Letter to the Editor

SPG4-related hereditary spastic paraplegia: frequency and mutation spectrum in Brazil

To the Editor:

Hereditary spastic paraplegia (HSP) is a heterogeneous group of neurodegenerative disorders characterized by progressive lower limb spasticity and weakness. Mutations in SPAST/SPG4 are the major cause of autosomal dominant (AD-HSP) in Europe accounting for 40–50% of all patients (1). However, there are few studies on SPG4-HSP from Latin America (2), which has a distinctive ethnic background (populations from European, African and Native American descent). Therefore, our aim was to investigate the frequency and mutation spectrum of SPG4-HSP in a cohort of 55 Brazilian patients with AD-HSP from 34 unrelated families.

Patients were recruited from three centers in the south and southeast of Brazil: Universidade Estadual de Campinas (23 families), Universidade Federal do Paraná (7) and Universidade Federal do Rio Grande do Sul (4). This study was approved by our institution Ethics Committee and written informed consent was obtained from all participants.

Genomic DNA was used in polymerase chain reactions with primers designed to cover the 17 exons of SPAST/SPG4 (3). Mutation screening was performed by automatic Sanger sequencing and multiplex-ligand probe amplification (MLPA). All variants found were checked in human single nucleotide polymorphism (SNP) and mutation databases: Ensembl (http://www.ensembl.org), Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and 1000 genomes project (www.1000genomes.org). New variants were subsequently screened in 200 control chromosomes using Sanger sequencing. To evaluate pathogenicity, we performed in silico analyses using POLYPHEN-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation taster (http://www.mutationtaster.org/), human splicing finder-v2.4.1 (http://www.umd.be/HSF/) and ESEfinder.

In our cohort, seven subjects from two unrelated families had a complicated phenotype (two with dementia and five with epilepsy/peripheral neuropathy). The remaining 48 patients had pure phenotypes, with the mean age at onset of 32 years. Twenty-four patients from 12 unrelated families presented mutations in SPAST/SPG4 (Table 1). All these subjects had a pure phenotype. Each mutation was found in a single pedigree. A synonymous SNP c.878G>A (rs145264166) was also found in one family. MLPA revealed no copy number variation (CNV) in our patients.

We show that mutations in SPAST/SPG4 are frequent in Brazilian families with AD-HSP (35% in this cohort). Our patients with SPAST/SPG4 mutations present a pure phenotype, with early adulthood onset. Eight of these mutations have been already described (3–8). The non-stop mutation c.1849C>T reported by Alvarez et al. (4) did segregate with the phenotype in family PR4. Since c.1849C>T abolishes the stop codon and results in a 46aa longer transcript, this suggests that a

### Table 1. Mutations identified in the SPAST/SPG4 gene in 12 Brazilian families with AD HSP

<table>
<thead>
<tr>
<th>Family</th>
<th>Age at onset (index case; men/women)</th>
<th>Mutation</th>
<th>Gene location</th>
<th>Protein change</th>
<th>Mutation type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR1</td>
<td>18 (1/0)</td>
<td>c.162C&gt;T</td>
<td>Ex 1</td>
<td>Ex1 skipping</td>
<td>Splice site</td>
<td>This study</td>
</tr>
<tr>
<td>PR2</td>
<td>29 (1/2)</td>
<td>c.839_840delAG</td>
<td>Ex 5</td>
<td>p.Q280Rfs289</td>
<td>Frameshift deletion</td>
<td>(7)</td>
</tr>
<tr>
<td>CP1</td>
<td>25 (2/0)</td>
<td>c.1255G&gt;T</td>
<td>Ex 10</td>
<td>p.G419X</td>
<td>Nonsense</td>
<td>This study</td>
</tr>
<tr>
<td>PA1</td>
<td>23 (1/0)</td>
<td>c.1267G&gt;T</td>
<td>Ex 10</td>
<td>p.V423L</td>
<td>Missense</td>
<td>(5)</td>
</tr>
<tr>
<td>CP2</td>
<td>7 (1/0)</td>
<td>c.1378C&gt;T</td>
<td>Ex 11</td>
<td>p.R460C</td>
<td>Missense</td>
<td>(5)</td>
</tr>
<tr>
<td>CP3</td>
<td>34 (1/0)</td>
<td>c.1413+5G&gt;A</td>
<td>In 11</td>
<td>Ex11skipping + frameshift</td>
<td>Splice site</td>
<td>(3)</td>
</tr>
<tr>
<td>PR3</td>
<td>1 (2/0)</td>
<td>c.1495C&gt;T</td>
<td>Ex 13</td>
<td>p.R499C</td>
<td>Missense</td>
<td>(3)</td>
</tr>
<tr>
<td>CP4</td>
<td>26 (1/1)</td>
<td>c.1535_1535delA</td>
<td>Ex 13</td>
<td>p.E512Gfs529</td>
<td>Frameshift deletion</td>
<td>This study</td>
</tr>
<tr>
<td>CP5</td>
<td>30 (3/1)</td>
<td>c.1651G&gt;C</td>
<td>Ex 15</td>
<td>p.A551P</td>
<td>Missense</td>
<td>(6)</td>
</tr>
<tr>
<td>CP6</td>
<td>32 (1/2)</td>
<td>c.1667_1668delCA</td>
<td>Ex 15</td>
<td>p.A566Gfs575</td>
<td>Frameshift deletion</td>
<td>This study</td>
</tr>
<tr>
<td>PA2</td>
<td>25 (1/0)</td>
<td>c.1741C&gt;T</td>
<td>Ex 17</td>
<td>p.R581X</td>
<td>Nonsense</td>
<td>(8)</td>
</tr>
<tr>
<td>PR4</td>
<td>30 (3/1)</td>
<td>c.1849T&gt;G</td>
<td>Ex 17</td>
<td>p.<em>616Eext</em>46</td>
<td>Non-stop change</td>
<td>(4)</td>
</tr>
</tbody>
</table>

aPatients evaluated by a neurologist at one of the centers.
gain-of-function mechanism may operate in this case if the extended protein is synthesized and toxic, although evidence so far is mostly consistent with a loss-of-function mechanism.

Patient CP2 had the known missense mutation c.1378C>T, but in apparent homozygosis (both parents were deceased, making it impossible to confirm their genotype). This patient had an otherwise mild phenotype, but with very early onset, thus suggesting that age at onset rather than disease severity may be associated to homozygous SPAST/SPG4 mutations.

We were able to identify four novel mutations in SPAST/SPG4. The c.162C>T variant is a synonymous SNP that is predicted to remove two exon splicing enhancer sites thus producing a transcript lacking exon1 (Figure 1). It would have been important to have additional family members to confirm segregation of the mutation; however, DNA samples of other relatives were not available. The remaining three novel mutations (c.1255G>T, c.1667_1668delCA and c.1535_1535delA) are all located in the highly conserved AAA ATPase domain and are predicted to result in early truncation of spastin (Fig. 1).

The only previous report on SPG4-HSP in Brazil is that of a large pedigree segregating a duplication spanning exons 10, 11 and 12 of SPAST/SPG4 (2). Despite this, we did not find any copy number aberrations in SPAST/SPG4 in our cohort. Further studies are needed to certify that CNV are indeed rare in Brazilian patients with SPG4-HSP.

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