 C>T in the *KIF5A* gene resulting in the P258L and P278L substitutions respectively. Both were located in the highly conserved kinesin motor domain of the protein which has previously been identified as a hot spot for *KIF5A* mutations. This study adds to the evidence associating the known occurrence of mild peripheral neuropathy in the adult onset SPG10 type of AD-HSP.

1. Introduction

Hereditary spastic paraplegias (HSP) are a group of clinically and genetically heterogeneous neurodegenerative disorders characterized by progressive spastic weakness of the lower limbs due to axonal degeneration of the corticospinal tract [1,2].

Clinically 'pure' HSP have isolated pyramidal signs and are defined by progressive lower limb spasticity, hyperreflexia and weakness, which may be associated with sphincter disturbances and mild sensory disturbance in the lower limbs [1]. 'Complex' HSP may show variable combinations of neurological and non-neurological symptoms in addition to spasticity [2–8].

HSP can be inherited as an autosomal-dominant (AD-HSP), recessive (AR-HSP), or X-linked recessive trait and corresponds to a wide range of mutated loci (SPG1-48 loci) and genes [9]. Between 70% and 80% of the cases of HSP reported in Europe are due to mutations in dominant loci, and among them mutations in a few specific genes. *SPAST* (SPG4), *ATL1* (SPG3A) and *REEP1* (SPG31) are responsible for the majority (50–60%) of AD-HSP cases [8,10]. SPG10 (OMIM 604187) is an unusual form of AD-HSP due to mutations in the neuronal kinesin heavy-chain *KIF5A* gene (OMIM 602821) involved in anterograde axonal transport [11–15]. Despite the rarity of these mutations, SPG10 may represent one of the major causes of complicated forms of AD-HSP in Europe [11,14–18]. In SPG10, the most frequently associated features are amyotrophy in the lower extremities and/or clinical or subclinical sensory–motor neuropathy [11,16–20]. Signs of spasticity in the upper limbs may also guide diagnosis toward the SPG10 form [16]. Identical *KIF5A* mutations can cause either moderate HSP or severe CMT2-like symptoms in different members of the same family [16], suggesting that environmental and genetic differences have a strong influence.

Here we describe two Spanish families with AD-HSP-associated mild sensory neuropathy due to two different novel mutations in *KIF5A*, both located in the highly conserved kinesin motor domain.

2. Material and methods

2.1. Clinical evaluation

Our study included four individuals from two Spanish families compatible with AD-HSP and diagnosed according to Harding's criteria [21,22]. Informed consent was obtained from members of both families. Clinical assessments, biological analysis and neurophysiological examinations were performed using standard techniques. Complex HSP was defined if, in addition to the LL pyramidal syndrome, the patient had other variable combinations of neurological and non-neurological symptoms [23,24]. Functional impairment was measured by the Spastic Paraplegia Rating Scale (SPRS) [25].

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2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood by using standard high-salt extraction methods. Mutation analysis of the *KIF5A* gene was performed by direct sequencing of the entire coding region, including splice site boundaries (RefSeq NM_004984.2), with specific primers designed using Primer 3 [26]. Molecular analysis of the *SPAST* (*SPG4*), *ATL1* (*SPG3A*) and *REEP1* (*SPG31*) genes had been previously carried out in all subjects. The new *KIF5A* mutations identified were confirmed on a second PCR performed on a second aliquot of patients' DNA. A hundred healthy individuals (200 chromosomes) from the Spanish population acted as controls and were checked for the mutations under examination. All mutations were described in accordance with the Human Genome Variation Society (HGVS) recommendations (http://www.hgvs.org/mutnomen).

Software tools were used to estimate the putative effect of amino acid changes on protein function by an in silico approach: SIFT (Sortin Intolerance from Tolerance; http://sift-jcvi.org), Align GVGD (http://agvgd.iarc.fr/), POLYPHEN (Polymorphism Phenotyping; http://genetics.bwh.harvard.edu/pph/) and Mutation Taster (http://doro.charite.de/MutationTaster). The evolutionary conservation of changed amino acids was evaluated using the ClustalW algorithm (www.ebi.ac.uk/clustalw/).

3. Results

3.1. Clinical evaluation

Proband PE-30 II:4 was a 70-year-old man who, since the age of 35, had complained of progressive walking difficulties due to rigidity in the lower limbs, and required two crutches to walk. Neurological examination revealed moderate spastic gait, markedly increased muscle tone in the lower limbs with severe pyramidal weakness, brisk proximal tendon reflexes with reduction of the distal reflexes in the four limbs, presence of Hoffman's sign and bilateral Babinski sign. Moreover, a slight sensory neuropathy was noted: neurophysiological studies revealed mildly reduced motor and sensory action potentials with relatively preserved nerve conduction in the lower limbs. Additionally, the patient showed hyperactive bladder with nocturia (Table 1). Clinical and neurophysiological evaluations were also performed in the offspring of the proband (Fig. 1). PE-30 III:3 presented mild spastic gait with brisk reflexes in the four limbs, bilateral Babinski sign and intention tremor, but no other symptoms or signs of lower motor involvement. PE-30 III:1 and PE III:2 showed no neurological abnormalities.

