A novel nonsense mutation in CACNA1A causes episodic ataxia and hemiplegia

J. Jen, MD, PhD; Q. Yue, MD; S.F. Nelson, MD; H. Yu, BS; M. Litt, PhD; J. Nutt, MD; and R.W. Baloh, MD

Article abstract—Objective: To identify the disease-causing mutation and to characterize penetrance and phenotypic variability in a large pedigree with episodic ataxia type 2 (EA-2) previously linked to chromosome 19. Background: Mutations in the CACNA1A gene on chromosome 19 encoding a calcium channel subunit cause EA-2, which is characterized by recurrent attacks of imbalance with interictal eye movement abnormalities. Methods: The authors used single-strand conformation polymorphism (SSCP) analysis to screen for point mutations, and direct sequencing to identify mutations in CACNA1A. Allele-specific oligonucleotides were designed to detect the presence of the diseased allele in members of their pedigree as well as in normal control subjects. Results: Reassessment of members of the pedigree revealed two notable clinical features. Diffuse weakness during attacks of ataxia was a prominent complaint. Two affected individuals had had episodic hemiplegia, one with typical migraine headaches. SSCP analysis revealed aberrant bands in exon 29 in affected members but not in normal control subjects. Direct sequencing of exon 29 identified a C-to-T change at position 4914 of the coding sequence of CACNA1A, predicting an early stop code at codon 1547. Two asymptomatic mutation carriers demonstrated the incomplete penetrance of this mutation. Conclusions: A nonsense mutation in CACNA1A causes episodic ataxia and complaint of weakness, and may be associated with hemiplegia.

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Familial episodic ataxia (EA) is an uncommon neurologic disorder characterized by episodes of ataxia and vertigo with minimal interictal neurologic deficits. Exertion, stress, and fatigue commonly precipitate ataxic episodes. The disorder is often dramatically responsive to acetazolamide, which prevents the attacks. Because several inherited episodic neurologic disorders responsive to acetazolamide have been found to involve ion channel gene mutations, a channelopathy had long been suspected to underlie EA.1 Indeed, mutations in genes encoding ion channels have been identified in two different types of EA. Mutations in KCNA1 on chromosome 12 encoding a potassium channel cause EA-1 (episodic ataxia with myokymia).2,3 Three different point mutations in CACNA1A on chromosome 19 encoding a calcium channel subunit cause EA-2 (episodic ataxia with interictal eye movement abnormalities).4,5 Several other neurologic disorders associated with mutations in CACNA1A have also been described. Four different missense mutations in CACNA1A were identified in pedigrees with familial hemiplegic migraine.5 A small expansion of CAG repeats in CACNA1A is associated with spinocerebellar ataxia type 6 (SCA-6), which is a slowly progressive, late-onset cerebellar syndrome.6 More recently, a missense mutation was identified in a family with early-onset progressive ataxia with superimposed episodic attacks of imbalance.8

CACNA1A encodes the α1A-subunit of the calcium channel, which is a 220-kd polypeptide that is the pore-forming and voltage-sensing component of the P/Q-type voltage-gated calcium channels expressed throughout the CNS but most abundantly so in the cerebellum.9 The α1A-subunit is a transmembrane protein with four homologous domains. Although the exact composition of the calcium channel complex in vivo is not known, the α1A-subunit associates with auxiliary subunits that include an intracellular β-subunit, a disulfide-linked largely extracellular α2δ-subunit, and a transmembrane γ-subunit.10,11 In addition to mediating neurotransmitter release centrally,12 the P/Q-type channels are the main presynaptic voltage-gated calcium channels responsible for triggering neurotransmission at the neuromuscular junction.13 Autoantibodies directed against the α1A-subunit of the P/Q channels underlie Lambert–Eaton syndrome.14

We now report the identification of a new mutation in CACNA1A in a large pedigree with EA-2 previously linked to chromosome 19p.15 The size of the
pedigree is ideal for assessing penetrance and phenotypic variability.

Methods. Figure 1 presents a large pedigree with clinical features that have been reported in detail. To assess the current disease status, we reexamined 10 family members within the past year, and interviewed 8 others. Individuals III-13 and III-14 could not be reached. We also examined three children in the fourth generation.

