Phenotypes of Spinocerebellar Ataxia Type 6 and Familial Hemiplegic Migraine Caused by a Unique CACNA1A Missense Mutation in Patients From a Large Family

Isabel Alonso, BSc; José Barros, MD; Assunção Tuna, MD; João Coelho, BSc; Jorge Sequeiros, MD, PhD; Isabel Silveira, PhD; Paula Coutinho, MD, PhD

Background: Different mutations in the α1A-subunit of the brain P/Q-type calcium channel gene (CACNA1A) are responsible for familial hemiplegic migraine (FHM), episodic ataxia type 2, and spinocerebellar ataxia type 6 (SCA6). Missense and splice site mutations have been found in FHM and episodic ataxia type 2, respectively, whereas a CAG repeat in the CACNA1A gene was found expanded in patients with SCA6.

Objective: To identify the disease causing mutation in a large family of patients with phenotypes of hemiplegic migraine with or without cerebellar signs or permanent cerebellar ataxia without migraine inherited in a dominant manner.

Patients and Methods: We examined 15 patients from a large family identified through a systematic survey of hereditary ataxias being conducted in Portugal. Linkage analysis was performed with CACNA1A intragenic markers, and mutation analysis was performed by single strand conformational polymorphism analysis and sequencing.

Results: Genetic linkage analysis with CACNA1A intragenic markers showed positive LOD scores. The maximal LOD score was obtained with the polymorphic CAG repeat (Zmax = 4.47, θ = 0). By single-strand conformational polymorphism analysis, a shift in exon 13 of the CACNA1A gene was detected in all patients. A G-to-A substitution was then identified, resulting in an arginine-to-glutamine change at codon 583 of this calcium channel α1A-subunit.

Conclusions: The disease-causing mutation in this family was identified, showing that a unique mutation in the CACNA1A gene causes several phenotypes, including those of SCA6 and FHM, thus suggesting that SCA6 and FHM are not only allelic diseases but are the same disorder with a large phenotypic variability.

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cated on chromosome 19p13. Missense and splice site mutations have been found in FHM and EA2, respectively, whereas a CAG repeat in the CACNA1A gene was expanded in patients with SCA6.2,21 A G293R missense mutation in the CACNA1A gene is also responsible for progressive cerebellar ataxia.24

To improve knowledge of the underlying mechanism involved in hemiplegic migraine and progressive cerebellar ataxia, we studied a large family in which patients presented with phenotypes of either hemiplegic migraine or progressive cerebellar ataxia, performed genetic linkage analysis with chromosome 19p13 markers, and performed mutation screening in the CACNA1A gene.

**METHODS**

We studied a Portuguese family ascertained during a systematic, population-based survey of hereditary ataxias and spastic paraplegias, initiated in 1993 and covering half of the Portuguese population (5.6 million people).25 This family consisted of 17 patients with hemiplegic migraine and/or progressive cerebellar ataxia in 4 consecutive generations. Fifteen patients were clinically examined by one of us (P.C., J.B., or A.T.) (Table 1). Age at onset ranged from 3 to 23 years for migraine episodes (mean, 13.4±7.2 years) and from 16 to 50 years for cerebellar ataxia (mean, 31.7±11.5 years). The age at examination varied from 8 to 71 years. Clinical manifestations were pleomorphic, including episodes of altered consciousness precipitated by minor head trauma, focal neurological deficits precipitated or not by minor head trauma, and migraine without aura, besides progressive late-onset cerebellar ataxia in a few patients. One of these patients (III-3) was studied by brain magnetic resonance imaging, which showed atrophy of the cerebellum.

Peripheral blood samples were collected from patients and their relatives after written informed consent was obtained. Genomic DNA was obtained from peripheral blood leukocytes by standard techniques.26 Molecular analyses of genetic markers, exons, and intronic sequences of the CACNA1A gene were performed by polymerase chain reaction (PCR) amplification using the published primer sequences.2,21 The PCR was performed with 1µM of each primer, 200µM deoxynucleotides, 1mM magnesium chloride, 10mM Tris (pH 9.0), 50mM potassium chloride, 1 U of Taq polymerase, and 2% formamide in a final volume of 12.5 µL. The PCR products of markers were radioactively labeled and analyzed on 6% polyacrylamide gels. Allele sizes were determined by comparing migration relative to an M13 sequencing ladder.

Polyomorph markers, within a 4-centimorgan (M) interval containing the CACNA1A gene tel-D19S840-19S1150- (CAG)-D19S226-cen, according to the Fondation Jean Dausset Centre d’Études des Polymorphismes (Paris, France) database, were selected for linkage analysis. Markers D19S1150 and the polymorphic CAG repeat are intragenic. Analysis was performed with the LINKAGE27 software program version 5.22. The disease was considered autosomal dominant with incomplete penetrance (93%) and with a disease gene frequency of 0.0001. The PCR products of exons and intronic sequences of the CACNA1A gene were screened for molecular variants by single strand conformational polymorphism analysis28 and electrophoresis in ×0.5 Mutation Detection Enhancement gels (Bio-Whittaker Molecular Applications, Rockland, Me) at 4°C. Conformational changes were confirmed by sequencing with Thermosequenase cycle-sequencing kit (Amersham Pharmacia Biotech, Uppsala, Sweden). Restriction analysis of exon 13 was performed by PCR amplification, and products were digested with the BanII restriction enzyme (New England BioLabs, Beverly, Mass) according to manufacturer instructions.

