Exon Deletions of SPG4 are a Frequent Cause of Hereditary Spastic Paraplegia

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J. Med. Genet. published online 10 Nov 2006; doi:10.1136/jmg.2006.046425

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Exon deletions of *SPG4* are a frequent cause of hereditary spastic paraplegia

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Running title: Exon deletions in *SPG4*

Key words: HSP, SPG4, SPAST, microdeletion, MLPA
ABSTRACT

Background: Point mutations in \textit{SPG4}, the gene encoding spastin, are a frequent cause of autosomal dominant hereditary spastic paraplegia (AD-HSP). Standard methods for genetic analyses miss, however, exonic microdeletions.

Objective and Methods: We screened 121 mutation-negative probands for rearrangements in \textit{SPG4} by multiplex ligation-dependent probe amplification (MLPA).

Results: We identified 24 patients with 16 different heterozygous exon deletions in \textit{SPG4} (20%) ranging from one exon to the whole coding sequence. Comparison with 78 patients with point mutations showed a similar clinical picture but earlier age at onset.

Conclusions: These results indicate that exon deletions in \textit{SPG4} are as frequent as point mutations and that \textit{SPG4} is responsible for 40\% of AD-HSP.

Key Points: 121 HSP patients negative for point mutations in SPG4 were screened for rearrangements in \textit{SPG4} by MLPA. We identified 20\% of exonic micro-deletions. Clinical features of patients with \textit{SPG4} deletions were similar to those with point mutations with an earlier age at onset.
Hereditary spastic paraplegias (HSP) are a genetically heterogeneous group of neurodegenerative disorders clinically characterized by progressive stiffness and weakness of the lower limbs that results from axonal neurodegeneration in the cortico-spinal tract. Pure and complicated forms of HSP have been described depending on whether spasticity occurs in the absence or in the presence of other clinical features such as cerebellar ataxia, neuropathy, retinal degeneration, cognitive impairment, dementia, or epilepsy. The clinical heterogeneity of HSP is partly explained by its genetic heterogeneity. To date, 35 loci have been identified, associated with autosomal dominant, autosomal recessive and X-linked modes of inheritance (for additional information, see the HUGO site at http://www.gene.ucl.ac.uk/nomenclature/).

The most common form of autosomal dominant HSP (AD-HSP) is caused by mutations in the \( \text{SPG4} / \text{SPAST} \) gene (MIM# 604277), encoding spastin, a member of the AAA family of ATPases. \(^1\) \( \text{SPG4} \) has been shown to account for 15 to 40% of all AD-HSP families, depending on the population. \(^2-5\) HSP due to \( \text{SPG4} \) mutations (MIM# 182601) is generally described as a pure form of the disease, i.e. as spastic paraparesis often associated with decreased vibration sense in the lower limbs and urinary problems, but has also occasionally been described in association with additional features, including cognitive impairment, peripheral neuropathy, cerebellar signs or epilepsy. \(^6-8\) Age at onset is highly variable, ranging from early infancy up to the eighth decade. More than 150 different mutations have been identified in all exons of the \( \text{SPG4} \) gene except for exon 4, which is alternatively spliced. All types of DNA alterations are observed, including missense, nonsense, splice site mutations and small insertions / deletions. \(^9\) This wide spectrum of mutation, combined with the observation that many mutations reduce the abundance of the normal full-length transcript and/or functionally normal spastin protein, suggests that the pathogenic mechanism is haploinsufficiency, i.e. the disease occurs once the level of functional spastin falls below a critical level. \(^10,11\) Although larger deletions in \( \text{SPG4} \) have been described in two cases \(^5,12\) and should theoretically lead to HSP, they are missed by standards screening methods such as direct sequencing and DHPLC and have not yet been systematically sought. In the present study, we screened 121 patients for micro-rearrangements in the \( \text{SPG4} \) gene by multiplex ligation-dependent probe amplification (MLPA) to assess their frequency in HSP-families.
METHODS

Patients
We selected 121 European, mostly French (105/121), probands with hereditary spastic paraplegia in whom mutations in SPG4 gene were not detected by DHPLC. Dominant transmission was observed in 120 families. The remaining family had two affected sibs. The large majority of the probands presented pure HSP (n=114). The complicated forms (n=7) included signs of peripheral neuropathy (n=3), and one each with cerebellar signs, mental retardation, cognitive impairment and parkinsonism. Informed written consent was obtained from each individual before blood sampling. This study was approved by the ethical committee Paris-Necker (CCPRPB, n°03-12-07, 2/10/2004).

