Mutation analysis of the spastin gene (SPG4) in patients with hereditary spastic paraparesis

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Abstract

Background—Hereditary spastic paraparesis is a genetically heterogeneous condition. Recently, mutations in the spastin gene were reported in families linked to the common SPG4 locus on chromosome 2p21-22.

Objectives—To study a population of patients with hereditary spastic paraparesis for mutations in the spastin gene (SPG4) on chromosome 2p21-22.

Methods—DNA from 32 patients (12 from families known to be linked to SPG4) was analysed for mutations in the spastin gene by single strand conformational polymorphism analysis and sequencing. All patients were also examined clinically.

Results—Thirteen SPG4 mutations were identified, 11 of which are novel. These mutations include missense, nonsense, frameshift, and splice site mutations, the majority of which affect the AAZA cassette. We also describe a nucleotide substitution outside this conserved region which appears to behave as a recessive mutation.

Conclusions—Recurrent mutations in the spastin gene are uncommon. This reduces the ease of mutation detection as a part of the diagnostic work up of patients with hereditary spastic paraparesis. Our findings have important implications for the presumed function of spastin and schemes for mutation detection in HSP patients.

Keywords: spastin; hereditary spastic paraparesis; mutation; recessive

Hereditary spastic paraparesis (HSP) comprises a group of inherited neurodegenerative disorders. The defining clinical feature is progressive lower limb weakness and spasticity, with a common pathological feature being degeneration of the corticospinal tracts.1-3 HSP is genetically heterogeneous. Autosomal dominant, autosomal recessive, and X linked inheritance have been described in seven families with the disease showed an autosomal dominant mode of inheritance, but which were too small for linkage analysis, or were the only known case in their family. At least one affected member of each family was examined and a detailed neurological history taken by one of the authors (CMD, KW, ER).

DNA extraction from whole blood was carried out using standard procedures. Primers were designed for detection by single strand conformational polymorphism (SSCP) analysis of the 17 exons of the SPG4 gene using the spastin cDNA sequence (GenBank accession AJ246001) and the genomic sequence (Genbank accession AJ246003). The sequences of the primers are shown in table 1.

PCR was performed using the annealing temperatures for the primers shown in table 1. SSCP was performed on two types of gels: 50% MDE™ (Flowgen) and 5% glycerol gels in 0.6% TBE and 8% polyacrylamide (49:1 acrylamide:bisacrylamide) gels in 0.6% TBE. The latter type of gel did not allow the detection of any changes than were not seen on the MDE gel, but this gel was better at separating the products of exon 1a and 1b amplification. PCR products showing SSCP changes were sequenced directly on an ABI377 DNA Sequencer. In addition, some changes were
confirmed by cloning into pGemTeasy (Promega) and sequenced. For each SSCP change detected in the patients, 100 normal chromosomes were screened under the same conditions.

Results

Mutations in the SPG4 gene were found in 14 families, nine of whom were already known to be linked to the SPG4 locus.

Clinical Examination of Patients

At least one affected member of each family was examined. A synopsis of the major points in the history of each family in whom a spastin mutation was identified is detailed below in the order in which the results are presented in table 2. The clinical features for each family are summarised in table 3. Clinical features for families C5, C7, C22, C24, C25, and C27 have been described in more detail previously.

N35

The single patient examined in this family was the only member of his family (in preceding or subsequent generations, including his three children and 11 grandchildren) with any neurological symptoms at all. He had two sibs, one of whom died at the age of 39 years from myocardial infarction and the other at the age of 81 years of “old age”. Neither had had any mobility problems. He was never able to run, but was otherwise asymptomatic until the age of 60 years when he began to notice slowness of his gait and stiffness of his lower limbs with a tendency to trip and difficulty climbing hills. These symptoms progressed slowly, but he remained ambulant at the age of 75 years. On neurological examination, he had mild pes cavus, an exaggerated lumbar lordosis, and a symmetrical, moderately severe spastic paraparesis with mild impairment of vibration sense in the lower limbs. A mild degree of urinary hesitancy was reported, but the upper limbs were normal.