**RESULTS**

The size of the CAG repeat, in the 3’ end of the CACNA1A gene, responsible for SCA6 was determined in all 25 subjects available for this study. The repeat size in 12 patients, 9 at-risk individuals, and 4 spouses, ranged from

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**Table 1. Clinical Features of the Patients**

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>Age at Onset, y</th>
<th>Focal Neurological Deficits Without Headache</th>
<th>Migraine Without Aura</th>
<th>Triggered Minor Head Trauma*</th>
<th>Coma or Impaired Consciousness</th>
<th>No. of HM Episodes per Year</th>
<th>HM Episodes, h</th>
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<tr>
<td>I-2/F/†</td>
<td>22</td>
<td>50</td>
<td>22</td>
<td>22</td>
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<td>III-1/M/50</td>
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<td>III-3/F/39</td>
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<td>III-9/F/29</td>
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<td>72</td>
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<td>IV-3/M/16</td>
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<tr>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>12</td>
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7 to 14 units, which is within the normal allele interval for SCA6. Further screening excluded SCA1, SCA2, Machado-Joseph disease, SCA7, SCA8, SCA10, SCA12, SCA17, and dentatorubropallidoluysian atrophy expansion.

Linkage analysis with polymorphic markers D19S840, D19S1150, the polymorphic CAG repeat, and D19S226 gave positive LOD scores (Table 2). The maximal LOD score was obtained with the intragenic CAG repeat (Zmax = 4.47, θ = 0). Haplotype construction with chromosome 19p markers showed a common haplotype shared by all patients of this family (Figure 1), except patient II-7, in whom a recombination occurred between the CAG repeat and marker D19S226, located 3 cM from the CACNA1A gene (Fondation Jean Dausset CEPH database). His affected offspring shared the same recombined haplotype.

Mutation detection was performed by single strand conformational polymorphism analysis after PCR amplification of each exon and exon-intron boundaries. A mobility variant was detected in the exon 13 fragment that showed a 3-band pattern (Figure 2A). By direct sequencing, a G-to-A substitution at position 2023 was identified (Figure 2B). This substitution produces an arginine-to-glutamine change at codon 583 in the CACNA1A gene. By restriction analysis with BanII restriction enzyme, this mutation was excluded in 100 control chromosomes from the Portuguese general population. After BanII digestion, fragments of 123, 122, and 67 base pairs (bp) were detected for healthy individuals, whereas in the patients an additional band of 245 bp was also present, resulting from the loss of a restriction site on the mutated allele.

In this study, we describe the first family to our knowledge in which patients presented phenotypes of hemiplegic migraine with or without cerebellar signs or permanent progressive cerebellar ataxia without migraine due to a unique missense mutation in the CACNA1A gene. The disease locus in this family showed strong linkage to intragenic markers in this gene. By mutation analysis, we identified an R583Q substitution in all available patients. This mutation had first been described in 2 affected members from a family with hemiplegic migraine and ataxia. We described a large family with 17 patients who presented with high clinical variability due to this R583Q mutation.

The α1A-subunit of the P/Q-type calcium channel gene is composed of 4 homologous domains (I-IV), each containing 6 putative transmembrane segments (S1-S6) and a pore-forming segment between S5 and S6. The missense mutation identified in this family is located in the S4 transmembrane segment of protein domain II, which is thought to be the voltage sensor of the channel.

Mutation R583Q replaces a conserved, polar, positively charged arginine by a neutral glutamine, which can increase hydrophobicity and reduce polarity in this voltage sensor segment. This mutation causes a shift in the activation and inactivation voltage dependence of the channel to more negative potentials. The hyperpolarization shift increases intracellular calcium levels by altering P/Q-type calcium channel activity at weak depolarizations in mutants with this substitution. Channel recovery from inactivation in R583Q mutants is slower, which can lead to an accumulation of inactivated channels during rapid depolarizations. Another HFM mutation due to an arginine-to-glutamine substitution also located in the S4 transmembrane segment, but of protein domain I at codon 192, also causes an excess of intracellular calcium due to altered gating properties. The abnormal calcium influx, mostly during high neuronal activity, would explain the paroxysmal character of FHM and the precipitation of episodes by sensory or emotional stimuli. Calcium overload causes excessive release of excitotoxic neurotransmitters such as glutamate, which can lead neurons to apoptotic death.