Multiplex ligation-dependent probe amplification (MLPA)
The autosomal dominant spastic paraplegia MLPA kit (P165) designed to search for deletions or duplications in SPG4 was purchased from MRC-Holland (Amsterdam, The Netherlands). MLPA reactions were carried out according to the manufacturer’s instructions. Electrophoresis of PCR products was performed using an ABI 3730 sequencer and MLPA data were analysed using the GeneMapper 4.0 software (PE Applied Biosystems, Foster City, CA). Relative ratios were calculated for each peak using the formula \( r = \frac{\text{mean (peak area } \text{patient} / \text{control area } \text{patient})}{\text{(peak area } \text{control individual} / \text{control area } \text{control individual})} \).

Statistical Tests
Frequencies were compared with the Chi-Square test or the Fischer exact test when appropriate. Quantitative variables were compared with Mann-Whitney test. Statistical analysis was performed using SPSS software. Means are expressed as values +/- standard deviation.
RESULTS

Results of MLPA analysis
We identified 24 index patients (22 French, one Portuguese and one Spanish) with microdeletions in the SPG4 gene. This corresponds to a frequency of 20% (24/121). There were 16 different exon deletions detected, spanning a single exon to the whole coding sequence of the gene (i.e. exons 1 to 17). Five of the deletions (exon 1, exons 1 to 17, exons 8 to 17, exon 13 and exon 16) were detected in more than one family, but the remaining 11 were private rearrangements. Segregation of the microdeletion with the disease was analyzed when DNA from other affected individuals was available (table 1). All affected relatives (n=32) of probands from 12 families also had the corresponding exon deletion, confirming that these rearrangements are responsible for the disease. In addition, we tested six asymptomatic relatives and found one deletion carrier examined who was still asymptomatic at age 46 years. These observations confirm that the penetrance is age-dependant and incomplete in patients with SPG4 deletions, as previously described for SPG4 point mutations.

<table>
<thead>
<tr>
<th>Family number</th>
<th>Deletion</th>
<th>Nomenclature</th>
<th>Predicted effect on protein</th>
<th>Segregation</th>
<th>Ages at onset*</th>
</tr>
</thead>
<tbody>
<tr>
<td>165</td>
<td>Exon 1</td>
<td>c.1-?_415+?del</td>
<td>Unknown</td>
<td>ND</td>
<td>31</td>
</tr>
<tr>
<td>172</td>
<td>Exon 1</td>
<td>c.1-?_415+?del</td>
<td>Unknown</td>
<td>Yes (n=5)</td>
<td>2, 2, 30</td>
</tr>
<tr>
<td>366</td>
<td>Exon 1</td>
<td>c.1-?_415+?del</td>
<td>Unknown</td>
<td>Yes (n=5)</td>
<td>10, 37, 47, 50, 50</td>
</tr>
<tr>
<td>774</td>
<td>Exons 1 to 4</td>
<td>c.1-?_682+?del</td>
<td>Unknown</td>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>628</td>
