# SPG10 is a rare cause of spastic paraplegia in European families

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## ABSTRACT

**Background:** SPG10 is an autosomal dominant form of hereditary spastic paraplegia (HSP), which is caused by mutations in the neural kinesin heavy chain *KIF5A* gene, the neuronal motor of fast anterograde axonal transport. Only four mutations have been identified to date.

**Objective:** To determine the frequency of SPG10 in European families with HSP and to specify the SPG10 phenotype.

**Patients and methods:** 80 index patients from families with autosomal dominant HSP were investigated for SPG10 mutations by direct sequencing of the *KIF5A* motor domain. Additionally, the whole gene was sequenced in 20 of these families.

**Results:** Three novel *KIF5A* mutations were detected in German families, including one missense mutation (c.759G>T, p.K253N), one in frame deletion (c.768_770delCAA, p.N256del) and one splice site mutation (c.217G>A). Onset of gait disturbance varied from infancy to 30 years of age. All patients presented clinically with pure HSP, but a subclinical sensory–motor neuropathy was detected by neurophysiology studies.

**Conclusions:** SPG10 accounts for approximately 3% of European autosomal dominant HSP families. All mutations affect the motor domain of kinesin and thus most likely impair axonal transport. Clinically, SPG10 is characterised by spastic paraplegia with mostly subclinical peripheral neuropathy.

Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of disorders that share the key symptom of lower extremity spasticity due to progressive degeneration of the corticospinal tract.[1 2] At present, at least 32 loci for HSP, termed SPG1-37, are known, including nine genes for autosomal dominant disease.[3] SPG3A (10%) and SPG4 (40–50%) are the most frequent forms, responsible for more than 50% of dominant HSP cases.[4 5]

SPG10 is an autosomal dominant HSP (adHSP) caused by mutations in the gene encoding kinesin heavy chain *KIF5A*, the motor of anterograde axonal transport.[6] To date, only four mutations have been described, three of them located in the *KIF5A* motor domain and one in the adjacent coiled coil domain.[6–9]

To determine the frequency of SPG10 in German, Dutch, Austrian and Serbian families with HSP and to define the phenotype of SPG10, we investigated index patients from families with adHSP by direct sequencing of the *KIF5A* gene.

## PATIENTS AND METHODS

Eighty index patients from German (n = 41), Dutch (n = 25), Austrian (n = 9) and Serbian (n = 5) families segregating spastic paraplegia in an autosomal dominant manner were included in this study following established diagnostic criteria.[10 11]

*Spastin* mutations (SPG4) had been excluded by direct sequencing or dHPLC in 51 of 80 patients (64%).

The study was approved by the local ethics committee (Vote 277/2004). Informed and written consent was obtained from all participants.

DNA was extracted from blood samples following standard procedures.

Direct sequencing of the kinesin motor domain, encoded by exons 1–11 of the *KIF5A* gene (NM004984), was performed in all index patients. In 20 of the German index patients, the whole coding sequence (exons 1–28) was analysed (see supplement online for experimental details and primer sequences).

Nerve conduction studies were performed according to standard techniques. Motor evoked potentials were recorded from the first dorsal interosseus and tibialis anterior muscle using a circular coil according to standard techniques.[12]

## RESULTS

### Sequencing of the KIF5A gene

Mutational screening in 80 index patients revealed three novel mutations in the KIF5A motor domain, including one missense mutation (c.759G>T, p.K253N), one in frame deletion (c.768_770delCAA, p.N256del) and one splice site mutation (c.217G>A). None of these sequence variations was present in 384 unrelated control alleles. Cosegregation with the disease was shown where possible (fig 1).

Interestingly, residue N256 that is deleted by the c.768_770delCAA mutation was shown to cause HSP when replaced by serine in a British family.[6] The c.217G>A nucleotide exchange affects a potential splice donor site and reduces splicing efficiency from 81% to 0%, as predicted by NNSPLICE 0.9.[13] The effect of the c.217 G>A mutation on mRNA splicing could not be tested experimentally as *KIF5A* is not expressed in peripheral blood and no other tissue samples were available from the affected patient.

All three novel mutations are predicted to affect protein function using programs designed to predict deleterious effects of nucleotide exchanges based on sequence homologies and physical amino acid properties (SIFT: http://blocks.fhcrc.org/sift/sift.html).