Genomic DNA was extracted from every consenting family member. All 47 exons and flanking introns of CACNA1A in members of this pedigree as well as normal control subjects were amplified by PCR and screened for mutation by single-strand conformation polymorphism (SSCP) analysis. Exon 29 in affected individuals produced aberrantly migrating bands on SSCP and was PCR amplified, gel purified, and subjected to direct sequencing analysis. CAG repeat repeat lengths were determined as described previously.

Allele-specific oligonucleotides (ASO) were designed to detect the presence of the diseased allele in members of this pedigree and in 96 normal control subjects. Exon 29 PCR products were transferred onto Hybond N membranes (Amersham Pharmacia, Piscataway, NJ) by a 96-pin replicator (V & P Scientific, San Diego, CA). After denaturation in 0.4 M NaOH and neutralization in 2× SSC, the air-dried filters were prehybridized in ExpressHyb solution (Clontech Laboratories, Palo Alto, CA) for 30 minutes at 37 °C then hybridized for 1 hour at 37 °C with either the wild-type ASO with the sequence 5'-CGCTGACCTGACACATG-3' or the mutated ASO 5'-CGCTGACCCGACACATG-3'. Oligonucleotides were end labeled by using T4 polynucleotide kinase (New England Biolabs, Beverly, MA) and [γ-32P] adenosine triphosphate. Filters were washed in 2× SSC and 0.5% sodium dodecyl sulfate (SDS) for 30 minutes at 37 °C, followed by a wash in 0.2× SSC and 0.5% SDS for 20 minutes at 50 °C, and were then exposed to film.

Results. Attacks of vertigo, truncal and limb ataxia, as well as slurred speech generally began in early childhood (table). Vigorous exercise and emotional stress often triggered these ataxic episodes. The frequency and duration of attacks varied among different affected individuals. Many reported exquisite sensitivity to alcohol. All except the most severely affected member (Individual III-1) responded well to acetazolamide, with a marked decrease in frequency and severity of attacks. Of note, Individual II-2 has never taken acetazolamide because she denied having similar symptoms as her brother and children; yet, she has recurrent episodes of imbalance treated with ticlopidine for presumed TIAs. Symptomatic individuals report diffuse weakness and severe headaches during attacks of ataxia. Nystagmus was present in several individuals since infancy. Neurologic examination revealed interictal cerebellar deficits including gaze-evoked and rebound nystagmus in most individuals.

Two affected members had hemiplegia during episodes of ataxia. Since infancy, Individual III-16 had nystagmus and attacks of vertigo and ataxia, often accompanied by debilitating, mostly right-side pounding headaches associated with nausea and photophobia. Acetazolamide reduced the frequency and severity of these spells markedly. She stopped taking acetazolamide during her recent pregnancy and developed a new type of spell, manifested as severe right-side weakness in addition to ataxia and headache. During these spells she was unable to write and had to drag her right leg while walking. The episodes of ataxia, hemiplegia, and migraine headache generally lasted for hours and occurred almost daily during her last trimester. Since she gave birth and resumed taking acetazolamide, she has only had rare attacks. Individual III-6 has had attacks of vertigo and ataxia since childhood. In recent years, she noted hemiparesthesias and an inability to move her right fingers or to lift her right arm during her ataxic spells, which generally last from hours to days. Unlike Individual III-16, she has not experienced headaches.

SSCP analysis revealed aberrantly migrating fragments in exon 29 of all affected members and two asymptomatic members (figure 2). Direct sequencing showed a C-to-T change at position 4914, resulting in a stop code at codon 1547. SSCP analysis of all exons detected no other mutation elsewhere in the coding sequence. Neither was there CAG repeat expansion to greater than 21 repeats. ASO hybridization analysis confirmed the presence of the mutation in all members with aberrant bands in exon 29 by SSCP analysis. The mutant allele was absent in 96 normal control subjects.

