

Progressive Ataxia Due to a Missense Mutation in a Calcium-Channel Gene

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Summary

We describe a family with severe progressive cerebellar ataxia involving the trunk, the extremities, and speech. The proband, who has prominent atrophy of the cerebellum, shown by magnetic resonance imaging, was confined to a wheelchair at the age of 44 years. Two sons have episodes of vertigo and ataxia that are not responsive to acetazolamide. Quantitative eye-movement testing showed a consistent pattern of abnormalities localizing to the cerebellum. Genotyping suggested linkage to chromosome 19p, and SSCP showed an aberrant migrating fragment in exon 6 of the calcium-channel gene CACNA1A, which cosegregated with the disease. Sequencing of exon 6 identified a G→A transposition in one allele, at nucleotide 1152, resulting in a predicted glycine-to-arginine substitution at codon 293. The CAG-repeat expansion associated with spinocerebellar ataxia 6 was not present in any family members. This family is unique in having a non-CAG-repeat mutation that leads to severe progressive ataxia. Since a great deal is known about the function of calcium channels, we speculate on how this missense mutation leads to the combination of clinical symptoms and signs.

Introduction

The number of abnormal genes associated with inherited cerebellar-ataxia syndromes is increasing rapidly; yet, there is still relatively little known about how different mutations lead to specific phenotypes (Junck and Fink 1996). There has been a surprisingly large clinical heterogeneity both within families with the same mutation and across families with mutations in the same gene (Higgins et al. 1996). A further complication is that most of the identified genes code for proteins for which nor-

mal function is poorly understood. When genotype-phenotype correlation is considered, one of the most interesting genes is the calcium-channel gene CACNA1A (previously called “CACNL1A4”), which codes for the main transmembrane component of a calcium channel expressed throughout the brain but expressed particularly heavily in the cerebellum (Mori et al. 1991; Volsen et al. 1995; Ludwig et al. 1997). Recently, Ophoff et al. (1996) reported four different missense mutations in conserved functional domains in four families with familial hemiplegic migraine (FHM) and two mutations disrupting the reading frame in two families with episodic ataxia type 2 (EA-2). Both of these disorders can have a mildly progressive interictal ataxia, particularly with EA-2, but patients are not affected severely and neither disorder shortens the expected life span. At almost the same time as Ophoff et al. (1996), Zhuchenko et al. (1997) reported that expansion of a CAG-repeat segment in CACNA1A (which normally has 4–16 repeats) to 21–27 repeats caused a moderately progressive cerebellar-ataxia syndrome that typically begins when patients are in their 30s–40s. They named this autosomal dominant syndrome “spinocerebellar ataxia 6” (“SCA-6”). However, unlike the other spinocerebellar-ataxia syndromes caused by expanded CAG repeats, the expanded repeats with SCA-6 were stable. There was no evidence of anticipation from generation to generation.

We now report another clinical phenotype due to a missense mutation in the highly conserved pore region of CACNA1A. Some patients in this family exhibited a severe progressive cerebellar-ataxia syndrome without episodic features, whereas others had a combination of episodic ataxia and progressive ataxia. Since a great deal already is known about the function of calcium channels and their distribution within the nervous system, the range of mutations in CACNA1A provides a unique model with which to begin the process of explaining clinical symptoms and signs, on the basis of specific mutations.

Subjects and Methods

Proband

Starting at the age of 15 years, the proband noted the gradual onset of imbalance and incoordination. The

Received June 30, 1997; accepted for publication August 28, 1997; electronically published October 29, 1997.

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0002-9297/97/6105-0012\$02.00

symptoms gradually progressed, and she first noted slurring of speech in her 20s. In her early 40s, her gait deteriorated more rapidly, and she experienced several falls, forcing her to use a walker. At the age of 44 years, she became confined to a wheelchair and has remained so for the past 10 years. She denied ever having any episodes of imbalance or vertigo and never experienced migraine headaches. When first seen at UCLA, at the age of 54 years, she could stand with a markedly widened base but could not take even a single step without support. She could not stand with her feet together, even with her eyes open. She exhibited a horizontal gaze-evoked nystagmus of equal amplitude to the right and to the left, with rebound nystagmus in the primary position after returning from either side. There was marked dysrhythmia and dysmetria in the upper extremities, with bilateral intention tremor (left greater than right). No brain-stem or spinal-cord signs were identified. Magnetic resonance imaging (MRI) showed prominent cerebellar atrophy with lesser diffuse cortical atrophy (fig. 1). The brain stem was normal.

