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De Novo Mutation in CACNA1A Caused Acetazolamide-Responsive Episodic Ataxia

Qing Yue,[1] Joanna C. Jen,[1] May Myo Thwe,[2] Stanley F. Nelson,[2] and Robert W. Baloh[1,3]*

[1]*Department of Neurology, UCLA School of Medicine, Los Angeles, California*
[2]*Department of Pediatrics/Hematology and Oncology, UCLA School of Medicine, Los Angeles, California*
[3]*Department of Surgery (Head and Neck), UCLA School of Medicine, Los Angeles, California*

With the recent report of mutations in the calcium channel gene CACNA1A in two families with episodic ataxia type 2, we investigated a patient with nonfamilial episodic vertigo and ataxia responsive to acetazolamide for similar mutations. Single-strand conformation polymorphism (SSCP) analysis of exon 23 identified an extra band in the patient that was not present in other relatives or in normal controls. Exon 23 of the patient showed a spontaneous C to T substitution at position 4410 resulting in an early stop codon. Patients with nonfamilial episodic ataxia may respond to acetazolamide and may have mutations in CACNA1A. Am. J. Med. Genet. 77:298–301, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: calcium channel gene; episodic ataxia; acetazolamide; de novo mutation

INTRODUCTION

Familial episodic ataxia, type 2 (EA-2) is characterized by bouts of ataxia, typically lasting hours, and interictal neurological findings, usually gaze-evoked nystagmus and truncal ataxia [Vahedi et al., 1995; Baloh et al., 1997]. Episodes of ataxia are triggered by exertion, emotional upset, and stress, and they are often dramatically relieved by acetazolamide [Griggs et al., 1978]. As other inherited episodic neurological diseases that respond to acetazolamide treatment are because of ion channel abnormalities, a channelopathy was suspected in EA-2 [Griggs and Nutt, 1995]. Several groups reported linkage to chromosome arm 19p in families with EA-2 [Vahedi et al., 1995; Kramer et al., 1995; Teh et al., 1995]. Ophoff et al. [1996] recently reported mutations in the calcium channel gene CACNA1A (previously CACNL1A4) in two families with EA-2. They also found mutations in the same gene in four families with hemiplegic migraine. CACNA1A codes for the major transmembrane subunit of a neuronal voltage-gated calcium channel heavily expressed in the cerebellum [Stea et al., 1995]. The point mutations in CACNA1A with EA-2 resulted in a premature stop codon and aberrant splicing, both of which predicted a truncated calcium channel subunit [Ophoff et al., 1996]. We now report a spontaneous mutation in CACNA1A in a patient that we have followed for 15 years with acetazolamide-responsive episodic ataxia.

CLINICAL REPORT

A 38-year-old man experienced recurrent episodes of ataxia dating back to infancy. His mother noticed spells during which he would stagger and appear extremely frightened, beginning at about age 18 months. As a boy, episodes of ataxia were typically triggered by exercise and he would often have severe nausea with vomiting that would last for several hours. The ataxia involved trunk and limbs and typically occurred with slurring of speech. The episodes recurred at least once a month. At age 21, the episodes began occurring several times a week, so he stopped working and was considered vocationally disabled. He was initially seen at UCLA in January of 1983 reporting at least one attack per week along with some mild interictal imbalance. His interictal examination at that time showed horizontal gaze-evoked nystagmus of equal amplitude to both sides with rebound nystagmus in the primary position after returning from either side. There was mild truncal ataxia with impaired tandem walking. Limb coordination and the remainder of the neurological status were normal. Quantitative eye movement testing showed severely impaired smooth pursuit and optokinetic nystagmus but normal vestibular and saccadic eye movements [Baloh et al., 1986]. A magnetic resonance image (MRI) of the brain was normal. Despite an unremarkable family history, he was treated with acetazolamide with a dramatic response. His episodes of ataxia stopped and he was able to return to regular work with only occasional mild symptoms after exer-

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*Correspondence to: Dr. Robert W. Baloh, UCLA Department of Neurology, Box 951769, Los Angeles, CA 90095-1769. E-mail: rwbaloh@ucla.edu

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cise or in a stressful situation. With these *breakthrough spells* he felt as though one of his regular attacks was coming on but it did not. He has been maintained on acetazolamide since 1983. His interictal neurological examination has remained unchanged with only gaze-evoked nystagmus and mild truncal ataxia present on his most recent examination. Quantitative eye movement findings are unchanged.

His father, mother, and brother were interviewed and examined, and none reported episodes of ataxia and, except for findings of benign essential tremor in his father, none showed abnormalities on neurological examination. He did not have children.

METHODS

Single-Strand Conformation Polymorphism

Polymerase chain reaction (PCR) products of exons in CACNA1A were screened for molecular variants by single-strand conformation polymorphism (SSCP) analysis [Orita et al., 1989; Ravnik-Glavak et al., 1994]. The published primers [Ophoff et al., 1996] were used to amplify all 47 exons from introns flanking each exon, except for exons 37 and 43 for which the primers had to be redesigned [Yue et al., 1997]. PCR was performed under conditions optimized for each individual primer pair. Products were labeled by inclusion of [α-^{32}P]dCTP in the PCR. PCR products were then denatured and loaded onto 0.5× MDE gel (AT Biochem, Malvern, PA) and electrophoresed at 4°C with or without 10% glycerol. After autoradiography of gels, conformers were identified by visual inspection.