Discussion. We report the incomplete penetrance of a nonsense mutation in CACNA1A in a large kindred affected variably with EA that may be associated with hemiplegia. How this newly identified stop mutation may affect the function of CACNA1A is not clear, but there are several possibilities. The exon containing a premature stop codon could be skipped during the splicing process, leading to deletion of amino acid residues without disrupting the reading frame. Alternatively, the premature stop codon

Figure 1. Pedigree of family with episodic ataxia. Filled symbols indicate symptomatic individuals. * = examined; △ = interviewed.
could lead to a truncated gene product, with only three of four domains. The truncated polypeptide is unlikely to form a functional channel. Yet, because it retains recognition sites for association with the auxiliary calcium channel β-subunit and the G protein βγ subunit, it could potentially interfere with normal channel membrane targeting and function by sequestering associated proteins. Of note, a 95-kd glycoprotein thought to be a short form of the α1A-subunit copurified with the P/Q-type calcium channel complexes. The role of this naturally truncated polypeptide in the normal function of P/Q-type channels is not understood.

We describe the coexistence of EA and hemiplegia in the same pedigree. One member (Individual III-16) had episodes with typical features of hemiplegic migraine, whereas another (Individual III-6) had hemiplegia not associated with headache. Hemiplegic migraine, which may be a form of basilar migraine, has features such as vertigo and ataxia that overlap with EA. Three previously reported mutations in EA-2 (a deletion mutation, a mutation in a splice site, and a stop mutation) were all predicted to lead to a premature stop in the open reading frame of CACNA1A. So far, four point mutations have been identified in pedigrees with familial hemiplegic migraine; all were missense mutations predicted to lead to single amino acid residue changes. Although there appears to be a tendency for missense mutations to be associated with hemiplegic migraine, nonsense mutations to be associated with EA, and CAG repeat expansions to be associated with progressive ataxia, there is some overlap in symptoms such that a strict genotype–phenotype correlation cannot be established. A missense mutation caused EA superimposed on a severe progressive decline in baseline cerebellar function. There have also been several reports of episodic features in SCA-6. We now report a nonsense mutation that is similar to previously reported EA-2-causing mutations but causes hemiplegia in addition to EA in two affected family members.

How does the same mutation in a calcium channel gene lead to such varied phenotypic expression from

Table Clinical profile of mutation carriers in this pedigree

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Age at onset of episodic ataxia, y</th>
<th>Headache meeting IHS criteria for migraine</th>
<th>Ictal weakness</th>
<th>Interictal nystagmus</th>
<th>Interictal truncal ataxia</th>
<th>Brain MRI</th>
<th>Acetazolamide Trial</th>
<th>Response</th>
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<td>F</td>
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</table>

IHS = International Headache Society; – = absent; + = present; ++ = severe; NA = not applicable.
episodic and progressive ataxia to hemiplegic migraine? Abnormal channel activity likely gives rise to transient cellular dysfunction, leading to episodic symptoms. Chronic abnormal calcium homeostasis could lead to progressive neuronal degeneration. Phenotypic variability among individuals in the same pedigree suggests that environmental, metabolic, gender, and other genetic factors likely contribute to the phenotypic expression of the mutation. Abnormal eye movements preceded other symptoms of ataxia by many years. The early manifestation of gaze-evoked nystagmus suggests selective involvement of the oculomotor centers in the cerebellum. That anxiety and stress rapidly trigger ataxic episodes suggests selective involvement of the phenotypic expression of the mutation. Abnormal channel activity likely gives rise to the calcium channels or downstream signaling functions of these calcium channels may be closely modulated by catecholamines. Hormonal levels may also play a role because a female member (Individual III-16) experienced exacerbation of her symptoms particularly during pregnancy. Furthermore, men appear to be more severely affected than women in this pedigree. Compared with their sisters, male Individuals II-4, III-1, and IV-1 all had earlier onset and more frequent and severe ataxic spells.

Although not described in other patients with EA, diffuse weakness during ataxic spells was a prominent complaint among affected individuals in this kindred. A favorable response to acetazolamide was discovered serendipitously when patients with EA were suspected to have periodic paralysis.26 Indeed, there may be a physiologic basis for diffuse weakness as part of the clinical manifestation of calcium channel mutations causing EA. Because the P/Q-type channels are the major presynaptic voltage-gated calcium channels critical for the release of acetylcholine at the neuromuscular junction, mutations in CACNA1A could interfere with normal calcium entry into the presynaptic nerve terminal, leading to impaired neurotransmitter release at the neuromuscular junction, and resulting in weakness.

References