C24

Clinical information was available for seven affected members from two generations of this family, all of whom had typical clinical features of pure spastic paraplegia. Age at onset of symptoms ranged from 2 to 33 years. Three family members used a walking stick and one, symptomatic for 30 years, used a wheelchair. One family member had bladder involvement, one family member suffered from constipation, and another from faecal urgency. The rate of disease progression was variable, though tended to be slow. Three patients had upper limb hyperreflexia. Three subjects had pes

Table 2. Novel mutations detected in the spastin gene

<table>
<thead>
<tr>
<th>Family</th>
<th>No of affected patients examined in family</th>
<th>Average age of onset of disease (range)</th>
<th>Mutation</th>
<th>Exon/intron</th>
<th>Nucleotide change</th>
<th>Protein change</th>
</tr>
</thead>
<tbody>
<tr>
<td>N35 1</td>
<td>60</td>
<td>Missense (present homozgyously)</td>
<td>Exon 1</td>
<td>256C&gt;T</td>
<td>S44L</td>
<td></td>
</tr>
<tr>
<td>C24 7</td>
<td>2–33</td>
<td>Frameshift</td>
<td>Exon 1</td>
<td>411delG</td>
<td>Frameshift aa96-159</td>
<td></td>
</tr>
<tr>
<td>N4 5</td>
<td>26.2 (&lt;5-37)</td>
<td>Nonsense</td>
<td>Exon 5</td>
<td>859G&gt;G</td>
<td>S245Stop codon</td>
<td></td>
</tr>
<tr>
<td>N5 11</td>
<td>28.1 (1–50)</td>
<td>Nonsense</td>
<td>Exon 5</td>
<td>859G&gt;G</td>
<td>S245Stop codon</td>
<td></td>
</tr>
<tr>
<td>N37 1</td>
<td>3 (2–5)</td>
<td>Splice</td>
<td>Intron 8</td>
<td>1298+1g&gt;a</td>
<td>Presumed missplicing (skipping of exon 8)</td>
<td></td>
</tr>
<tr>
<td>N2 5</td>
<td>11.7 (1.5-20)</td>
<td>Missense/nonsense</td>
<td>Exon 10</td>
<td>139T&gt;A</td>
<td>R424G</td>
<td></td>
</tr>
<tr>
<td>C27 9</td>
<td>20.3 (&lt;10–27)</td>
<td>Frameshift/nonsense</td>
<td>Exon 10</td>
<td>1406delT</td>
<td>Frameshift aa47-436</td>
<td></td>
</tr>
<tr>
<td>C5 8</td>
<td>31.4 (&lt;5-50)</td>
<td>Splice</td>
<td>Intron 11</td>
<td>1538+3c</td>
<td>Presumed missplicing (skipping of exon 11)</td>
<td></td>
</tr>
<tr>
<td>N36 1</td>
<td>40 (&lt; not seen)</td>
<td>Splice</td>
<td>Intron 11</td>
<td>1538+3del(aag)</td>
<td>Presumed missplicing (skipping of exon 11)</td>
<td></td>
</tr>
<tr>
<td>C7 6</td>
<td>21.4 (&lt;2-33)</td>
<td>Splice</td>
<td>Intron 12</td>
<td>1618+2c&gt;</td>
<td>Presumed missplicing (skipping of exon 12)</td>
<td></td>
</tr>
<tr>
<td>C22 5</td>
<td>18.5 (&lt;2-30)</td>
<td>Splice</td>
<td>Intron 13</td>
<td>1661+2c&gt;</td>
<td>Presumed missplicing (skipping of exon 13)</td>
<td></td>
</tr>
<tr>
<td>N37 1</td>
<td>11 (—)</td>
<td>Splice</td>
<td>Intron 15</td>
<td>1812+2c&gt;</td>
<td>Presumed missplicing (skipping of exon 15)</td>
<td></td>
</tr>
<tr>
<td>C25 11</td>
<td>26.4 (15–41)</td>
<td>Splice</td>
<td>Intron 16</td>
<td>1853+2c&gt;</td>
<td>Presumed missplicing (skipping of exon 16)</td>
<td></td>
</tr>
<tr>
<td>N8 3</td>
<td>40 (40–40)</td>
<td>Missense</td>
<td>Exon 17</td>
<td>1875G&gt;C</td>
<td>D584H</td>
<td></td>
</tr>
</tbody>
</table>
Mutation analysis of the spastin gene (SPG4)