DNA Sequence Analysis

Exon 23 was PCR amplified from each member of this family. PCR products were separated by electrophoresis on 1.5% low melting agarose gel. Single bands were excised from the gel and treated with GENECLEAN® kit (BIO 101 Inc). The sequence of the purified DNA samples was generated by Thermo Sequenase cycle sequencing kit, using manufacturer's recommended conditions (Amersham). Nucleotide and exon numbering of CACNA1A are according to GenBank accession X99897 and Z80119.

(CAG)n Repeats Analysis

Determination of the number of CAG repeats in CACNA1A was described previously [Zuchenko et al., 1997]. In brief, PCR products were electrophoresed in denaturing polyacrylamide gels and compared to a sequencing ladder. Homozygous alleles were sequenced to confirm the number of CAG repeats.

RESULTS

All 47 exons and flanking introns in CACNA1A were subjected to SSCP analysis in the patient, his family, and 15 normal controls. The patient showed aberrantly migrating fragments in exon 23 that were not observed in other relatives or in controls (Fig. 1). Sequencing of exon 23 from the patient showed a C-to-T substitution in one allele at nucleotide 4110 of CACNA1A open reading frame (Fig. 1). This alteration predicts a premature stop codon at codon 1279. This mutation was not seen in either parent, his brother, or in any of the controls. Paternity was confirmed by typing seven microsatellite markers on chromosome arm 19p in all four relatives [Baloh et al., 1997]. Because of the possible phenotype overlap between our patient and SCA-6 patients, the CAG repeat length at the 3′ end of CACNA1A was determined [Zuchenko et al., 1997]. Both alleles in our patient had 13 CAG repeats (normal range is 4 to 16 CAG repeats).

DISCUSSION

Although there was no family history of a similar disorder, our patient had all of the typical manifestations of EA-2. As with other patients with EA-2, his episodes of ataxia were precipitated by stress, emotional upset, and exercise [Vahedi et al., 1995; Baloh et al., 1997]. The interictal oculomotor findings of gaze-evoked and rebound nystagmus along with severe pursuit and optokinetic deficits are localizing to the cerebellum. Although CACNA1A is expressed in neurons throughout the brain, it is most heavily expressed in the cerebellum [Mori et al., 1991]. Hybridization with CACNA1A-specific riboprobes in rat found labeling of Purkinje neurons, granule cells, and neurons in the molecular layer [Ludwig et al., 1997].

The four homologous domains (I-IV) of the α1A calcium channel subunit each have six putative alpha-helical membrane spanning segments (S1-S6) (Fig. 2) [Catterall, 1995]. The central pore is lined by four P regions (pore loops) interconnecting putative membrane spanning segments S5 and S6 of each domain. The S4 segment is thought to be the voltage sensor because the highly conserved sequence containing positively charged amino acids at every third position is ideally structured to sense changes in the membrane electrical field. Mutations that neutralize any of the positive residues in the S4 sequence alter the voltage dependence. The calcium channel is thought to be composed of the integral membrane segments (S1-S6) arranged in a ring like the staves of a barrel about a central pore [MacKinnon, 1995]. The four pore loops reach into the barrel and confer ion conduction properties. Channel gating arises from conformational changes in the transmembrane barrel staves of the subunits.

The mutation causing the clinical syndrome in our patient resulted in a premature stop codon and a predicted truncated protein with only the first two domains intact. The location and the nature of the mutation in our patient with de novo EA-2 is similar to the two prior mutations identified in two families with EA-2 (Fig. 2)[Ophoff et al., 1996]. Although the truncated gene products with EA-2 may not be translated at all, the fact that patients are symptomatic would suggest that these gene products are made and that they interfere with cell function. They might exert a dominant negative effect by interfering with the functioning gene product from the normal allele or by sequestering auxiliary subunits [Ophoff et al., 1996]. Less likely, they might be unstable and cause haploinsufficiency, or they could join together and form units with altered function.

