Hereditary Spastic Paraplegia 3A Associated With Axonal Neuropathy

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Objective: To study the frequency and distribution of mutations in SPG3A in a large cohort of patients with hereditary spastic paraplegia.

Design: We screened a large cohort of 182 families and isolated cases with pure or complex hereditary spastic paraplegia phenotypes, which were negative for mutations in SPG4.

Results: In 12 probands (6.6%), we identified 12 different SPG3A mutations (11 missense and 1 insertion/frameshift) of which 7 were novel and 3 were de novo. We found incomplete penetrance in 1 family (G482V). In most cases, SPG3A mutations were associated with an early age at onset (mean, 3 y); however, in 1 family (R495W mutation), symptoms started later (mean, 14 y) with clear intrafamilial variability (8-28 y). Six patients with an SPG3A mutation (F151S, Q191R, M408T, G469A, R495W) originating from 5 unrelated families presented with a complex form of hereditary spastic paraplegia associated with a neuropathy (17%). Our electrophysiological and pathological findings confirmed an axonal sensory-motor neuropathy. There was no correlation between the genotype and the presence of a neuropathy.

Conclusions: We conclude that mutations in SPG3A represent an important cause of patients in the overall hereditary spastic paraplegia population. SPG3A is more often associated with a neuropathy than previously assumed. Therefore, patients with a bipyramidal syndrome and a neuropathy should be screened for mutations in SPG3A.

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In the SPG3A families (Figure 1 and Table 1), we performed standard neurological and technical examinations for the probands and their relatives. To indicate the severity of disease, we used the following scale: grade 0, asymptomatic; grade 1, hyperreflexia in lower limbs (LL) without unequivocal gait abnormalities (mild); grade 2, obvious gait abnormalities (moderate); grade 3, walking aid necessary (severe); and grade 4, requiring a wheelchair (very severe). We obtained

Figure 1. SPG3A hereditary spastic paraplegia pedigrees. Open diamond indicates unaffected; filled diamond, affected; half-filled diamond, probably affected; slashed symbols, deceased; ?, status unknown; inf, onset in infancy; n, normal allele; m, mutant; *, neuropathy; E, epilepsy; arrow, propositus; and §, de novo. The sex is not shown to preserve confidentiality. For each affected individual, age at onset, age at examination (in years), and disease severity score are indicated.
motor and sensory nerve conduction velocities (NCVs) and electromyography by standard techniques in 15 patients belonging to 8 different families (family SL-41: patient II.1; SL-53: II.1; SL-258: III.3; SL-121: II.2, III.2, III.3, IV.2, and IV.3; SL-6: II.2, III.1, and III.3; SL-166: II.1). Somatosensory evoked potentials were performed in 9 patients (SL-41: II.1; SL-53: II.1; SL-121: II.2, III.2, and III.3; SL-6: II.2, III.1, and III.3; SL-166: II.1), and motor evoked potentials (MEP) in 7 patients (SL-41: II.1; SL-121: II.2, III.2, and III.3; SL-6: II.2, III.1, and III.3). Interestingly, in the latter 3 patients belonging to family SL-6, MEP testing was performed twice in a time interval of 11 years by the same electrophysiologist (R.M.). A sural nerve and muscle biopsy was obtained in patient II.1 of family SL-166. Furthermore, a brain magnetic resonance imaging scan was obtained in 5 patients (SL-53: II.1; SL-121: III.2 and IV.3; SL-166: II.1; HSP-6: III.5) and a spine magnetic resonance imaging scan in 3 patients (SL-41: II.1; SL-53: II.1; SL-166: II.1). In addition, we performed electroencephalography for 2 patients (HSP-6: III.5; SL-166: II.1).

MOLECULAR GENETIC STUDIES

We isolated genomic DNA from total blood samples obtained from patients with HSP and controls using standard extraction procedures. SPG3A mutation screening was performed by polymerase chain reaction amplification of all 14 coding exons and the intron/exon boundaries using HotGoldStar DNA polymerase (Eurogentec, Seraing, Belgium). Polymerase chain reaction products were sequenced on an ABI3730 DNA Analyzer (Applied Biosystems, Foster City, Calif) using the BigDye Terminator Cycle Sequencing Kit 3.1 (Applied Biosystems). The DNA sequence data were collected and analyzed using the ABI DNA Sequencing Analysis software 5.0 and Seqman II 5.07 (DNASTAR Inc, Madison, Wis). When a mutation was detected in a proband, additional family members were analyzed to determine cosegregation with the disease. In families with a de novo mutation, paternity was confirmed using 2 panels of 18 short tandem repeat markers each dispersed over different chromosomes. The numbering of the SPG3A codons was based on the published amino acid sequence (National Center for Biotechnology Information accession numbers NP_056999 for the protein sequence and NM_015915 for the messenger RNA sequence). The SPG3A mutations were defined according to standard guidelines.

