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 AAA 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 has been described with 13 published loci to date,19 20 The majority of families with dominantly inherited HSP (40-60%)21 22 show linkage to this locus. Spastin, like paraplegin, belongs to the AAA protein family, although these two proteins share little homology outside the AAA motif. Unlike paraplegin which is a mitochondrial protease, spastin has been proposed to have a nuclear localisation. Little is known about the function of spastin although it is highly homologous to the 26S proteosome subunit and may participate in the assembly or function of protein complexes.19 In this paper we report 13 mutations in the gene for spastin, including one which is present homozygously.

Methods

A total of 32 unrelated British patients with HSP were involved in this study. Twelve were affected members of families where linkage to the SPG4 locus had previously been shown and the remainder were either from families where 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 mutation 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).9 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 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.

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</td>
<td>1</td>
<td>60</td>
<td>Missense (present homozgyously)</td>
<td>Exon 1</td>
<td>256C&gt;T</td>
<td>S44R</td>
</tr>
<tr>
<td>C24</td>
<td>7</td>
<td>2–33</td>
<td>Frameshift</td>
<td>Exon 1</td>
<td>411delG</td>
<td>Frameshift aa96-159</td>
</tr>
<tr>
<td>N4</td>
<td>5</td>
<td>26.2 (&lt;5–37)</td>
<td>Nonsense</td>
<td>Exon 5</td>
<td>859G&gt;C</td>
<td>S245Stopcodon</td>
</tr>
<tr>
<td>N5</td>
<td>11</td>
<td>28.1 (1–50)</td>
<td>Nonsense</td>
<td>Exon 5</td>
<td>859G&gt;C</td>
<td>S245Stopcodon</td>
</tr>
<tr>
<td>N3</td>
<td>1 (2 others affected but not examined)</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>
</tr>
<tr>
<td>N2</td>
<td>5</td>
<td>11.7 (1.5–20)</td>
<td>Frameshift/nonsense</td>
<td>Exon 10</td>
<td>139TA&gt;G</td>
<td>R424G</td>
</tr>
<tr>
<td>C27</td>
<td>9</td>
<td>20.3 (&lt;10–27)</td>
<td>Frameshift/nonsense</td>
<td>Exon 10</td>
<td>1406delT</td>
<td>Frameshift aa427-436</td>
</tr>
<tr>
<td>C5</td>
<td>1 (6 not seen)</td>
<td>31.4 (&lt;5–50)</td>
<td>Splice</td>
<td>Intron 11</td>
<td>1538+3T&gt;a</td>
<td>Presumed missplicing (skipping of exon 11)</td>
</tr>
<tr>
<td>N36</td>
<td>1 (6 not seen)</td>
<td>30 (&lt;30)</td>
<td>Splice</td>
<td>Intron 11</td>
<td>1538+3del(aag)</td>
<td>Presumed missplicing (skipping of exon 11)</td>
</tr>
<tr>
<td>C7</td>
<td>6</td>
<td>21.4 (&lt;2–33)</td>
<td>Splice</td>
<td>Intron 12</td>
<td>1618+2T&gt;a</td>
<td>Presumed missplicing (skipping of exon 12)</td>
</tr>
<tr>
<td>C22</td>
<td>5</td>
<td>18.5 (&lt;2–30)</td>
<td>Splice</td>
<td>Intron 13</td>
<td>1661+2T&gt;c</td>
<td>Presumed missplicing (skipping of exon 13)</td>
</tr>
<tr>
<td>N37</td>
<td>1</td>
<td>11 (&lt;–)</td>
<td>Splice</td>
<td>Intron 15</td>
<td>1812+2Tg</td>
<td>Presumed missplicing (skipping of exon 15)</td>
</tr>
<tr>
<td>C25</td>
<td>11</td>
<td>26.4 (15–41)</td>
<td>Splice</td>
<td>Intron 16</td>
<td>1853+2T&gt;c</td>
<td>Presumed missplicing (skipping of exon 16)</td>
</tr>
<tr>
<td>N8</td>
<td>3</td>
<td>40 (40–40)</td>
<td>Missense</td>
<td>Exon 17</td>
<td>1875G&gt;C</td>
<td>D584H</td>
</tr>
</tbody>
</table>