Affected Family Members

The proband (II-6; fig. 2A) reported that her mother (I-2) had experienced a similar gradually progressive ataxia syndrome beginning before the age of 20 years and progressing to profound ataxia in her late 40s. She died in her early 60s of unknown cause. Two maternal aunts and a maternal uncle had exhibited a similar progressive ataxia syndrome, all dying in their 60s of unknown causes. The proband did not know of any episodic features in her mother, aunts, or uncle. The proband had seven children by four different husbands. Three of the children, each from a different father, exhibit an ataxia syndrome. At the age of ~15 years, her 34-year-old son (III-4) began having dizzy spells lasting several hours, characterized by vertigo, nausea, and vomiting, with ataxia and slurring of speech. These spells occurred as frequently as two or three times per month, but he then could go as long as 6 mo without a spell. Between the spells, there was a gradual deterioration in balance, with incoordination of the extremities and slurring of speech, that has progressed gradually over the past 20 years. The proband's 31-year-old daughter (III-6) has noted a gradually progressive ataxia syndrome, beginning at the age of ~10 years and progressing over the years to involve the coordination of her upper extremities and the development of slurred speech. She has never experienced any episodes of vertigo or ataxia but does have episodic headaches that meet the International Headache Society (IHS) criteria for migraine without aura. Since the age of 5 years, another son (III-3) has experienced severe recurrent attacks of vertigo, nausea, and vomiting, with ataxia of the trunk and extremities. These episodes last for as long as 1 d

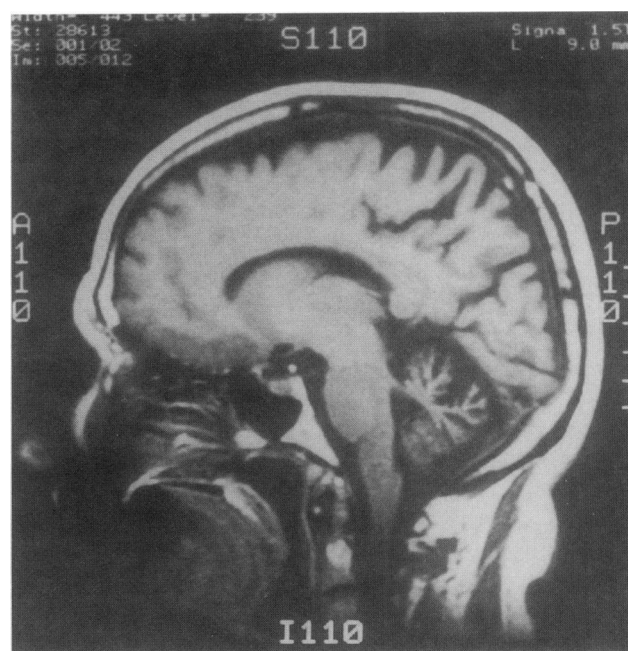


Figure 1 MRI of the cortex and cerebellum of the proband (II-6). The sagittal section through the vermis shows prominent atrophy. T₁ is weighted.

and recently have been occurring two or three times per week so that he is unable to work. With his recent attacks, he has noted painful dysesthesias in his right hand and right foot. Between attacks, he has had a slowly progressive truncal and extremity ataxia, with slurring of speech. He also has headaches that meet the IHS criteria for migraine without aura. On examination, all three of the proband's affected children exhibited truncal ataxia, ataxia of the upper extremities, and slurring of speech. They also each had horizontal gaze-evoked nystagmus, with rebound nystagmus in the primary position. None exhibited brain-stem or spinal-cord signs. Neither son with episodic ataxia responded to treatment with acetazolamide. The study was approved by our institutional review board, and appropriate informed consent was obtained from all subjects.

Eye-Movement Recordings

Eye movements were recorded with direct-current electro-oculography. Detailed descriptions of the recording system, data analysis, and normative data have been reported elsewhere (Baloh et al. 1980; Moschner et al. 1994). With the subject seated and the head fixed by a brace, saccades were generated by a laser target moving in a square wave pattern of pseudorandom frequency, direction, and amplitude. Smooth pursuit was generated with the same laser target moving in a sinusoidal pattern (0.2 and 0.4 Hz; peak velocities of 23°/s and 45°/s, respectively). Optokinetic nystagmus (OKN) was induced

