**Objectives:** To characterize the nature of CACNA1A mutation in a previously unreported family with episodic ataxia type 2 (EA2) and to better delineate EA2 clinical features.

**Background:** Episodic ataxia type 2 is an autosomal dominant disorder characterized by the recurrence of acetazolamide-responsive spells of cerebellar ataxia, usually starting during childhood or adolescence. The mutated gene, CACNA1A, is located on chromosome 19 and encodes the α1A subunit voltage-dependent calcium channel. So far, most CACNA1A mutations detected in patients with EA2 have led to a truncated CACNA1A protein, whereas missense mutations cause familial hemiplegic migraine.

**Methods:** All 47 exons of CACNA1A were screened by a combination of single-strand conformation polymorphism and sequencing analysis.

**Results:** A CACNA1A missense mutation, Glu 1757 Lys, was identified. It was absent in 200 control chromosomes. It is predicted to result in an amino acid substitution at a highly phylogenetically conserved position, within a domain that plays a major role in the function of the channel.

**Conclusions:** The Glu 1757 Lys missense mutation is likely to be pathogenic, causing episodic ataxia within a family whose phenotype is indistinguishable from EA2 except for a slightly later age of onset. These data strongly suggest that additional work is needed to fully establish genotype/phenotype correlations for CACNA1A mutations.

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Episodic ataxia type 2 is caused by mutations within the α1A subunit of a P/Q-type voltage-dependent calcium channel gene, CACNA1A. P/Q-type channels, which are expressed throughout the brain and at the neuromuscular junction, are implicated in the control of membrane excitability and neurotransmitter release. So far, 11 EA2 mutations have been reported, most of them leading to a truncated CACNA1A protein. Interestingly, distinct types of CACNA1A mutations have been reported in other autosomal dominant neurological conditions. Chromosome 19-linked familial hemiplegic migraine is caused by missense mutations. Small expansions of the CAG repeat located within the 3' coding end of CACNA1A cause spinocerebel-
lar ataxia type 6,\textsuperscript{14} a late-onset, moderate to severe progressive cerebellar ataxia, without paroxysmal event. However, these strong genotype/phenotype correlations may not be absolute. Two families with a permanent progressive cerebellar ataxia, associated with paroxysmal ataxic episodes, and 1 family with pure episodic ataxia were shown to harbor CAG repeat expansions.\textsuperscript{15,16} More recently, a CACNA1A missense mutation was shown to cause a severe progressive cerebellar ataxia with early onset in several members of a family.\textsuperscript{17}

We report herein a missense CACNA1A mutation causing episodic ataxia within a family whose phenotype is indistinguishable from EA2 except for a slightly later onset.

**REPORT OF CASES**

**PEDIGREE**

This family included 4 symptomatic members (Figure 1, individuals I-2, II-1, II-5, and III-2). Detailed clinical information was obtained from patient III-2 and 2 of his 3 sons who were clinically examined. Clinical information regarding patients I-2, II-1, II-5 was obtained from patient III-2 (Figure 1).

**PROBAND**

Proband III-2 (Figure 1), a 53-year-old man, experienced recurrent episodes of paroxysmal cerebellar ataxia since he was 40 years old. His medical history was unremarkable except for a strabismus, which needed surgical repair at age 22 years. Ataxic spells were strongly stereotyped. Onset was sudden with brief bilateral parasthesias in upper and lower extremities, diffuse weakness, and heat sensations rapidly followed by generalized ataxic symptoms. Attacks always included severe truncal and limb ataxia with dysarthria, vertigo, and oscillopsia and diplopia sometimes associated with nausea, vomiting, and blurred vision. The patient reported headaches fulfilling International Headache Society criteria for migraine without aura, both during and between ataxic spells. Duration of ataxic episodes usually ranged from half an hour to 4 hours. They were precipitated by emotional and physical stress and spontaneously resolved with rest or sleeping.

This patient suffered from 3 to 4 attacks per month, up to 1 per day in stressful periods. He first presented to us in 1996 at age 50. Findings of interictal examination disclosed an isolated, gaze-evoked nystagmus. The remainder of his neurological examination results were normal. Brain magnetic resonance imaging revealed a moderate vermian cerebellar atrophy. Findings of electroencephalographic and electromyelographic studies were normal. The patient began treatment with 250 mg of acetazolamide twice a day, and reported a marked decrease in severity and frequency of attacks (once a month) during 1 year, but no improvement on isolated migraine episodes. In 1997, he stopped treatment during 2 months and experienced an outbreak of attacks; frequency of these attacks decreased with the reinstatement of treatment. Two years later, interictal neurologic examination results disclosed a gaze-evoked nystagmus and a mild statokinetic cerebellar ataxia.