Positive family history for gait abnormalities in PE-32 II:1 suggested an AD-HSP (Fig. 1a): the deceased father and three of the proband's six siblings complained of rigidity in the lower limbs with gait disturbances and intention tremor or Parkinsonism. The other three siblings were asymptomatic. PE-32 II:1 presented an adult onset complicated form of HSP. Disease progression was mild, given that the patient was still able to have unaided walk at the time of examination. Neurological examination showed mild spastic paraparesis with unsteadiness associated with extrapyramidal signs, marked LL hyperreflexia with augmented symmetric patellar clonus and ankle reflexes, as well as increased bilateral plantar reflexes. PE-32 II:1 suffered from postural and action tremor of the hands. Brain/spinal cord MRI showed diffuse panencephalic atrophy and severe cervicoarthrosis with secondary stenosis of the spinal canal. Sensory deficits or signs of lower motor involvement were absent. There was no evidence of ataxia, retinal involvement, epilepsy or sphincter impairment (Table 1).

3.2. Mutation analysis

Genetic testing of PE-30 and PE-32 families was negative for *SPAST* (SPG4), *ATL1* (SPG3A) and *REEP1* (SPG31). Mutation screening of the *KIF5A* gene revealed two novel heterozygous *missense* mutations located in exon 10 (c.833 C>T, p.P278L) and in exon 9 (c.773 C>T, p.S258L) respectively (Fig. 1b). Both substitutions affect highly conserved residues distributed throughout the kinesin motor domain of the protein. They were not detected in a panel of 200 chromosomes from a healthy Spanish population used as controls; nor were they reported in any of the well-known single-nucleotide polymorphism (SNP) databases. Segregation studies were only possible in the PE-30 family, in which the P278L *missense* mutation was found to segregate in the affected individual PE-30 III:3 (Fig. 1b).

4. Discussion

Mutations in *KIF5A* have been shown to cause a late onset, mild-to-moderate form of slowly progressing AD-HSP (SPG10) [11,13–16]. SPG10 patients may also present sensory–motor peripheral neuropathy [11,16–20]. The clinical features of the families described in this study, both of which included paraparesis complicated by mild sensory neuropathy, conform closely to the phenotypic range so far associated to the SPG10 form (Table 1). The proband PE-30 II:4 showed a mild sensory neuropathy that was more prevalent in the lower limbs, with mild clinical manifestations, while PE-30 III:3 had intention tremor. Index patient P32 II:1 also suffered from postural and action tremor of the hands and had a family history of gait disturbances due to rigidity in the lower limbs and intention tremor (Table 2).

KIF5A is one of the major plus-end-directed microtubule anterograde motors for the slow axonal transport of neurofilaments [9,27,28]. A defect in this kinesin cargo delivery at the axon tip is the likely reason for the degeneration of the peripheral nerves which has been proposed as the pathogenic mechanism in SPG10 [17,27–29]. Most of the *KIF5A* mutations reported are located close to or inside the highly conserved kinesin motor domain, a known mutational hot spot in SPG10 patients [14,15].

The two mutations found in this study were located inside the microtubule-binding site included in the switch II cluster within the kinesin motor domain of KIF5A [11,13–16,18,19,30,31] (Fig. 1c–d). S258L is located directly in the α4 domain next to loop 11, which connects the microtubule and ATP-binding sites (Fig. 1c–d) in the motor domain. P278L substitution is located directly in the loop 12 domain [30,31]. Both changes should block the conformational change within the two loops needed for kinesin physiological activity [32,33]. Moreover, they affect highly evolutionarily conserved residues within human members of the different kinesin families, as well as among *KIF5A* homologues from different species (Fig. 1c).

In silico analysis of these *missense* mutations reveals that they are likely to damage the structure and function of the corresponding kinesin motor domain (Table 2). Furthermore, the two novel mutations were not identified in 200 control chromosomes from the healthy Spanish population or found in well-known single-nucleotide polymorphism (SNP) databases, suggesting that they were unlikely to be rare polymorphisms.

In conclusion, the results of this study add to the wide range of neurological impairment reported in the previously published *KIF5A* mutations [16,17]. Although SPG10 is a rare form of AD-HSP, clinicians should be on the lookout for signs of peripheral neuropathy as a recurrent feature in SPG10.

Conflict of interest

All authors declare no conflict of interest.

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The project conformed to the tenets of the Declaration of Helsinki and written consent was obtained.

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