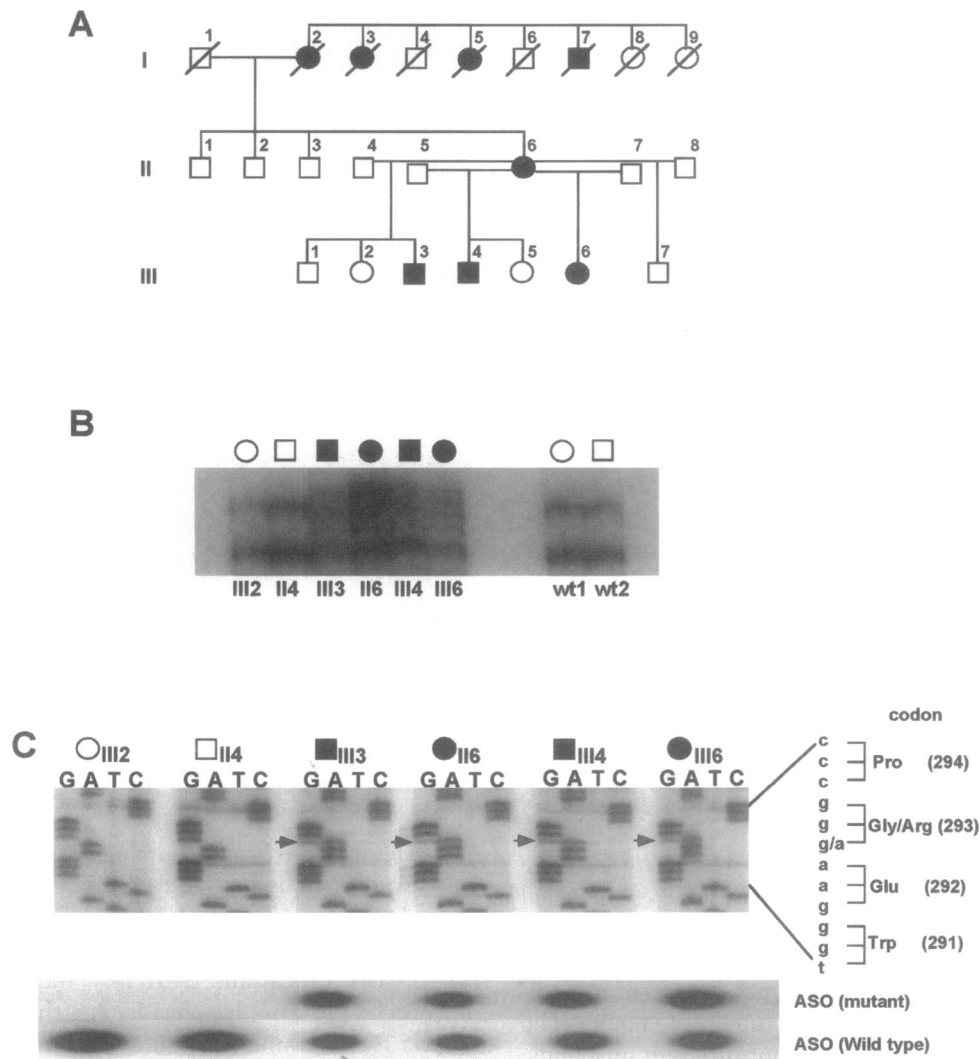


Figure 2 A, Pedigree of family with progressive ataxia. Blackened symbols indicate affected individuals. B, Results of SSCP analysis of exon 6, showing aberrantly migrating fragments in affected family members but not in unaffected family members or in normal controls (lanes wt1 and wt2). C, Sequence of exon 6 (top), showing a G→A transposition in one allele (indicated by the arrows), at nucleotide 1152, in affected family members, and ASOs (bottom), showing that the mutant oligonucleotide detected the mutant allele in affected but not in unaffected individuals. The normal (wild-type) allele detected all individuals.

with the subject seated inside a cloth drum 1 m in diameter. The drum was made of black cloth with 3°-wide vertical stripes spaced at intervals of 15.6°. The drum was rotated at a constant velocity of 30°/s clockwise and counterclockwise as well as sinusoidally (0.05 Hz; peak velocity of 60°/s). The vestibulo-ocular reflex (VOR) was tested by sinusoidal rotations (0.05 Hz; peak velocity of 60°/s), with the subject's eyes open in darkness. Fixation suppression of the VOR (VOR-F) was tested by sinusoidally rotating (0.05 Hz; peak velocity of 60°/s) the subject in the dark, with a small fixation light attached to the chair, directly in front of the subject.

