Large CACNA1A Deletion in a Family With Episodic Ataxia Type 2

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**Background:** Episodic ataxia (EA) is an ion channel disorder that manifests as paroxysmal attacks of imbalance and incoordination. Episodic ataxia type 2 (EA2) is characterized by prolonged episodes of ataxia with interictal nystagmus and is caused by mutations in CACNA1A. All mutations identified thus far (to our knowledge) are nonsense or missense point mutations.

**Objective:** To describe a family with EA2 having a novel mutation deleting several exons of CACNA1A.

**Design:** Clinical and molecular study of a family manifesting EA2 attacks.

**Setting:** Academic research.

**Patients:** DNA was extracted from blood samples of 3 family members.

**Main Outcome Measures:** Microsatellite genotyping of CACNA1A, quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF), and sequencing were performed.

**Results:** Genotyping of CACNA1A showed nonmendelian inheritance of a CAG repeat located at the 3' end of the gene in a mother and daughter, suggesting a deletion event, which was subsequently confirmed by QMPSF analysis and sequencing. This 39.5-kilobase deletion removes the last 16 coding exons of the gene.

**Conclusion:** Deletion of several exons of CACNA1A may cause EA2 and should be assessed in patients having EA2 without a CACNA1A point mutation.

*Arch Neurol.* 2008;65(6):817-820

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**EPISODIC ATAXIA TYPE 2** (EA2) (OMIM 108500) is a rare autosomal dominant neurologic disorder characterized by attacks of ataxia lasting hours to days and interictal nystagmus. These attacks can be triggered by fatigue or emotional stress and are usually responsive to acetazolamide treatment. Episodic ataxia type 2 is caused by mutations in CACNA1A. CACNA1A encodes the Cav2.1 subunit, which is the pore-forming and voltage-sensing subunit of a neuronal P/Q voltage-activated calcium channel expressed throughout the central nervous system but most abundantly in the cerebellum. Episodic ataxia type 2 has been shown to be associated with CACNA1A point mutations, most often with mutations leading to a premature stop codon and sometimes with missense mutations (OMIM 601011). More than 40 different mutations have been reported, and 19 others have been identified in our laboratory (F.R., unpublished data, May 2007). However, in some typical families with EA2, no mutation is detected in CACNA1A, suggesting the existence of other types of mutations undetectable by exon sequencing or by denaturing high-performance liquid chromatography (DHPLC) screening or the existence of additional and unidentified EA2 genes. Herein, we describe a large CACNA1A deletion in a family with EA2.

**METHODS**

**CLINICAL DATA**

Four patients in this family (II-3, III-1, III-2, and III-3) were examined.

**Patient II-3**

Patient II-3, born in 1941, was evaluated in 1997. Since adolescence, she had experienced paroxysmal gait unsteadiness several times a
week. Precipitating factors included sudden noise and emotional stress. These paroxysmal ataxic episodes lasted for minutes to hours and were accompanied by pallor, blurred vision, and phonophobia, and their severity was increased by physical exertion. Clinical examination disclosed multidirectional nystagmus and cerebellar ataxia. Brain magnetic resonance (MR) imaging showed moderate cerebellar vermian atrophy. An interictal electroencephalogram demonstrated mild slowing of background activity.

Patient III-1

Patient III-1, born in 1977, was evaluated in 1999. Since adolescence, he had experienced ataxic episodes lasting for 2 to 6 hours, preventing his ability to stand. These episodes were sometimes associated with migrainous headache. Clinical examination revealed interictal horizontal nystagmus. Additional signs were noted, including short stature, hypertrophy of calves and leg muscles, and a degree of facial dysmorphism. All investigations, including brain MR imaging, electroencephalogram, and muscle biopsy specimen, were normal or negative.

Patient III-2

Patient III-2, born in 1972, was evaluated in 2000. She reported stereotyped episodes of ataxia lasting for 15 minutes to 3 hours. Her clinical examination findings were normal except for pes cavus. Cerebral MR imaging was normal.