Table 3 Clinical features observed in families with spastin gene mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>No of affected</th>
<th>m&amp;AO (range)</th>
<th>Severity: use of stick/wheelchair</th>
<th>Vibration sense</th>
<th>Bladder disturbance</th>
<th>Ptus cavius</th>
<th>Distal amyotrophy</th>
<th>Upper limb hyperreflexia</th>
<th>Cognition</th>
<th>Other comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>N3</td>
<td>1</td>
<td>60</td>
<td>0 0 1 1 1 0 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>Lumbar lordosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C24</td>
<td>7</td>
<td>23.7 (2–33)</td>
<td>1 2 1 3 2 3 2 3</td>
<td>3 3</td>
<td>3 3</td>
<td>Slow steady progression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>5</td>
<td>26.2 (5–37)</td>
<td>1 0 3 1 0 0 0 0</td>
<td>4 0</td>
<td>4 0</td>
<td>Unusual “tauopathy” described at necropsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td>11</td>
<td>28.1 (1–50)</td>
<td>1 3 4 8 4 4 4 4</td>
<td>1 1</td>
<td>1 1</td>
<td>Choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>3</td>
<td>3 (2–5)</td>
<td>0 1 — — 1 — — —</td>
<td>0 0</td>
<td>0 0</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>5</td>
<td>10.6 (1–18)</td>
<td>1 2 1 2 0 1 2 2</td>
<td>2 2</td>
<td>2 2</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C27</td>
<td>9</td>
<td>20.3 (10–29)</td>
<td>1 3 2 5 6 1 6 0</td>
<td>0 0</td>
<td>0 0</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>8</td>
<td>31.4 (5–50)</td>
<td>2 1 5 3 5 2 4 0</td>
<td>4 0</td>
<td>4 0</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N36</td>
<td>5</td>
<td>40</td>
<td>0 0 1 1 0 0 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>6</td>
<td>21.4 (2–37)</td>
<td>1 2 1 3 1 0 0 0</td>
<td>5 0</td>
<td>5 0</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C22</td>
<td>5</td>
<td>18.5 (2–30)</td>
<td>1 0 2 3 3 0 1 0</td>
<td>1 0</td>
<td>1 0</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N37</td>
<td>1</td>
<td>11</td>
<td>0 0 1 0 0 0 0 0</td>
<td>1 0</td>
<td>1 0</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N27</td>
<td>11</td>
<td>26.4 (15–41)</td>
<td>4 5 7 5 6 3 2 0</td>
<td>2 2</td>
<td>2 2</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N8</td>
<td>4</td>
<td>40</td>
<td>0 1 0 0 0 4 4 4</td>
<td>4 4</td>
<td>4 4</td>
<td>choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Eight affected members from three generations were examined. Age at onset of symptoms ranged from 5 to 50 years and one affected family member was asymptomatic at the age of 28 years. Two family members required a

N4
Five affected family members have been seen in this family, between 12 and 70 years of age. Onset in the youngest child was before the age of 5 years, but in the older family members ranged from 20 to 37 years. With the exception of one female family member, now in her late 30s, who has needed a wheelchair within three years of diagnosis, the progression has been very slow. Upper limb involvement is minimal (brisk reflexes only in 4/5 members), three have bladder involvement, and the findings in the lower limbs are of a pure spastic paraparesis without wasting or sensory changes. No cognitive changes are reported in any family members.

N5
Eleven affected subjects are known in this large family, ranging in age from 27 to 72. Two of the younger family members were reported as having symptoms by the age of 5 years. The onset in the older family members was between 20 and 50 years. One obligate gene carrier remains asymptomatic at 38 years. Three subjects are wheelchair users, all after at least 15 years of symptoms. As with family N4 (now known to have the same mutation, see above), all findings were consistent with a pure hereditary spastic paraplegia, with no cognitive problems, though one person was said to have a paranoid affective disorder.