Genotyping

Genomic DNA from each individual was extracted from peripheral blood, with a DNA-extraction kit (Gentra system). Microsatellite markers on chromosome 19p were typed by use of the standard PCR protocol, except that [α -³²P]-dCTP (ICN Biomedicals) was used (Vignal et al. 1993). PCR products were resolved on 6% denaturing polyacrylamide gels. Pedigree allelotypes were inferred after autoradiography. The markers used for this study were D19S413, D19S865, D19S914, D19S221, D19S226, D19S841, and D19S253 and are

listed in order, from distal to proximal on 19p (Dib et al. 1996; Ducros et al. 1996).

SSCP Analysis

PCR products of exons in CACNA1A were screened for molecular variants, by SSCP analysis (Orita et al. 1989; Ravnik-Glavac et al. 1994). The published primers (Ophoff et al. 1996) were used to amplify all 47 exons from the introns flanking each exon, except for exons 37 and 43, for which the primers had to be redesigned. The sequences of the redesigned primers are as follows: 37F, 5'-CGTGTGAACCCATTGCCTGCA-3'; 37R, 5'-CCCAAGCCACACTCATTCCA-3'; 43F, 5'-TTGTGATCTGGAGGTCAGGCC-3'; and 43R, 5'-AGTGCAGAGTGAGGGTCCAGC-3'. PCR was performed under conditions optimized for each individual primer pair. All 47 exons were amplified in patients II-6 and III-3 and in 15 normal controls, and exon 6 was amplified in the entire subject family. Products were labeled by inclusion of [α -³²P]-dCTP in the PCR. PCR products then were denatured and loaded onto 0.5 × mutation-detection enhancement gels (AT Biochem) and were electrophoresed at 4°C, with or without 10% glycerol. After autoradiography of the gels, conformers were identified by visual inspection.

DNA Sequence Analysis

Exon 6 was PCR amplified for each member of this family. PCR products were separated by electrophoresis on 1.5% low-melting agarose gels. Single bands were excised from the gel and were treated with the GENE-CLEAN® kit (BIO 101). The sequences of the purified DNA samples were generated by the Thermo Sequenase cycle-sequencing kit (Amersham), by use of the manufacturer's recommended conditions. Nucleotide and exon numbering of CACNA1A are according to GenBank accession numbers X99897 and Z80119 (<http://www.ncbi.nlm.nih.gov/>).

Allele-Specific Hybridization

To analyze more unaffected individuals, allele-specific oligonucleotides (ASOs) were developed, and exon 6 was amplified as described, for family members and for 88 normal controls, in a 96-well plate (Thermowell; Corning Costar). The PCR conditions consisted of 5 min of 95°C denaturation followed by 35 cycles of 45 s at 94°C, 35 s at 55°C, and 1 min at 72°C and by a final elongation step of 7 min at 72°C. PCR products then were transferred onto the Hybond N⁺ membranes, by a 96-pin replicator (V & P Scientific). After denaturation in 0.4 M NaOH and neutralization in 2 × SSC, the air-dried filters were prehybridized in ExpressHyb solution (Clontech Laboratories) for 30 min at 37°C and then were hybridized for 1 h at 37°C, with either the wild-

type ASO with the sequence 5'-ACTGGGAAGGGC-CCAAC-3' or the mutated ASO 5'-ACTGGGAAAGGGC-CCAAC-3'. Oligonucleotides were end-labeled by use of T4 polynucleotide kinase (New England Biolabs) and [γ -³²P]-ATP. Filters were washed in 2 × SSC and 0.5% SDS for 30 min at 37°C, followed by a wash in 0.2 × SSC and 0.5% SDS for 20 min at 52°C, and then were exposed to film.

(CAG)_n-Repeat Analysis

Determination of the number of CAG repeats in CACNA1A has been described elsewhere (Zhuchenko et al. 1997). In brief, PCR products were electrophoresed in denaturing polyacrylamide gels and were compared with a sequencing ladder. Homozygous alleles were sequenced, to confirm the number of CAG repeats.

Results

Clinical Data

The age at onset of ataxia varied within the range of 5–15 years. In both of the proband's affected sons, episodes were triggered by emotional upset and physical exertion. Two of four reported headaches met the IHS criteria for migraine without aura. A uniform pattern of eye-movement abnormalities was found in all affected individuals (table 1). All affected individuals had gaze-evoked and rebound nystagmus, and all had profound impairment of smooth pursuit, OKN, and fixation suppression of vestibular nystagmus. Saccades and VOR responses were consistently normal.

Haplotype Analysis

Microsatellite-marker typing at chromosome 19p13 confirmed that, from marker D19S914 to marker D19S841, all three affected children shared the same haplotype with their affected mother. Recombination was observed between D19S865 and D19S914 on the telomeric side and between D19S841 and D19S253 on the centromeric side, defining an interval of ~9 cM. Linkage analysis was not feasible, because of the size of this family; however, the allelic segregation from this family suggested a possible genomic colocalization with EA-2/FHM (Baloh et al. 1997).