<td>Exons 1 to 17</td>
<td>c.1-?_1851+?del</td>
<td>Absence of protein</td>
<td>ND</td>
<td>14</td>
</tr>
<tr>
<td>202</td>
<td>Exons 1 to 17</td>
<td>c.1-?_1851+?del</td>
<td>Absence of protein</td>
<td>ND</td>
<td>26</td>
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<tr>
<td>253</td>
<td>Exons 1 to 17</td>
<td>c.1-?_1851+?del</td>
<td>Absence of protein</td>
<td>Yes (n=3)</td>
<td>10, 14, 35</td>
</tr>
<tr>
<td>282</td>
<td>Exons 1 to 17</td>
<td>c.1-?_1851+?del</td>
<td>Absence of protein</td>
<td>ND</td>
<td>29</td>
</tr>
<tr>
<td>237</td>
<td>Exons 4 to 17</td>
<td>c.587-?_1851+?del</td>
<td>Absence of protein</td>
<td>ND</td>
<td>38</td>
</tr>
<tr>
<td>742</td>
<td>Exons 5 to 6</td>
<td>c.683-?_1004+?del</td>
<td>p.Glu228Val/fsX237</td>
<td>Yes (n=4)</td>
<td>40</td>
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<tr>
<td>530</td>
<td>Exons 5 to 7</td>
<td>c.683-?_1098+?del</td>
<td>p.Glu228Val/fsX233</td>
<td>Yes (n=2)</td>
<td>40, 77</td>
</tr>
<tr>
<td>167</td>
<td>Exon 6</td>
<td>c.871-?_1004+?del</td>
<td>p.Gly291Trp/fsX295</td>
<td>Yes (n=2)</td>
<td>1, 15</td>
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<tr>
<td>374</td>
<td>Exons 8 to 12</td>
<td>c.1099-?_1493+?del</td>
<td>p.Leu367AlafsX379</td>
<td>ND</td>
<td>37</td>
</tr>
<tr>
<td>629</td>
<td>Exons 8 to 17</td>
<td>c.1099-?_1851+?del</td>
<td>Unknown</td>
<td>ND</td>
<td>40</td>
</tr>
<tr>
<td>152</td>
<td>Exons 8 to 17</td>
<td>c.1099-?_1851+?del</td>
<td>Unknown</td>
<td>Yes (n=5)</td>
<td>25, 30, 32, 52</td>
</tr>
<tr>
<td>157</td>
<td>Exon 9</td>
<td>c.1174-?_1245+?del</td>
<td>p.Ala492_Tyr415del</td>
<td>ND</td>
<td>17</td>
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<tr>
<td>235</td>
<td>Exons 9 to 12</td>
<td>c.1174-?_1493+?del</td>
<td>p.Lys393Phe/fsX404</td>
<td>ND</td>
<td>40</td>
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<tr>
<td>335</td>
<td>Exons 10 to 16</td>
<td>c.1246-?_1728+?del</td>
<td>p.Val416_Glu576del</td>
<td>ND</td>
<td>33</td>
</tr>
<tr>
<td>12</td>
<td>Exon 13</td>
<td>c.1494-?_1536+?del</td>
<td>p.Arg499Gln/fsX515</td>
<td>Yes (n=5)</td>
<td>14, 18, 25, 54</td>
</tr>
<tr>
<td>333</td>
<td>Exon 13</td>
<td>c.1494-?_1536+?del</td>
<td>p.Arg499Gln/fsX515</td>
<td>Yes (n=3)</td>
<td>1, 26</td>
</tr>
<tr>
<td>236</td>
<td>Exon 16</td>
<td>c.1688-?_1728+?del</td>
<td>p.Glu563Asp/fsX570</td>
<td>Yes (n=2)</td>
<td>1, 53</td>
</tr>
<tr>
<td>803</td>
<td>Exon 16</td>
<td>c.1688-?_1728+?del</td>
<td>p.Glu563Asp/fsX570</td>
<td>Yes (n=4)</td>
<td>20, 36</td>
</tr>
<tr>
<td>193</td>
<td>Exons 16 to 17</td>
<td>c.1688-?_1851+?del</td>
<td>Unknown</td>
<td>ND</td>
<td>30</td>
</tr>
<tr>
<td>168</td>
<td>Exon 17</td>
<td>c.1729-?_1851+?del</td>
<td>Unknown</td>
<td>Yes (n=4)</td>
<td>30, 50</td>
</tr>
</tbody>
</table>