A necropsy was carried out on one member of this family and the findings were entirely consistent with pure HSP, showing pathology limited to the corticospinal tracts. Myelin pallor and loss of axons was especially pronounced in the lateral and ventral corticospinal tracts. Myelin loss in the dorsal column was most prominent in the cervical spinal cord, but the corticospinal pathways in the cerebral peduncle were well preserved.

N3
Relatively little information is available on this family. However, reported onset in all subjects where this was recorded was before the age of 5 years. One person requires a wheelchair.

N2
This family has been reported previously. Onset in the youngest generation was in the first two years of life and in the late teens in previous generations. In all but one family member, the phenotype was consistent with pure HSP. However, one of the older family members had a history of progressive cognitive decline in the two years before his death. Necropsy showed diffuse tau related cortical changes. Another affected family member had a cognitive problem with generalised impairment of verbal learning, non-verbal learning, and memory, detected as learning problems in childhood. The relevance of these difficulties to her HSP is not known.

C27
Nine affected members from three generations of this family were examined. Age at onset of symptoms ranged from 10 years to 29 years. Two clinically affected subjects were asymptomatic aged 23 and 25 years. Rate of disease progression was variable, although tended to be slow. One subject required a walking stick and three, all symptomatic for more than 30 years, used a wheelchair. Five patients had bladder involvement and three of these required a urinary catheter. Three patients suffered from constipation and two of the male subjects were impotent. Six subjects had upper limb hyperreflexia. Lower limb examination showed signs typical of spastic paraparesis in all cases. Six subjects had foot deformity, one had mild distal lower limb wasting, and three had absent lower limb vibration sensation. No family members had overt cognitive impairment.

C5
Eight affected members from three generations were examined. Age at onset of symptoms ranged from 5 to 50 years and one affected family member was asymptomatic at the age of 28 years. Two family members required a

cavus, two had mild to moderate distal limb amyotrophy, and two had altered lower limb vibration sensation. No family members had overt cognitive impairment.
walking stick and one family member, sympto-
matic for 47 years, used a wheelchair. Rate of
disease progression was variable, though was
generally slow. Bladder involvement was
present in three subjects, with one requiring an
indwelling catheter, and five family members
suffered from constipation. Typical signs of
lower limb spastic paraparesis were found in
every affected subject. Four family members had
upper limb hyperreflexia, five subjects had pes
cavus, and two had mild distal lower limb
amyotrophy. Five family members had de-
creased lower limb vibration sensation and two
had diminished lower limb pain sensation. No
overt cognitive impairment was reported in
any family member. Three family members
also suffered from choroideremia, which was
segregating in the family independently of
HSP.

N36
Only one member of this three generation fam-
ily was seen, and the onset of his disease was at
40 years. The condition has been slowly
progressive and 12 years later he remains
ambulant. Clinical examination is consistent
with a pure HSP phenotype, with some minor
distal wasting and sensory loss to pinprick and
vibration in his lower limbs. Neuropsychomet-
ric testing showed a mild impairment of verbal
learning. Complicating his assessment is a his-
tory of polio and TB meningitis as a child. His
mother, who was presumed from history to be
affected with HSP, had a late onset dementia,
as did a maternal aunt.

C7
Six affected family members from two genera-
tions of this family were seen. Age at onset for
symptomatic patients ranged from 2 to 37
years, and one affected subject was asympto-
matic at 22 years. One family member used
walking sticks and two, both symptomatic for
more than 20 years, used a wheelchair. Rate of
disease progression was variable, though
tended to be slow. Three family members had
bladder involvement. Five family members had
upper limb hyperreflexia and lower limb exam-
ination findings typical of spastic paraparesis
were present in all cases. Two patients had foot
deformity and one had diminished lower limb
vibration and joint position sensation. None of
the subjects was reported to have cognitive
impairment.