The kinesin motor domain, corresponding to amino acid residues 1–324, is highly conserved within the human kinesin family as well as

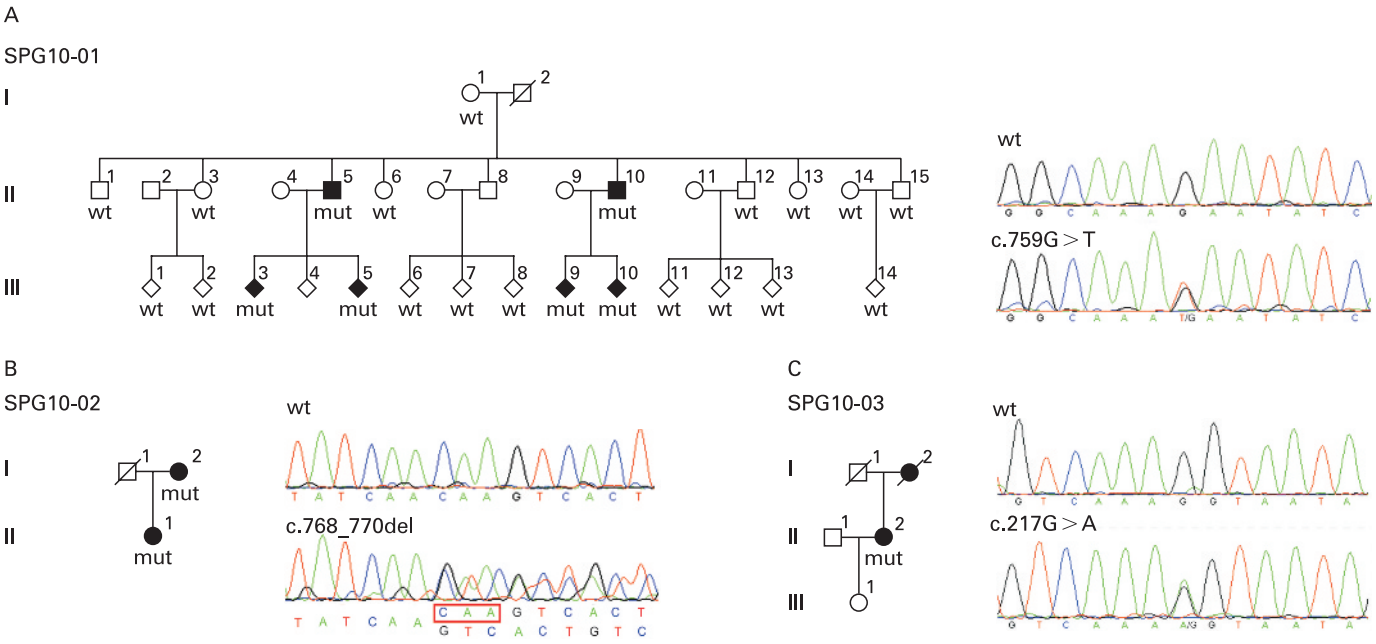


**Figure 1** Pedigrees of SPG10 families; electropherograms and cosegregation of the mutations. (A) Pedigree of family SPG10-01, modified to ensure confidentiality. The c.759G>T mutation was verified in all six living affected family members. No unaffected family members carried the mutation. This mutation leads to replacement of lysine by asparagine at amino acid position 253 (K253N). (B) Pedigree of family SPG10-02. The index patient (II-2) as well as her affected mother carry a heterozygous three base pair deletion at position 768_770 of the coding sequence, resulting in an inframe deletion of asparagine at amino acid position 256. (C) Pedigree of family SPG10-03. A nucleotide exchange at position 217 of the coding sequence leads to loss of a splice donor site in the index patient (II-2). Filled symbols denote affected individuals, open symbols denote unaffected individuals; deceased family members are marked by a slash.

between species (see supplementary fig 1 online). The three novel mutations found in this screen all affect highly conserved amino acid residues in the motor domain (see supplementary fig 2 online). No mutations outside the motor domain (exons 1–11) were detected in the 20 subjects in whom the whole *KIF5A* gene was sequenced.