Patient III-3

Patient III-3, born in 1965, was evaluated in 1997. She had the same symptoms as those reported by her mother (II-3). Clinical examination disclosed horizontal nystagmus and permanent invalidating ataxia.

The conditions of patients II-3, III-1, III-2, and III-3 were improved by acetazolamide treatment.

Patient I-1

Patient I-1, a 98-year-old woman, had experienced attacks of paroxysmic dizziness associated with headaches. The frequency decreased after age 70 years, and she developed progressive ataxia.

Patient II-2

Patient II-2 declined clinical evaluation. According to his relatives, during 30 years he experienced attacks associated with headache, dizziness, and ataxia lasting up to 1 day. The frequency of the attacks decreased at about age 55 years and disappeared at age 60 years.

GENOTYPING

Blood samples were obtained from patients II-3, III-1, and III-2, and genomic DNA was extracted using standard protocols. All 3 patients underwent genotyping with 6 polymorphic markers spanning the CACNA1A locus; these included 2 intragenic markers (D19S1150 and exon 47 CAG repeat) and 4 telomeric markers (D19S221 and 3 dinucleotide repeats, including a GT repeat located 219 kilobase [kb] from the 3’ end of CACNA1A [chr19:12,960,084-12,960,121], an AC repeat at 60 kb [chr19:13,118,894-13,118,927], and an AT repeat at 8.7 kb [chr19:13,170,349-13,170,389] [University of California Santa Cruz Genome Browser, March 2006 Assembly; available at http://genome.ucsc.edu/]). Primers are available on request from the author.

LONG-RANGE POLYMERASE CHAIN REACTION AND SEQUENCING

Long-range polymerase chain reaction (PCR) amplifications were performed (TripleMaster PCR System; Eppendorf International, Westbury, New York) using primers located in intron 30 and after the stop codon of CACNA1A. Sequencing was performed with a primer located in intron 31 using standard protocols (ABI 3130 DNA analyzer; Applied Biosystems, Norwalk, Connecticut). Primers are available on request from the author.

QUANTITATIVE MULTIPLEX PCR OF SHORT FLUORESCENT FRAGMENTS

The quantitative multiplex PCR of short fluorescent fragments (QMPSF) method is described in detail elsewhere. Oligonucleotide primer pairs for amplification of short fluorescent fragments corresponding to CACNA1A exons were designed using commercially available software (Primer PREMIER 5 Software; PREMIER Biosoft International, Palo Alto, California). Two multiplex PCRs were set up to estimate the copy number of CACNA1A exons. The first one (QMPSF1) screened exons 2, 4, 11, 16, 21, 25, 30, 34, 39, and 45, and the second one (QMPSF2) screened exons 30, 31, 32, 34, and 33. An additional primer set that amplified a short sequence of exon 13 in the hydroxymethylbilane synthase gene (HMBS; OMIM 608060), uninvolved in EA2, was used as an internal control. Primer sequences are available on request from the author. All DNA samples were reextracted using a commercially available kit (QIAamp DNA Blood Mini Kit; Qiagen, Study City, California). Multiplex PCR was performed as previously described. Control DNA was amplified simultaneously with patient DNA. Polymerase chain reaction products were separated by capillary electrophoresis using a DNA analyzer (ABI 3130; Applied Biosystems). Quantification of the area of peaks corresponding to the tested exons and to the internal HMBS control was determined using commercially available analysis software (GeneMapper version 4.0, Applied Biosystems). Data for each exon were expressed using the following ratio: (the area of the peak corresponding to a tested exon for the patient divided by the area of the peak corresponding to HMBS for the patient) to (the area of the peak corresponding to the same tested exon for the control DNA divided by the
The family was first screened for linkage using 2 intragenic polymorphic markers located in intron 7 (D19S1150) and in exon 47 (polymorphic CAG repeat). Patient II-3 was homozygous at D19S1150, and patients III-1 and III-2 were heterozygous; the 3 patients shared a common allele. An apparent nonmendelian inheritance was observed for the CAG polymorphic marker, as patient III-2 did not inherit any allele from her affected mother (II-3), suggesting the existence of a null allele (Figure 1). Patients II-3 and III-2 had no incompatible genotype with a panel of 9 polymorphic microsatellite markers (AmpFlSTR Profiler kit, Applied Biosystems), eliminating inaccurate maternity information or sampling error. Sequencing of the region, including the CAG repeat and the hybridization sites for primers, excluded polymorphisms. Ratios obtained by QMPSF1 analysis were close to 1 for exons 2, 4, 11, 16, 21, 25, and 30 and were close to 0.5 for exons 34, 39, and 45. These results were consistent with a deletion of the C-terminal exons of CACNA1A. The results of the second QMPSF analysis indicated that the 5′ boundary was located within intron 31 (Table 1 and Table 2).