C22
Five affected family members from two genera-
tions of this family were examined. Age at onset
for symptomatic patients ranged from 2 to 30
years, and one affected subject was asympto-
matic at 23 years. One family member required
a walking stick although none required a
wheelchair. Three family members had bladder
involvement and one complained of constipa-
tion. Rate of disease progression tended to be
slow, though was variable. Typical clinical
examination findings of spastic paraparesis
were present in all cases. One family member
had upper limb hyperreflexia, three subjects
had pes cavus, and three subjects had altered
fine touch, vibration, or joint position sensa-
tion. Cognitive impairment was not reported in
any family members.

N37
This patient is the only known case of HSP in
his family. Disease onset was at 11 years and
has been very slowly progressive. The only
signs of disease in the upper limbs are brisk
tendon reflexes. In the lower limbs, he has mild
distal impairment of temperature and vibration
sensation. He has mood problems, but no other
associated features.

C25
Twelve affected members from three genera-
tions of this family were examined. Age at onset
of symptoms ranged from 16 to 41 years, and
one subject with signs on examination was
asymptomatic at 30 years. Four of the family
members required a walking stick and five, all
asymptomatic for at least 30 years, required a
wheelchair. Five patients had bladder involve-
ment, one patient suffered from constipation,
and another from faecal urgency. Rate of
disease progression was variable, but generally
slow. Two patients had mild upper limb hyper-
reflexia, in one accompanied by slight upper
limb weakness. In all cases there were typical
signs of spastic paraparesis in the lower limbs.
Six patients had pes cavus and three pes
planus. Mild to moderate lower limb amyotro-
ymyopathy was present in three cases. Seven patients
had lower limb sensory abnormalities, involv-
ing fine touch, pinprick sensation, or joint
position sense. One affected family member
aged 80 had a late onset dementia, although
further clinical details regarding this are not
available.

N8
Two clinically affected family members were
seen. One 70 year old gene carrier is asympto-
matic. Both affected family members had
disease onset at 40 years, and one requires a
wheelchair many years later. All subjects have
distal muscle wasting and pes cavus. No
bladder involvement was reported. In an 86
year old affected family member, there has
been a four year history of progressive demen-
tia with mild word finding difficulties and fron-
tal lobe release signs on the right. Whether or
not this relates to a complication of her HSP or
is related to another coincidental disease proc-
ess has not been established with certainty.

GENETIC ANALYSIS
Using SSCP analysis we identified 13 novel
mutations in the SPG4 gene. None of these
mutations was present in a control panel of 100
normal chromosomes. Only one of these
mutations (859C>G) was present in more than
one of our families. The two families N4 and
N5 were not known to be related, but as both
families originate from the same area of
England and have the same haplotype for
markers D2S2203 and D2S2347 either side of the
SPG4 gene, the most probable explanation
is that both families share a common ancestor.
Two mutations 1298+1g>a and 1538+3del (aagt) have been described previously by Fonknechten et al. The remaining mutations are novel.

The majority of mutations (7/13) are splice mutations, all of which affect the donor splice site. These mutations would be expected to result in exon skipping of the preceding exon and would cause a frameshift in the remaining sequence, except in the case of the mutation involving exon 8 (patient N3). All of these mutations would result in severe disruption of the conserved AAA motif.

Three mutations (859C>G, 411delG, and 1406delT) lead directly or indirectly to a premature stop codon and would result in the production of a truncated protein.

The two missense mutations which behave in a dominant manner are both located in the conserved AAA cassette region and both affect amino acids that are highly conserved between spastin and closely related proteins. The mutation in family N2 replaces arginine, a basic amino acid with glycine, which is much smaller and neutral. The mutation in N8 replaces aspartic acid, an acidic amino acid with histidine, a basic amino acid with an aromatic side chain. It seems likely that these changes would severely reduce or abolish the function of the protein. There is little to distinguish these patients phenotypically from those with nonsense/splice mutations. The age of onset is late (40) in family N8, but as can be seen from table 2, the general age of onset in these families is very variable even within a family. In each of the families N2 and N8, an older member was described with dementia, though these were single subjects in each family.