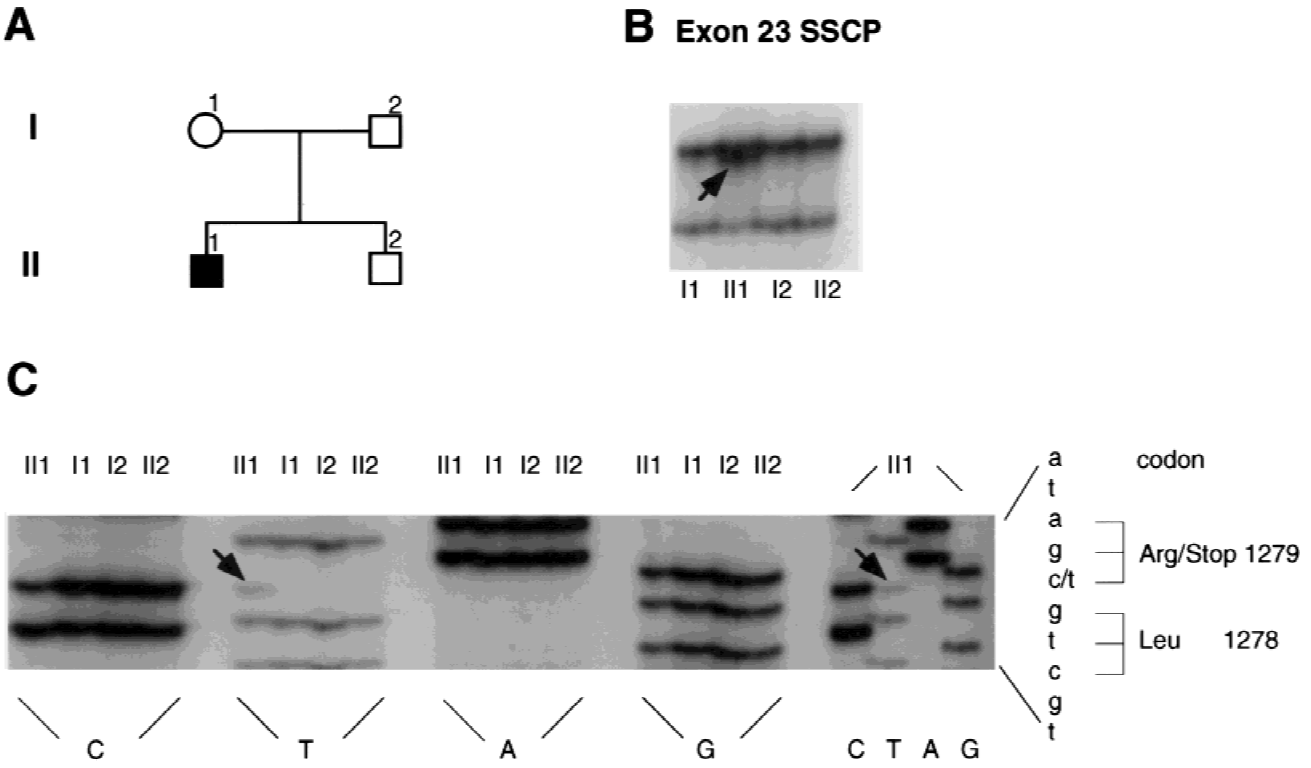

Fig. 1. **A:** Pedigree of patient with de novo episodic ataxia. **B:** SSCP of exon 23 showing aberrantly migrating fragment (arrow) in patient not seen in unaffected family members. **C:** Sequence of exon 23 showing C-to-T transposition in one allele (arrows) at nucleotide 4110 in the patient.

Acetazolamide presumably works by stabilizing the abnormal ion channels through changes in extracellular pH [Bain et al., 1992]; pH is known to have a prominent effect on most ion channels [Hille, 1992]. Decreasing pH (increasing the number of free protons) strongly inhibits ion permeation through open calcium channels as well as reducing channel opening. Recently it was shown that the protonation site in the L-type voltage-regulated Ca^{2+} channel lies within the pore [Chen et al., 1996]. It is formed by a combination of conserved region glutamates, the amino acid shown to be the key to calcium selectivity of these channels [Heinemann et al., 1992]. These data provide a simple molecular explanation for the modulatory effect of H^+ ions on open channel flux and the competition between H^+ ions and divalent cations. By increasing extracellular proton concentration in the cerebellum, acetazolamide presumably stabilizes the mutant channels that fail to inactivate properly. The mildly progressive interictal ataxia seen with EA-2 probably results from chronic excess entry of calcium into neurons leading to activation of calcium's intracellular signaling pathways ultimately leading to cell death (apoptosis) [Koh and Cottman, 1996]. By stabilizing (blocking) the abnormal channels, acetazolamide may not only prevent the episodes of ataxia but also the interictal progressive ataxia.

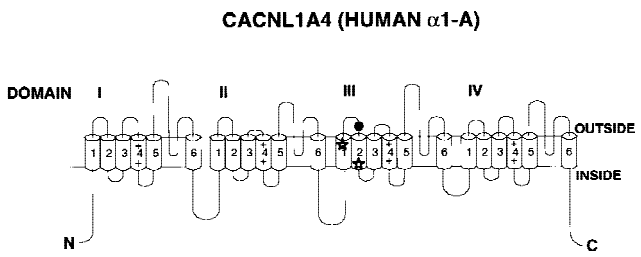

Fig. 2. Schematic drawing of α1A subunit of a calcium channel coded for by CACNA1A. Each of the four domains (I to IV) has six transmembrane segments (1 to 6). The pore forming regions are between segments 5 and 6. The black dot indicates the location of the predicted stop code in our patient; the stars indicate the position of the two previously reported mutations with EA-2 [Ophoff et al., 1996].

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