ND: not determined; n indicated the number of affected patients tested; ages in bold correspond to those of the index cases. * Ages at onset were undetermined for 12 patients.
Clinical features of patients with deletions

Ages at onset in patients with SPG4 deletions, including the 24 index cases and 32 affected relatives, ranged from 1 to 77 year (mean age at onset 28.7± 17.4 years, n=44). Age at onset could not be determined for 12 patients, including three who presented minor signs only detected on examination. All index cases with exon deletions had pure HSP. However, cognitive impairment was present in a single affected relative. One index patient (FSP-774) had cerebellar atrophy on cerebral MRI but no cerebellar signs at examination. The 4 index patients with the largest deletion spanning exons 1 to 17, had ages at onset between 4 and 35 years, similar to the mean age of the whole group.

Comparison of probands with microdeletions (n=24) and probands with point mutations (n=78, personal unpublished data) showed that the former were significantly younger at onset (25.4± 12.0 versus 31.8± 16.8, p=0.033) (table 2). All other features were strikingly similar in both groups, except for a tendency towards less severe walking disability in the deleted group compared to the other (9% versus 14%) despite similar disease durations.

Table 2 Clinical comparison of index patients with SPG4 exon deletions and SPG4 point mutations

<table>
<thead>
<tr>
<th></th>
<th>SPG4 exon deletions (n=24)</th>
<th>SPG4 point mutations (n=78)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at examination (years)</td>
<td>45.4± 15.9, 18-76</td>
<td>51.2 ± 14.9, 6-85</td>
<td>0.075</td>
</tr>
<tr>
<td>Mean age at onset (years)</td>
<td>25.4± 12.0, 1-40</td>
<td>31.8± 16.8, 1-63</td>
<td>0.033</td>
</tr>
<tr>
<td>Women: Men</td>
<td>8: 16, 24</td>
<td>34: 44, 78</td>
<td>0.479</td>
</tr>
<tr>
<td>Severe disability walking (needs two canes or is wheelchair bound)</td>
<td>9%, 2/23</td>
<td>14%, 10/71</td>
<td>0.501</td>
</tr>
<tr>
<td>Gait spasticity</td>
<td>87.5%, 21/24</td>
<td>96%, 71/74</td>
<td>0.134</td>
</tr>
<tr>
<td>Spasticity at rest</td>
<td>86%, 19/22</td>
<td>85.5%, 47/55</td>
<td>0.918</td>
</tr>
<tr>
<td>Increased reflexes LL</td>
<td>87.5%, 21/24</td>
<td>87.3%, 62/71</td>
<td>0.982</td>
</tr>
<tr>
<td>Increased reflexes UL</td>
<td>33%, 8/24</td>
<td>34%, 23/68</td>
<td>0.965</td>
</tr>
<tr>
<td>Extensor plantar reflex</td>
<td>91%, 21/23</td>
<td>90%, 63/70</td>
<td>0.847</td>
</tr>
<tr>
<td>Proximal motor deficit LL</td>
<td>42%, 10/24</td>
<td>58%, 42/72</td>
<td>0.156</td>
</tr>
<tr>
<td>Distal wasting LL</td>
<td>17%, 4/24</td>
<td>13%, 9/69</td>
<td>0.659</td>
</tr>
<tr>
<td>Decreased vibration sense at ankles</td>
<td>59%, 13/22</td>
<td>59%, 40/68</td>
<td>0.982</td>
</tr>
<tr>
<td>Decreased touch and prick sense LL</td>
<td>0%, 0/22</td>
<td>3%, 2/68</td>
<td>0.416</td>
</tr>
<tr>
<td>Urinary urgency</td>
<td>39%, 9/23</td>
<td>56%, 39/70</td>
<td>0.167</td>
</tr>
</tbody>
</table>
DISCUSSION

We have identified 24 patients with partial exonic deletions in SPG4 in 121 patients who were negative for point mutations. These findings demonstrate that a large proportion (20%) of mutation-negative HSP patients in fact carry SPG4 microdeletions and confirm that haploinsufficiency of SPG4 is a major cause of AD-HSP.

Previous estimates of the proportion of patients with SPG4 mutations who had family histories of the disease ranged between 15 and 40%. The percentage of patients with point mutations in SPG4 in our large series of French HSP families (that include patients with unknown linkage status) is, however, approximately 25% (AD, CD and AB, personal communication), a proportion also confirmed in another large European series. When taking into account only the French families with AD inheritance and excluding families with SPG4 point mutations in this study, the proportion of cases with exon deletions reaches 16% of AD-HSP. These results indicate that SPG4 could indeed be responsible for 40% of HSP families in the French population. This proportion might even be greater considering that mutations or deletions in the SPG4 promoter have not yet been systematically sought.

Many different combinations of exon deletions are observed, indicating that dosage should be applied to all SPG4 exons. This can easily be done in a single reaction using MLPA. A few deletions were found more than once, and it will be interesting to determine whether they descend from a common founder or are recurrent mutations.

Globally, the clinical picture of the patients with exon deletions is similar to that of patients with point mutations: most patients present pure HSP that includes, in addition to spasticity in the lower limbs, brisk reflexes, decreased vibration sense at ankles, proximal or generalized weakness in the lower limbs as well as frequent sphincter disturbances as part of the “SPG4 phenotype”. However, the age at onset appeared to be significantly earlier in patients with microdeletions who tended to be less severely affected than patients with point mutations. Disease progression had indeed been previously shown to be more rapid in late-onset than in early onset cases. Patients with a deletion of the whole gene also had a clinical phenotype and an age at onset similar to patients with other deletion types or point mutations. The functional consequence of SPG4 “mutation” is therefore the loss of spastin function.

This study confirms that HSP is caused by SPG4 haploinsufficiency, attested by the similar phenotypes associated with different types of mutations including whole gene deletions. The fact that exon deletions are almost as frequent as point mutations in this large series of families with AD-HSP justifies the inclusion of gene dosage experiments in future genetic tests for SPG4 so that appropriate genetic counselling can be given.

ACKNOWLEDGEMENTS

The authors thank the bank of IFR70 for DNA extraction, especially Christiane Penet for her kind participation. We are very grateful to the families for participating, to the SPATAK network and to the clinicians who referred their patients: Perrine Charles, Bertrand Fontaine, Richard Levy, Olivier Lyon-Caen, Alain Lagueny, Robert-Thierry Ghnassia, Jean-Marc Visy, Valérie Drouin Garraud, Jean-Pierre Salles, Christine Tranchant, Tanya Stojkovic, Pierre Labauge, Laurent Magy, Isabelle Penisson-Besnier and Isabelle Desguerre. This work was financially supported by the VERUM foundation and the Programme Hospitalier de Recherche Clinique AP-HP (n°AOM03059, to AD).
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