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

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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.

alcohol, and cigarettes. By his mid-30s, attacks were characterized by ataxia, dysarthria, diplopia, and vertigo that lasted 6 to 8 hours and occurred 2 to 6 times per week. He was eventually diagnosed as having EA2 and was treated with acetazolamide, 150 mg/d, which significantly reduced the frequency of attacks. At age 59 years, he developed dystonia that involved the right side of his neck and the right arm. The dystonia responded well to clonazepam and carbamazepine; however, these medications resulted in worsening of the interictal dysarthria and ataxia. Neck dystonia initially responded to botulinum toxin, but less benefit was seen with subsequent injections.

The patient’s physical examination revealed impaired smooth-pursuit movements, horizontal nystagmus in the direction of gaze, normal finger-nose and heel-shin test results, a broad-based gait, and absent dysdiadochokinesis. The patient was unable to tandem walk, and he walked with a wide-based gait. Dystonia manifested as head rotation and flexion to the right, elevation of the right shoulder, and extension of the elbow and wrist. Results of magnetic resonance imaging of the head were normal.

The proband’s parents were asymptomatic, but the paternal grandmother (I:2) had experienced attacks of dizziness and headache. The proband’s son (IV:3) developed attacks of dizziness and ataxia with exertion at age 15 years. These attacks lasted a few hours and occurred once per week. When the son got older, he noticed that in addition to exertion, anxiety and cigarettes would also trigger an attack. His symptoms improved considerably with acetazolamide.

**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 these medications included acetazolamide, diazepam, phenytoin, carbamazepine, propanolol, hydrochloride, medazine hydrochloride, nortriptyline hydrochloride, clonazepam, clordiazepoxide 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.

**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 CACNA1A 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 sequencer (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 CACNA1A 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 CACNA1A gene, a PCR reaction was performed using the forward primer 5’-CCATCCCCGGTCTCCCTTTTCT-3’ and the reverse primer 5’-GGTATGAGCAGGCGGTACC-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.
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 interfacial 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 II: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. Positron emission tomography studies have demonstrated increased metabolic activity in the cerebellum of patients with DYT1 dystonia who have sustained dystonia at rest. The genetically dystonic rat has been demonstrated to result from biochemical, metabolic, and functional abnormalities of the cerebellum. Dystonia is a recognized component of the phenotype in the tottering mutant mouse (CACNA1A<sup>−/−</sup>).<sup>9</sup> 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 acetylcholine. 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 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.

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