**OTHER AFFECTED FAMILY MEMBERS**

The proband reported that his father (individual II-1, Figure 1) experienced similar attacks of episodic ataxia since his 40s, with an average frequency initially close to twice a month and increasing with age. Attacks usually lasted 2 hours, precipitated by physical exercise or emotional stress, and disappeared with sleep. Acetazolamide treatment had never been tried. He had not developed any progressive severe ataxia or gait disorder by the time of his death at age 76 years (of prostate cancer). The proband's grandmother (Figure 1, I-2) and paternal aunt (Figure 1, II-5) also exhibited paroxysmal attacks of generalized ataxia with late onset (after age 30 years) and without permanent severe gait disorders.

The proband had 3 sons, aged 12 (Figure 1, IV-1), 20 (Figure 1, IV-2), and 21 (Figure 1, IV-3) years. All 3 were asymptomatic. Findings from clinical examination of patients IV-1 and IV-2 were normal. Patient IV-3 was not examined.

**GENETIC ANALYSIS**

Samples of DNA from the proband (Figure 1, III-2) and 2 of his 3 sons (Figure 1, IV-1 and IV-2) were extracted from peripheral blood using standard procedures. In addition, DNA samples from 100 unrelated healthy subjects (white French individuals, to match the proband family) were also available for the study. All 47 exons of CACNA1A were screened using a combination of single-strand conformer polymorphism\textsuperscript{18} and sequencing analysis as previously described.\textsuperscript{11}

**RESULTS**

A missense mutation was identified within exon 35 in the proband's DNA. This G$\rightarrow$A substitution at codon 1757 (GAA$\rightarrow$AAA, Figure 2 and Figure 3) leads to the replacement of a highly conserved preexisting glutamic acid for a lysine. This mutation was absent in the panel of 200 normal chromosomes, as well as in the 2 asymptomatic sons of the proband (Figure 1, IV-1 and IV-2). The number of CAG repeats at the 3’ coding end of the gene was in the normal range (7/13).
Clinical manifestations observed within affected members of this family (namely, recurrent paroxysmal acetazolamide-responsive attacks of generalized cerebellar ataxia associated with interictal permanent cerebellar symptoms as well as the cerebellar atrophy evident on magnetic resonance imaging) are strongly suggestive of EA2. None of these family members suffered from hemiplegic migraine. The only subtle difference from previously reported families with EA2 was a later age of onset. Whereas in most patients with EA2 clinical onset occurs during childhood or adolescence, initial symptoms in the 4 affected members of this family occurred after age 30 years. However, clinical onset after age 30 years has been reported in a few members of families with EA2.2

CACNA1A screening revealed a missense mutation, Glu 1757 Lys, while most previously described EA2 mutations led to a truncated CACNA1A protein.6-9 Multiple arguments strongly suggest that this amino acid substitution caused the disease of our patient. First, it was not detected in 200 control chromosomes, strongly suggesting that it is not a rare polymorphism. Second, this mutation affects a highly conserved amino acid located within the pore loop, which plays a major role in the function of the channel pore.

The α1A calcium channel subunit encoded by CACNA1A is formed by 4 homologous domains (Figure 2). Each domain contains 6 membrane-spanning segments (S1-S6). The central pore of the channel is delineated by the 4 pore-loop regions, which interconnect the fifth and sixth segment membrane spanning each domain. The glutamates located within each pore loop are key players for calcium selectivity.19,20 Substitution within this pore loop of a negatively charged glutamic acid for a positively charged lysine would most likely be very deleterious. In addition, glutamic acid at codon 1757 is a highly conserved amino acid from Drosophila to man. For these reasons, despite the fact that DNA from other members was not available for cosegregation analysis, we think that this missense mutation most likely caused the disease observed in this family.

To our knowledge, there is only 1 family harboring a CACNA1A missense mutation, although not affected with familial hemiplegic migraine. This family included 8 affected members who suffered from a severe progressive cerebellar ataxia,17 which confined some of them to wheelchairs by their 40s. Interestingly, 2 of these members had, in addition to this severe progressive ataxia, acetazolamide-resistant paroxysmal episodes of vertigo and ataxia. Mutation in this family substituted an uncharged glycine for a positively charged arginine within the pore-loop of the first domain of CACNA1A.

Although in most cases families with EA2 harbor truncating mutations in CACNA1A whereas in familial hemiplegic migraine missense mutations occur, the fam-
ily reported herein is an example of overlap between episodic neurological conditions due to CACNA1A missense mutations. These data strongly suggest that additional work is needed to fully establish genotype/phenotype correlations. The mechanisms leading from these various types of mutations to these phenotypes are not understood at present, and electrophysiological studies are strongly needed.

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