RESULTS

MOLECULAR GENETIC ANALYSIS

We identified 12 different SPG3A mutations (absent in 340 control chromosomes) in 12 probands, resulting in a diagnostic yield of 6.6% (12/182) (Table 1 and Figure 2). Eleven probands had a single nucleotide sub-
A492fsX522) (Table 1 and Figure 2), who affected highly conserved amino acids. Interestingly, the G482V mutation was also observed in 1 asymptomatic individual (HSP-6: II.3) (Figure 1, Table 2 and Table 3), indicating incomplete penetrance of the disease. The other identified SPG3A mutations have already been reported in the literature (Figure 2 and Table 1): F151S (c.452T>C; exon 4; SL-41),2 L157W (c.470T>G; exon 4; SL-53),3 R239C (c.715C>T; exon 7; SL-121),2,4,5,7,8 H258R (c.773A>G; exon 8; SL-6),4 and R495W (c.1483C>T; exon 12; SL-108).3,9

**CLINICAL FINDINGS**

The mean age at onset in the probands was 3 years. All patients had an early onset of symptoms, before 10 years of age, except for the patients of family SL-108 (R495W), in which the mean onset age was 14 years. Only in the latter family (SL-108) we observed intrafamilial variability in age at onset, varying from 8 to 28 years. Age and symptoms at onset, clinical signs and disease severity at the time of the most recent examination, and pure or complex HSP phenotype are given for the probands and their affected family members in Figure 1 and Tables 2 and 3.
In 6 SPG3A patients, a polyneuropathy was found on NCV studies and electromyography (Table 4 and Figure 1). The proband of family SL-41 (F151S) and 1 patient (III.7) of family SL-108 (R495W) had an axonal, predominantly motor polyneuropathy. In both patients, compound motor action potentials of the peroneal and tibial nerves were reduced and NCVs were decreased in the axonal range. In the left sural nerve of both patients, the sensory nerve action potential was reduced with a normal NCV. Two patients (II.3, II.5) of family SL-109 (G469A) also had an axonal motor neuropathy with reduced compound motor action potentials and NCVs for the peroneal and tibial nerves. In patient II.3 of this family, there was a slight decrease in NCVs of the right sural nerve with normal sensory nerve action potential. The index patient of family SL-258 (Q191R) showed absent responses for both sural nerves and decreased compound motor action potentials and NCVs for the peroneal nerve. The proband of family SL-166 (M408T) had reduced compound motor action potentials and slowed NCVs of the tibial nerve and diminished sensory nerve action potential and decreased NCVs of the sural nerve.
Other possible causes for a neuropathy, such as diabetes, were excluded in these patients. A sural nerve biopsy in patient II.1 of family SL-166 revealed diffuse chronic axonal neuropathy with moderate to severe diffuse axonal loss affecting both small and large myelinated fibers. There were no signs of active demyelination or remyelination (Figure 3A). The biopsy from the anterior tibial muscle in the same patient revealed advanced neurogenic muscular atrophy with group atrophy of muscle fibers with presence of nuclear clumps and an increase of interstitial connective tissue (Figure 3B).

The central motor conduction time in patients II.2, III.1, and III.3 of family SL-6 was normal in the upper limbs but 10 to 17 milliseconds above the upper normal limit in the LL (Figure 4). There was no significant change in the MEP results compared with the measurements of 11 years before. In patient II.1 of family SL-41 and patients II.2, III.1, and III.3 of family SL-121, the central motor conduction time was normal in the upper limbs and borderline normal to slightly delayed in the LL (Figure 4). Although in families SL-6 and SL-121 2 patients each had severity scale scores of 2, there was a clearly different pattern of MEP results in the LL. Somatosensory evoked potentials were normal in all tested patients except for patient II.1 of SL-53, who showed delayed cortical responses for stimulation at the LL. In this patient, somatosensory evoked potentials in the upper limbs were normal. Magnetic resonance imaging scans of brain and spine were normal except for patient III.5 of HSP-6 with epilepsy in whom a large arteriovenous malformation in the frontal lobes (more pronounced on the left side) was observed. Electroencephalography in this patient revealed focal paroxysmal activity in the left frontal region.

