Two Novel CACNA1A Gene Mutations Associated With Episodic Ataxia Type 2 and Interictal Dystonia

Sian D. Spacey, MD; Luke A. Materek, BSc; Blajez I. Szczygielski, BSc; Thomas D. Bird, MD

Background: Episodic ataxia type 2 (EA2) is an autosomal dominant condition that results from mutations in the CACNA1A gene. It is characterized by episodes of ataxia and nystagmus that typically last hours.

Objective: To describe the clinical and genetic features of 2 unrelated patients who developed EA2 in childhood and late-onset dystonia.

Design: Pedigree study.

Setting: University academic teaching hospital.

Patients: Two unrelated patients with childhood-onset EA2 and adult-onset dystonia were identified through a neurogenetics clinic. The CACNA1A gene was screened by heteroduplex analysis and sequencing for mutations.

Main Outcome Measure: Mutations in the CACNA1A gene.

Results: Novel mutations in the pore-forming subunit of the P/Q-type calcium channels were found in both pedigrees. None of the family members carried an expansion of the CAG sequence that is found in the carboxy terminus of the CACNA1A gene.

Conclusions: Truncating mutations are the most common mutations to cause EA2. We have identified 2 novel truncating mutations that are associated with interictal dystonia. The dystonia is a late feature in this disease and may be a manifestation of a degenerative cerebellar process.


Episodic ataxia type 2 (EA2), an autosomal dominant condition that demonstrates incomplete penetrance and variable expressivity both between and within families, is characterized by episodes of nystagmus and ataxia that last hours to days and is associated with vertigo, diplopia, nausea, vomiting, and headache.1 Attacks can be provoked by exercise, emotional stress, alcohol, and caffeine and relieved by acetazolamide.2 Onset of EA2 occurs in childhood or early adolescence (age range, 2-32 years). The frequency of attacks can range from 3 to 4 times per week to 1 to 2 times per year. During the interictal period, patients may initially be asymptomatic but eventually develop interictal nystagmus and ataxia.3

Episodic ataxia type 2 results from mutations in the CACNA1A gene on chromosome 19p13. The CACNA1A gene codes for the α1A-subunit, the pore-forming subunit of the voltage-dependent P/Q-type calcium channel.1 The primary structure of the α1A-subunit predicts the presence of 4 homologous domains, each consisting of 6 transmembrane segments and a pore-forming P loop (Figure 1). Most mutations responsible for EA2 are nonsense mutations that result in a truncated protein product and a nonfunctional channel.1 The EA2 mutations appear to result in a severe reduction in P/Q-type channel activity.

We describe 2 novel CACNA1A gene mutations that predict a truncated protein product. Both of these unique mutations are associated with EA2 and interictal dystonia. Like the interictal ataxia typically seen in this condition, the interictal dystonia is unresponsive to acetazolamide.

Methods

Patients

Pedigree 1

The proband (III:2) was a 64-year-old man from a 4-generation family (Figure 2) who first developed episodes of diplopia with exercise at age 15 years. Progression of the episodes into dizziness and vomiting could be prevented if he lay down and rested. With age, the frequency of attacks increased and could be triggered by mental and physical stress, caffeine,
Novel mutations in the pore-forming subunit of the P/Q-type calcium channels were found in both pedigrees. In the proband from pedigree 1 (III:2), a C-to-T substitution at exon 29 (c.4963C→T) resulted in the creation of a stop codon in place of glutamine (Q1561X) and the subsequent truncation of the protein between domains IIIS6 and IVS1 (Figure 1) (GenBank accession No. nm_023035 //www.ncbi.nlm.nih.gov/SNP/) of the CA1A gene. Samples that harbored mutations were cloned into pGEM-T easy vectors (Promega, Madison, Wis) and sequenced as described herein, using T7 and SP6 primers. To determine the number of CAG repeats at the 3′ end of the CA1A gene, a PCR reaction was performed using the forward primer 5′-CCAATCCCGTGTCCTCCTTTG-3′ and the reverse primer 5′-GGTAGTAGCAGCATGGTGCG-3′. The PCR products were subsequently sequenced as described previously.

RESULTS

Novel mutations in the pore-forming subunit of the P/Q-type calcium channels were found in both pedigrees. In the proband from pedigree 1 (III:2), a C-to-T substitution at exon 29 (c.4963C→T) resulted in the creation of a stop codon in place of glutamine (Q1561X) and the subsequent truncation of the protein between domains IIIS6 and IVS1 (Figure 1) (GenBank accession No. nm_023035 was used as the reference sequence). The mutation was identified in individuals III:2, IV:1, and IV:3.

GENETIC ANALYSIS

After patients consented, genomic DNA was isolated from peripheral blood from family members using standard techniques. Each of the 47 exons of the CA1A gene and their flanking intron-exon boundaries were polymerase chain reaction (PCR) amplified using primers previously described and were run on a heteroduplex gel. If any migrational differences were detected, the PCR product was sequenced on an ABI 377 automated DNA sequence machine (Applied Biosystems, Foster City, Calif) and compared with the human genomic sequence and single nucleotide polymorphism database (available at http://www.ncbi.nlm.nih.gov/SNP/) of the CA1A gene. Samples that harbored mutations were cloned into pGEM-T easy vectors (Promega, Madison, Wis) and sequenced as described herein, using T7 and SP6 primers.