The 3′ boundary was refined using 4 microsatellite markers for which at least 1 of 3 patients was heterozygous (Figure 1 and Figure 2). To determine the size and breakpoints of the deletion, we performed a long-range PCR amplification using a set of primers located in intron 30 and after the stop codon of the gene. We amplified a 2557-base pair (bp) fragment. The sequencing of this PCR product revealed that the deletion size was 39,567 bp. The deletion removes 8078 bp of intron 31, exons 32 to 47, and 3071 bp after the stop codon (Figure 3). Sequence analysis of the deletion boundaries suggests that this deletion arose through homologous recombination of Alu sequences. There were no known additional genes within the deleted fragment (University of California Santa Cruz Genome Browser).

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Figure 2. Genetic map of the CACNA1A locus. The open circle indicates the hemizygous CAG repeat allele; solid circles, microsatellites that were heterozygous in at least 1 affected individual; open triangles, exons for which the quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF) ratio was close to 0.5; and solid triangles, exons for which the QMPSF ratio was close to 1.0.
RNA instability. The consequence of this mutation is most often an absence of the protein caused by messenger RNA splicing in episodic ataxia type–2 are caused by mutations in the Ca2+ channel gene CACNL1A4. The deleted allele is predicted to lead to a truncated protein or to an absence of the protein caused by messenger RNA instability. The consequence of this mutation is most likely identical to that of previously reported premature stop codons observed in families with EA2.

All CACNA1A mutations reported thus far (to our knowledge) in EA2 are point mutations, including nonsense, missense, and small deletions or insertions. However, in some typical patients with EA2, routine sequencing or DHPLC screening of all 47 exons is negative, suggesting chromosomal rearrangements or regulatory mutations involving CACNA1A or genetic heterogeneity. Herein, we showed that large deletions involving CACNA1A might occur in patients with EA2. This type of mutation has recently been reported in another channelopathy, severe myoclonic epilepsy of infancy due to mutations in SCN1A (OMIM 182389), a gene coding for a sodium channel.

The systematic screening of intragenic deletions by methods such as multiplex QMPSF or multiplex ligation–dependent probe amplification will probably expand the spectrum of CACNA1A deletions in EA2. These findings should be extended to larger cohorts to estimate the frequency of these deletions and their possible recurrence.

We describe a 39.5-kb deletion that removes the last 16 coding exons at the 3′ end of CACNA1A in 3 patients of a family with an EA2 phenotype. The existence of this deletion is supported by a polymorphic marker genotyping approach and a QMPSF analysis.

The deleted allele is predicted to lead to a truncated protein or to an absence of the protein caused by messenger RNA instability. The consequence of this mutation is most likely identical to that of previously reported premature stop codons observed in families with EA2.

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Accepted for Publication: October 31, 2007.

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Financial Disclosure: None reported.

Additional Contributions: Claire Ploton, CMIT, and Christelle Lescoat, CMIT, helped in sequencing and in performing QMPSF. Nicole Philip, MD, assisted in the clinical analysis of patient III-1. We thank the family members for their participation in this study.

REFERENCES