SPG10 mutations were found in 3 of 41 (7%) German adHSP families. No mutations were detected in Dutch, Austrian or Serbian families. In the total cohort, the frequency of SPG10 mutations was 3/80 (4%).

### Clinical description and electrophysiological characteristics of SPG10 patients

#### SPG10-01

The six affected members of family SPG10-01 presented with a pure form of HSP with onset of gait disturbance between early infancy and adulthood (mean age at onset 15.7 years (range 2–30)). All patients were still ambulant at the time of examination despite disease duration of more than 50 years in one family member (II-5). Three affected family members suffered from postural and action tremor of the hands consistent with the diagnosis of essential tremor that segregated independently in this family.

Apart from mild reduction of vibration sense distally in the legs, no sensory deficits were observed. Other symptoms or signs of lower motor involvement were absent. Neurophysiological examination, however, revealed subclinical peripheral neuropathy of the sensor–motor type with axonal and demyelinating features (table 1).

#### SPG10-02

The index patient of family SPG10-02 (II-1) developed a progressive spastic gait disturbance at 19 years of age. At age 29 years she was diagnosed with multiple sclerosis because of left-sided hemiparesis responding to cortisone pulse therapy, immunoreactive CSF syndrome and multiple hyperintense, partially contrast enhancing, predominantly periventricular but also spinal lesions on MRI. Additionally, sensory–motor neuropathy was noted. Sural nerve biopsy revealed chronic axonal neuropathy without signs of inflammation. Family history was positive with the mother also being affected by spastic gait. On examination, visual acuity was reduced on the right eye with optic atrophy on fundoscopy. Sensory deficits included impaired vibration and joint position sense. Further findings are presented in table 1.

#### SPG10-03

The index patient of family SPG10-03 (II-2) reported onset of a spastic gait disturbance at the age of 51 years. On inquiry, however, she recognised a Trendelenburg-like gait, particularly during running, since young adulthood. Only after replacement of both hip joints due to arthritis at the age of 51 years did this gait abnormality became more obvious in everyday life and was noted to be progressive. In addition to spastic paraplegia, subject II-2 suffered from bladder and bowel urge incontinence. No signs and symptoms of complicated disease were noted.

## DISCUSSION

SPG10 was initially regarded as an infantile onset form of HSP.[6 7] In agreement with two previously described SPG10 families[8 9] however, our families demonstrated that age at onset is actually quite variable and ranges from early childhood until the third decade of life.

The classification of HSP into pure and complicated forms is based on clinical criteria.[10] Using these criteria, SPG10 presents as a clinically pure form of HSP with only mild amyotrophy

**Table 1** Clinical and neurophysiological features of SPG10 patients

| | SPG10-01 | | | | | SPG10-02 | SPG10-03 |
|---|---|---|---|---|---|---|---|
| | II-5 | II-10 | III-3 | III-9 | III-10 | II-1 | II-2 |
| Clinical characteristics | | | | | | | |
| Age at examination (y) | 56 | 47 | 21 | 25 | 19 | 32 | 58 |
| Sex | M | M | M | F | F | F | F |
| Age at onset (y) | 2 | 30 | 21 | 4 | 15 | 19 | Young adulthood |
| Disease duration (y) | 54 | 17 | 0.5 | 21 | 4 | 13 | >30 |
| Degree of disability* | (3) | (1) | (1) | (1) | (1) | (2) | (3) |
| Weakness (LL) | + | + | + | + | + | + | + |
| Muscle wasting | – | – | – | – | – | – | – |
| Spasticity (UL) | – | – | – | – | – | – | – |
| Spasticity (LL) | + | + | + | + | + | + | + |
| Hyperreflexia (LL) | + | + | + | + | + | + | + (PSR ↑) |
| Hyporeflexia (LL) | – | – | – | – | – | + (ASR missing) | + (ASR missing) |
| Babinski's sign | + | – | + | + | + | + | + |
| Impaired vibration sense (LL) | + | + | – | | | + | + |
| Urinary urgency | + | + | – | + | + | + | + |
| Neurophysiology | | | | | | | |
| MNCV tibial nerve (N >41 m/s) | 40.0 | 33.1 | 39.9 | | | 36.7 | 40 |
| CMAP tibial nerve (N >10 mV) | 2.3 | 1.0 | 4.6 | | | 2.2 | 22.1 |
| F latency tibial nerve (N <55 ms) | 46.7 | No F waves | 53.3 | | | No F waves | 51.4 |
| SNCV sural nerve (N >45 m/s) | 52 | 48 | 40 | | | | 52 |
| SNAP sural nerve (N >10 μV) | 2.1 | 1.0 | 3.2 | | | No SNAP | 5.9 |
| EMG TA | | Chronic neurogenic | | | | Chronic neurogenic | |
| CMCT TA left/right (N <16 ms) | 21.9/23.4 | 25.8 | 25.0/29.7 | | | No MEP | |
| CMCT FDI left/right (N <8 ms) | 8.6/8.9 | 9.0 | 9.8/10.6 | | | 7.8/7.8 | |