Pedigree 2

The proband (II:1) (Figure 2) was a 52-year-old man who from age 5 years had episodes of ataxia several times a day to several times per week. In later years, the episodes were associated with dysarthria. Typically, he would sit or lie down until the episode passed. Episodes could be precipitated by stress and anxiety. There was no consistent benefit from medications, but medications were usually taken at low doses for short periods; these medications included acetazolamide, diazepam, phenytoin, carbamazepine, propranolol hydrochloride, medazine hydrochloride, nortriptyline hydrochloride, clonazepam, clordiazopoxide hydrochloride, and verapamil hydrochloride. At age 47 years, he developed bilateral blepharospasm, which was treated with botulinum injections. These treatments were initially effective but became less beneficial with repeated use.

His only examination abnormality was persistent horizontal and vertical nystagmus. There was no evidence of interictal ataxia, dysarthria, or tremor. Magnetic resonance imaging of the brain at age 36 years showed mild midline atrophy of the cerebellar vermis. Several past electroencephalograms had revealed a variable amount of bilateral bursts of medium-voltage θ and δ activity in the frontal and temporal regions. No other family members were affected; however, little was known about his father.

Table 1. The primary structure of the αCa2+ subunit and the location of the novel mutations (c.4963C→T and c.3772delC). NH3+ indicates amino terminal; COOH−, carboxy terminal.

<table>
<thead>
<tr>
<th>Position</th>
<th>Mutation</th>
<th>Description</th>
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<tbody>
<tr>
<td>4963</td>
<td>C→T</td>
<td>Germinal stop (Q1561X)</td>
</tr>
<tr>
<td>3772</td>
<td>delC</td>
<td>Germinal truncation</td>
</tr>
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In the proband from pedigree 2 (II:1), the deletion of a C in exon 20 (c.3772delC) resulted in a frameshift and a predictive truncation of the putative protein at the start of exon 21 at c.3839. Parental DNA was not available. Therefore, it was not possible to determine if this was a de novo mutation or if it was inherited from one of the parents in whom the condition was nonpenetrant.

None of the family members carried an expansion of the CAG sequence that is found in the carboxy terminus of the CACNA1A gene. Therefore, an enlarged expansion size was not responsible for the dystonic phenotype. The impact of the c.4963C→T and c.3772delC mutations on channel function was not assessed; however, because previous EA2-truncating mutations have resulted in nonfunctional channels, we would expect these mutations to have a similar effect.

We have described 2 novel truncating mutations within the CACNA1A gene. Although many truncating mutations have been described in patients with EA2, these 2 mutations are interesting because they are both associated with interictal dystonia.

The Q1561X mutation was associated with interfamilial clinical variability and reduced penetrance in pedigree 1. Individuals III:2 and IV:3 both had onset of episodic symptoms (vertigo and nausea) in their early teens; however, individual II:2 did not develop ataxia as part of the episodes until his 30s, whereas individual IV:3 developed episodes of ataxia at age 15 years. Genetic studies demonstrate that the disease is not fully penetrant in this family. Individual I:2 had a history of symptoms, but her son (II:1) was asymptomatic and passed the disease on to his own son (III:2). Individual IV:1 is in her 30s and, despite harboring the Q1561X mutation, has not experienced any neurologic symptoms.

Dystonia appears to be a late-onset feature in our 2 pedigrees (ages 59 and 47 years, pedigrees 1 and 2, respectively), manifesting as torticollis and segmental dystonia in pedigree 1 (III:2) and blepharospasm in pedigree 2 (II:1). Although the occurrence of dystonia in the setting of ataxia in these 2 cases may be coincidental, it is also possible that the dystonia is a manifestation of the cerebellar condition that evolves in EA2. Dystonia has been described with progressive ataxias, and mutations have been found in the SCA3, SCA6, SCA7, and SCA12 genes. Electrophysiologic and functional neuroimaging studies have demonstrated a role of the cerebellum in dystonia. 7-9 Positron emission tomography studies have demonstrated increased metabolic activity in the cerebellum of patients with DYT1 dystonia who have sustained dystonia at rest. 7 The genetically dystonic rat has been demonstrated to result from biochemical, metabolic, and functional abnormalities of the cerebellum. 7 Dystonia is a recognized component of the phenotype in the tottering mutant mouse (CACNA1A<sup>Q5</sup>). 10 a mouse model that results from homozygous mutations in the mouse CACNA1A gene. Therefore, it is not surprising that dystonia is observed in humans as a result of mutations in the human CACNA1A gene. Although there is accumulating evidence to suggest a role of the cerebellum in the generation of dystonia, in this case the dystonia may be due to expression of the mutated channel in the basal ganglia.

The dystonia in the patients described herein developed later in the disease course, occurred in the interictal period, and was unresponsive to acetazolamide. These characteristics are similar to the interictal ataxia and nystagmus seen in EA2. The development of dystonia in these 2 patients paralleled the development of persistent interictal cerebellar dysfunction. Because the site of the disease in EA2 is the cerebellum, the generation of dystonia in these patients may be through cerebellar mechanisms.

Truncating mutations are the most common mutation to cause EA2. We have identified 2 novel truncating mutations that are associated with interictal dystonia. The dystonia is a late feature in this disease and may be a manifestation of a degenerative cerebellar process.

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