**COMMENT**

We identified 12 different SPG3A mutations in 12 probands in a large cohort of 182 index patients who had distinct HSP phenotypes and were negative for SPG4 mutations. The diagnostic yield of SPG3A screening was 6.6%, which is lower compared with the 8% to 39% reported in other studies. This can be explained by the fact that we screened a heterogeneous population including both pure and complex HSP phenotypes, whereas in the other studies, screening focused on pure HSP phenotypes or early-onset HSP. Most previously reported

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### Table 4. NCVs in Patients With SPG3A Mutation and Associated Neuropathy

<table>
<thead>
<tr>
<th>Patient*</th>
<th>AA Change</th>
<th>Age at Examination, y</th>
<th>R/L</th>
<th>Peroneal Motor</th>
<th>Tibial Motor</th>
<th>Sural Sensory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Amp, mV</td>
<td>NCV, m/s</td>
<td>Amp, mV</td>
</tr>
<tr>
<td>Normal†</td>
<td></td>
<td></td>
<td></td>
<td>&gt;6.0</td>
<td>&gt;44.0</td>
<td>&gt;10.0</td>
</tr>
<tr>
<td>SL-41/II.1</td>
<td>F151S</td>
<td>52</td>
<td>L</td>
<td>0.3‡</td>
<td>31.0‡</td>
<td>0.3‡</td>
</tr>
<tr>
<td>SL-258/II.3</td>
<td>G191R</td>
<td>39</td>
<td>R</td>
<td>1.1‡</td>
<td>39.0‡</td>
<td>...</td>
</tr>
<tr>
<td>SL-108/II.7</td>
<td>R495W</td>
<td>34</td>
<td>R</td>
<td>1.0‡</td>
<td>33.0‡</td>
<td>11.9</td>
</tr>
<tr>
<td>SL-109/II.3</td>
<td>G469A</td>
<td>40</td>
<td>L</td>
<td>1.9‡</td>
<td>38.0‡</td>
<td>6.2‡</td>
</tr>
<tr>
<td>SL-109/II.5</td>
<td>G469A</td>
<td>29</td>
<td>R</td>
<td>2.1‡</td>
<td>41.0‡</td>
<td>2.3‡</td>
</tr>
<tr>
<td>SL-166/II.1</td>
<td>M408T</td>
<td>4</td>
<td>R</td>
<td>0.6‡</td>
<td>45.0</td>
<td>2.9‡</td>
</tr>
</tbody>
</table>

Abbreviations: A, absent response at both sides; AA, amino acid; Amp, amplitude; NCV, nerve conduction velocity; ellipses, not measured; R/L, right/left.

*Patient refers to the family and patient number in the corresponding pedigree.
†Normal indicates normal values for the laboratory.
‡Abnormal values.
SPG3A patients have an early disease onset,\textsuperscript{4,5,7,11,12} although a smaller number of patients was described with a late symptom onset.\textsuperscript{6,10} Our results correspond with these data as the mean age at onset was 3 years. All families had an early onset (<10 y) except for 1 family (SL-108, R495W) with mean onset age of 14 years. The same R495W mutation has previously been reported in 6 unrelated families,\textsuperscript{5,11} all associated with an early disease onset (<10 y). Furthermore, in this family (SL-108) we observed intrafamilial variability in age at onset varying from 8 to 28 years, which was not reported in the other families with the R495W mutation.

Most patients with an SPG3A mutation presented with a pure HSP phenotype, corresponding to previous studies.\textsuperscript{1,8,11} However, 6 SPG3A patients (17\%) belonging to 5 unrelated families had a complex form of HSP, associated with an axonal, predominantly motor neuropathy. The neuropathy was proven by NCV studies in all patients and by a nerve and muscle biopsy in 1 patient. The neuropathy was associated with the R495W, F151S, M408T, G469A, and Q191R mutations. The R495W mutation has been reported previously both with\textsuperscript{5} and without a peripheral neuropathy.\textsuperscript{5,11} The F151S mutation has been described in 1 pure SPG3A family.\textsuperscript{2} In our study, there was no correlation between the site or type of mutation and the clinical severity or the presence of a neuropathy in the SPG3A patients. Pes cavus was present in 67\% of the patients with a neuropathy and is therefore indicative for the complex form of SPG3A. In contrast, amyotrophy of the distal LL appeared also in patients without associated neuropathy. The MEP results obtained in 2 families with a different mutation (SL-6, H258R; SL-121, R239C) differed significantly. The central motor conduction time might therefore be a useful parameter in the genotype-phenotype differentiation of SPG3A patients.

Eleven of 12 SPG3A mutations observed in the present study were missense mutations, corresponding with previous studies in which all except 1 of 22 different mutations were missense mutations. One insertion mutation causing a truncated protein was reported.\textsuperscript{10} We also identified 1 insertion (c.1474_1475insG, A492fsX522) resulting in a premature stop. We found a mutation in each of the 2 known mutational hot spots (R239C, R495W) of SPG3A.\textsuperscript{13} We observed incomplete penetrance in 1 family (HSP-6, G482V). Reduced penetrance has been described in several SPG3A families with a mutation distinct from G482V.\textsuperscript{5,12} We found a de novo mutation in 25\% of the patients, suggesting that screening of SPG3A mutations is also useful in sporadic cases. We conclude that SPG3A is more often associated with a neuropathy than previously expected. When a patient is found to have a bipyramidal syndrome and a neuropathy or pes cavus, screening for SPG3A mutations should be performed.

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