In this family, the mean age at onset for hemiplegic migraine symptoms was in the second decade and approximately 20 years earlier than that for the cerebellar signs. This onset of migraine symptoms is close to that reported in other clinical descriptions of FHM due to mutations in the CACNA1A gene. The 2 patients previously described as having mutation R583Q began migraine episodes at 17 and 40 years, respectively, whereas cerebellar signs were first noticed in both patients when they were in their 60s.

Emotional stress was the most frequent triggering factor of hemiplegic migraine in families with mutations in the CACNA1A gene as described in a previous study. In the present family, patients with hemiplegic migraine did not refer to emotional stress as a triggering factor, whereas minor head trauma was referred to in approximately 4 patients (44%). However, this family is unique in which cerebellar progressive ataxia was also triggered by mild head trauma.

Expansion of a CAG repeat in the CACNA1A gene causes not only SCA6 but also EA2 phenotypes in patients from the same family. On the other hand, in a family described by Yue et al, a point mutation in this gene originates severe progressive ataxia in some patients and episodic ataxia in others. Moreover, some families had members with either hemiplegic migraine accompanied by cerebellar signs or episodic ataxia with headache due to a point mutation in the CACNA1A gene. In this family, we found patients who only had symptoms of progressive cerebellar ataxia, patients affected by hemiplegic migraine only, and patients with both hemiplegic migraine and symptoms of progressive cerebellar ataxia. Thus, the R583Q mutation causes phenotypes of SCA6 and FHM. These results, in addition to those referred to herein, offer evidence that patients with cerebellar signs and migraine due to a point mutation in the CACNA1A gene may present with a unique neurological syndrome characterized by cerebellar ataxia and hemiplegic migraine.

### Table 2. Linkage Relationships Between the Disease Locus and Chromosome 19p13 Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>0.0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>Zmax</th>
<th>θ</th>
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<tbody>
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<td>D19S840</td>
<td>2.29</td>
<td>1.94</td>
<td>1.43</td>
<td>0.83</td>
<td>0.24</td>
<td>2.29</td>
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<td>D19S1150</td>
<td>2.67</td>
<td>2.20</td>
<td>1.61</td>
<td>0.94</td>
<td>0.29</td>
<td>2.67</td>
<td>0.0</td>
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<tr>
<td>CACNA1Ant2023</td>
<td>2.92</td>
<td>2.40</td>
<td>1.77</td>
<td>1.07</td>
<td>0.35</td>
<td>2.92</td>
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<tr>
<td>(CAG)n</td>
<td>4.47</td>
<td>3.80</td>
<td>2.95</td>
<td>1.96</td>
<td>0.80</td>
<td>4.47</td>
<td>0.0</td>
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<tr>
<td>D19S226</td>
<td>-7.22</td>
<td>2.69</td>
<td>2.25</td>
<td>1.53</td>
<td>0.96</td>
<td>2.74</td>
<td>0.069</td>
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</table>

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suggest that EA2, SCA6, and FHM are not only allelic diseases but are the same disorder with a large phenotypic variability. The presence of several different phenotypes strongly suggests the involvement of modifying polymorphisms in either this or other genes.

Mutations in the $\alpha_{1A}$-subunit orthologous mouse gene are responsible for 2 phenotypes: the tottering (tg) and the leaner (tgla). The tgla mouse phenotype presents severe progressive ataxia caused by a mutation in a splicing consensus sequence, which gives rise to CACNA1A aberrant transcripts. $^{35}$ On the other hand, the tg mutant mice phenotype is caused by an amino acid substitution in the pore-forming region of mice $\alpha_{1A}$ protein domain II. $^{31}$ This mutant expresses a milder phenotype and shows less functional changes. $^{38}$ The tg and tgla mutated channels exhibit a reduced calcium influx in Purkinje cells. $^{36-38}$

In conclusion, the mutation R583Q in the CACNA1A gene causes a large variety of clinical phenotypes, including hemiplegic migraine, permanent ataxia, and coma.
Mutations not only in the pore-forming segments but also in the voltage sensor transmembrane segments alter the gating properties of neuronal P/Q-type calcium channels, causing alterations in calcium influx through neurons.

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Author contributions: Study concept and design (Drs Silveira and Coutinho); acquisition of data (Ms Alonso, Drs Barros, Tuna, Silveira, and Coutinho, and Mr Coelho); analysis and interpretation of data (Ms Alonso, Drs Barros, Tuna, Sequeiros, Silveira, and Coutinho, and Mr Coelho); drafting of the manuscript (Ms Alonso, Drs Barros, Tuna, Silveira, and Coutinho, and Mr Coelho); critical revision of the manuscript for important intellectual content (Ms Alonso and Drs Sequeiros, Silveira, and Coutinho); obtained funding (Dr Silveira); administrative, technical, and material support (Ms Alonso, Drs Barros and Tuna, and Mr Coelho); study supervision (Drs Sequeiros, Silveira, and Coutinho).

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REFERENCES