*(1) able to walk unassisted >500 m; (2) able to walk >500 m with walking aid; (3) able to walk <500 m; (4) not able to walk.
Numbering of individuals corresponds to fig 1.
ASR, Achilles' tendon reflex; CMAP, compound muscle action potential; CMCT, central motor conduction time; EMG, electromyography; FDI, first dorsal interosseus muscle; LL, lower limb; MEP, motor evoked potential; MNCV, motor nerve conduction velocity; N, normal values; PSR, patellar tendon reflex; SNAP, sensory nerve action potential; SNCV, sensory nerve conduction velocity; TA, tibialis anterior muscle; UL, upper limb.
+, present; –, absent.

occurring occasionally in the later stages of the disease. However, subclinical involvement of the sensory and motor peripheral nervous system was noted in all SPG10 patients examined neurophysiologically. This is in accordance with pan-neuronal expression of *KIF5A*, that has been shown in mice.[14] As *KIF5A* mutations are most likely to affect axonal transport, it is plausible that neurons with particularly long axonal processes such as those constituting the corticospinal tract, dorsal columns and peripheral nerves would be affected in SPG10.

In all SPG10 families reported to date and also in the large German family described here, *KIF5A* mutations showed 100% penetrance. SPG10 is slowly progressive and follows a rather benign course; none of our patient has lost the ability to walk even after more than 50 years into the disease.

The frequency of SPG10 among adHSP has not been investigated previously. We screened 80 index patients of adHSP families from central Europe for *KIF5A* mutations and detected three mutations (~4%). Spastin mutations had been previously excluded in 51 of our patients. As about 60% of adHSP are SPG4 negative,[15] correction for this inclusion bias yields a SPG10 frequency of ~3% in European adHSP families (3/(29+51/0.6)). It has to be noted, however, that no mutations were detected in Dutch, Austrian or Serbian families.

None of the four previously published *KIF5A* mutations[6–9] were identified in our sample. It is remarkable, however, that five of the now seven known *KIF5A* mutations, including K253N and N256del identified in this study, are located in the switch cluster, encoded by exons 9 and 10 of the *KIF5A* gene. The switch cluster senses the χ-phosphate in bound nucleotide and triggers nucleotide dependent conformational changes in the motor.[16] Replacement of the KIF5B homologue of K253 (K252) by alanine has been shown to interfere with microtubule dependent ATPase activation and ATP turnover rate in vitro.[17] The N256 mutations, as indicated by Reid *et al*, might cause decoupling of nucleotide and microtubule binding of the motor.[18] The putative splice site mutation c.217G>A is predicted to result in omission of exon 3 which would lead to loss of the p-loop (N1) that is essential for the interaction of kinesin motor and ATP. This suggests that ATP hydrolysis and microtubule binding of *KIF5A* might be key targets of HSP pathogenic mutations.

Pure adHSPs differ little in their clinical presentation and the discriminating features that might exist are often lost in the noise of phenotypic variability of specific adHSP subtypes. With nine dominant HSP genes known to date, containing nearly 16 kB of coding sequence, pragmatic guidelines for genetic testing are warranted. We suggest genetic testing for SPG10 in families with pure forms of HSP and onset before 45 years of age in all affected family members. Although no clusters of SPG10 mutations have been found, all private mutations identified so far are located in the kinesin motor domain.

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**Competing interests:** None.

**Ethics approval:** The study was approved by the local ethic committee (Vote 277/2004).

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