Patient N35 was homozygous (fig 1) for the mutation in exon 1 which replaces a serine (a polar amino acid) at position 44 with a leucine, a non-polar amino acid. The finding of the polar amino acid at position 256.

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Three mutations (859C>G, 411delG, and 1406delT) lead directly or indirectly to a premature stop codon and would result in the production of a truncated protein.
by Northern blotting, owing to its low abundance. In an independent study predating the involvement of this gene in HSP, the spastin cDNA was isolated from brain by Kikuno et al and the protein named KIAA1083. This cDNA transcript lacks exon 4, so it is possible that further splice variants remain to be characterised.

The number of different mutations found, together with the low yield of mutations in the small families and sporadic cases studied, also suggest that mutation detection in spastin may not by itself be a logical way forward for molecular diagnosis in HSP for some time. In addition, the clinical features described in these families with spastin mutations do not provide many useful clues for detecting clinically which patients to target for spastin mutation analysis. HSP has been traditionally classified into “pure” and “complicated” forms depending on whether spasticity is the only feature or whether there are other symptoms, such as epilepsy, dementia, or ataxia. Most families with a mutation in spastin had a pure HSP phenotype, with remarkable variation in age at onset and severity of the disease, though overall relatively mild disease was the norm. Of 66 affected subjects examined or known of in these families, only 18 required a wheelchair, most late in the course of their disease. Minor signs only (brisk reflexes) were usually found in the upper limbs, with sensory changes detected in the lower limbs in a minority of patients. Bladder involvement was variable. The significance of reports of cognitive decline, mood problems, or affective disorders in occasional family members is difficult to assess; the history of late onset dementia in family N2 is clearer, and corresponds to previous reports of cognitive problems in SPG4 families. Overall, however, in keeping with previous linkage based reports, it is hard to discern specific features that would lead to clinical suspicion of spastin involvement in a single case.

The majority of mutations detected so far in SPG4 are predicted to result in either a truncated protein or a severely altered protein, confirming that haploinsufficiency is the cause of the abnormal phenotype. Splice site mutations account for seven out of 13 mutations that we found and this high frequency of splice site mutations is also reported by Fonknechten et al. The two dominant missense mutations we report affect amino acids in the conserved AAA domain in common with the 11 missense mutations described by Fonknechten et al suggesting that this region is crucial for protein function.

The missense mutation in exon 1, which is outside the conserved AAA motif, is present homozygously in the patient we studied. This patient has no affected relatives with HSP. Clinically, he has very mild disease with late onset. This sequence change was not present in our control population; unfortunately no other members of this family were available for study. Providing that this is not a rare polymorphism with the clinical manifestation of HSP in this person having an alternative cause, this is the first recessive mutation in spastin to be described. Autosomal recessive HSP (ARHSP) is rare and consanguineous families linked to chromosomes 8q, 16q, and 15q have been described. So far no families with linkage to the SPG4 locus. This mutation, which replaces a serine with a leucine residue at position 44, is in the early part of the protein, a region which shows little conservation between spastin and related proteins. In fact, in the closely related yeast protein SAP1, there is a leucine at this position. The most likely hypothesis is that this mutation is behaving as a hypomorph, an allele which produces a reduced amount or activity of product. If this mutation is only on one allele, the amount of spastin is not reduced enough to cause symptoms, but subjects with two copies of the mutant allele have insufficient spastin. The possibility that a “threshold effect” for spastin levels may be critical has already been suggested. If these conjectures are correct, this would represent an unusual explanation of the dominant and recessive effects of different mutations in the same gene. This hypothesis was recently also postulated to explain dominant and recessive mutations in the connexin31 gene (a gene causing non-syndromic hearing loss), although as both dominant and recessive mutations in this gene were missense and it was not clear whether the dominant mutations caused the disease through haploinsufficiency or dominant negative effects. If the latter is the case then the recessive mutations in this gene are likely to be null mutations. The discovery of an apparently recessive mutation in SPG4 means that this protein should potentially be considered in patients with ARHSP as well as dominant disease. It will be interesting to see if other recessive mutations are discovered and if these map to the early part of the gene. Such mutations may provide important clues to the function of the spastic protein.