*Requires the addition of 2 mol/l Betaine (Sigma) to the PCR reaction.
Table 3 Clinical features observed in families with spastin gene mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>No of affected</th>
<th>mKOA (range)</th>
<th>Severity of use of stick/wheelchair</th>
<th>Vibration sense</th>
<th>Bladder disturbance</th>
<th>Ps 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>4</td>
<td>4.0 (1-5)</td>
<td>0 0 0 0 0 4 4 4 4 0 0 0 0 0 4 4 4 4</td>
<td>4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4</td>
<td>N3</td>
<td>Lumbar lordosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N4</td>
<td>5</td>
<td>2.0 (2-3)</td>
<td>1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>N4</td>
<td>Slow steady progression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N5</td>
<td>11</td>
<td>1.0 (1-5)</td>
<td>1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1</td>
<td>N5</td>
<td>Incomplete clinical details</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>5</td>
<td>10.0 (1-5)</td>
<td>1 2 1 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>N2</td>
<td>Unusual “tusopathy” described at necropsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C27</td>
<td>9</td>
<td>20.0 (1-5)</td>
<td>1 3 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>C27</td>
<td>Choroideremia in 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>8</td>
<td>31.0 (5-5)</td>
<td>2 1 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>3 3 5 3 5 3 5 3 5 3 5 3 5 3 5 3 5 3 5 3 5</td>
<td>C5</td>
<td>Only one clinically evaluated, complicated by TB and polio as child.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N36</td>
<td>5</td>
<td>40.0 (1-5)</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>N36</td>
<td>2 with impaired fine touch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>6</td>
<td>21.0 (2-5)</td>
<td>1 2 1 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>C7</td>
<td>2 with impaired fine touch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C22</td>
<td>5</td>
<td>18.0 (2-5)</td>
<td>1 0 2 3 3 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>C22</td>
<td>2 with impaired fine touch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N37</td>
<td>11</td>
<td>26.0 (1-5)</td>
<td>4 5 7 5 6 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3</td>
<td>4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4</td>
<td>N37</td>
<td>All have impairment of joint position</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N8</td>
<td>4</td>
<td>40.0 (1-5)</td>
<td>0 0 0 0 0 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4</td>
<td>0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td>N8</td>
<td>vascular dementia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

N8
Four affected family members have been seen in this family, with onset in the youngest child being before the age of 5 years. Upper limb involvement is minimal (brisk reflexes only in 2/4 members), one has 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.
walking stick and one family member, symptomatic 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 all affected subjects. Four family members had upper limb hyperreflexia, five subjects had pes cavus, and two had mild distal lower limb amyotrophy. Five family members had decreased 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 family 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. Neuropsychometric testing showed a mild impairment of verbal learning. Complicating his assessment is a history 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 generations of this family were seen. Age at onset for symptomatic patients ranged from 2 to 37 years, and one affected subject was asymptomatic 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 examination 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 generations of this family were examined. Age at onset for symptomatic patients ranged from 2 to 30 years, and one affected subject was asymptomatic 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 constipation. 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 sensation. 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 generations 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 involvement, 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 hyperreflexia, 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 amyotrophy was present in three cases. Seven patients had lower limb sensory abnormalities, involving 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 asymptomatic. 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 dementia with mild word finding difficulties and frontal lobe release signs on the right. Whether or not this relates to a complication of her HSP or is related to another coincidental disease process 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. and 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 homozygous change was confirmed using a non-polar amino acid. The finding of the mutation in exon 1 which replaces a serine (a polar amino acid) with a histidine, a non-polar amino acid, at position 256. There are few 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.

In our study, this may reflect the limited yield of mutations seen from the spastin gene in 142 subjects analysed.

Discussion

Molecular analysis of the gene for spastin in this panel of British HSP patients has shown 13 mutations, 11 of them novel. Each family tested (with the exception of two families known to be linked to the SPG4 locus) had a different mutation. This confirms that mutation detection in this gene in patients with spastic paraplegia will be a more difficult task than if a smaller number of recurrent mutations had been identified. Further complicating this task, we have evidence that not all SPG4 mutations may be detected using these routine mutation detection techniques, as mutations were not found in three families where linkage to the SPG4 locus had been shown. This incomplete yield of mutations even in families known to be linked to the SPG4 locus was also reported by Fonknechten et al. In our study, this may reflect the limitations of SSCP which routinely detects only approximately 80% of mutations. Alternatively, it may mean that sequence outside the coding region needs to be studied, such as the promoter or polyadenylation site. A further possibility, especially as so many of the mutations described so far affect splicing, could be that sequence changes in the introns not detected using these sets of primers might cause disease by activating an intronic cryptic splice site. It is not yet known if alternatively spliced forms of spastin exist, which might potentially contain additional exon sequence, as the RNA transcripts have not been visualised.
by Northern blotting, owing to its low
abundance. In an independent study predat-
ing the involvement of this gene in HSP, the
spastin cDNA was isolated from brain by
Kikuno et al21 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 epi-
lepsy, dementia, or ataxia.22 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 over-
all 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 signifi-
cance 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,20 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 muta-
tions 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.20 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.20
suggesting that this region is crucial for protein
function.

The missense mutation in exon 1, which is
outside the conserved AAA motif, is present
homozgyously 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.14-16 It is not far from 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.19 In fact, in the
closely related yeast protein SAP1, there is a
leucine at this position. The most likely
hypomorph, an allele which produces a re-
duced 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.23 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 domi-
nant and recessive mutations in the connexin
31 gene24 (a gene causing non-syndromic
hearing loss), although as both dominant and
recessive mutations in this gene were missense
it was not clear whether the dominant muta-
tions caused the disease through haploinsuffi-
ciency 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 poten-
tially be considered in patients with ARHSP as
well as dominant disease. It will be interesting
to see if other recessive mutations are discov-
ered and if these map to the early part of the
gene. Such mutations may provide important
cues to the function of the spastic protein.

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Mutation analysis of the spastin gene (SPG4)


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