Mutation Detection

All 47 exons and flanking introns were subjected to SSCP analysis of PCR-amplified genomic DNA. The affected individuals showed aberrantly migrating fragments in exon 6, which were not observed in unaffected individuals from the same family or in controls (fig. 2B). Sequencing of exon 6, for each affected individual within this family, revealed a G→A transposition in one allele, at nucleotide 1152 of the CACNA1A open reading

Table 1**Results of Quantitative Oculomotor Testing of Four Patients with a Mutation in CACNA1A**

PATIENT	SACCADES			PURSUIT ^d	OKN ^e	VOR ^f	VOR-F ^g
	Velocity ^a (°/s)	Accuracy ^b	Delay ^c (ms)				
II-6	375	86	185	.21	.16	.34	.31
III-3	300	75	190	.15	.09	.55	.40
III-4	350	75	181	.07	.10	.39	.25
III-6	325	73	168	.40	.11	.33	.15

^a Average peak velocity of 20° saccades; minimum of 10 saccades. Normal >280°/s.

^b Target displacement/eye displacement × 100; average of 40 saccades. Normal 75-100.

^c Between target displacement and saccade initiation; average of 40 saccades. Normal <230 ms.

^d Gain = peak eye velocity/peak target velocity, 0.4 Hz, 45°/s. Normal >.58.

^e Gain = peak slow-phase eye velocity/peak stimulus velocity, 0.05 Hz, 60°/s. Normal >.50.

^f Gain = peak slow-phase eye velocity/peak stimulus velocity, 0.05 Hz, 60°/s. Normal >.30.

^g Gain = peak slow-phase eye velocity/peak stimulus velocity, 0.05 Hz, 60°/s. Normal <.13.

frame (fig. 2C). This alteration predicts a substitution of glycine by arginine, at codon 293. In the controls, we also identified two new polymorphisms, in exon 4 (g854a) and in exon 46 (t6938c), that have not been reported previously. ASO hybridization analysis showed that the mutant oligonucleotide detected the mutant allele in affected individuals but not in unaffected individuals in the family (fig. 2C). Furthermore, no hybridization was detected among 88 DNA samples from normal controls. In contrast, there were strong signals when the normal oligonucleotide was used as a hybridization probe for all samples. We therefore confirmed the complete segregation of this mutation with the disease phenotype.

CAG Expansion

Because of the phenotypic overlap between the family in this study and SCA-6 patients, the CAG-repeat length at the 3' end of CACNA1A was determined. There were two alleles in our patients; one had 11 CAG repeats (mutant allele), and the other had 13 CAG repeats (the normal range is 4-16 CAG repeats). The haplotypes in the patients were as follows: for II-6, 11/13; for III-3, 11/13; for III-4, 11/11; and, for III-6, 11/13.

Discussion

Clinical Features

Although this family has clinical features in common with other reported families with mutations in CACNA1A (Ophoff et al. 1996; Zhuchenko et al. 1997), there also were several unique features. The proband, her mother, and her daughter exhibited a relentlessly progressive cerebellar-ataxia syndrome, with clinical

signs suggesting involvement of the cerebellar vermis and the hemispheres. This is the first report of a missense mutation resulting in such a severe progressive ataxia syndrome. The clinical course is similar to that of families reported to have CAG-repeat syndromes, such as SCA-1, SCA-2, and SCA-3 (Junck and Fink 1996). However, unlike patients with these spinocerebellar-ataxia syndromes, the members of the family in this study did not exhibit any extracerebellar signs. Interestingly, the proband's two sons, each by a different father, had severe episodes of vertigo and ataxia, with progressive interictal ataxia. Unlike other patients with EA-2 (Baloh et al. 1997), however, neither of them responded to acetazolamide. As with other patients with EA-2, both sons noted that episodes of vertigo and ataxia could be precipitated by stress, emotional upset, and exercise. Finally, for the two patients with progressive ataxia and for the two patients with episodic and progressive ataxia, quantitative measurement of interictal eye movements showed the same overall pattern of abnormality reported elsewhere for EA-2 patients (Baloh et al. 1997).

The generalized ataxia involving the limbs and trunk, with slurring of speech, indicates diffuse cerebellar dysfunction in this family. The interictal oculomotor findings of gaze-evoked and rebound nystagmus, along with severe pursuit and optokinetic deficits, also are localizing to the cerebellum. The profound deficit in visual tracking and in visual-vestibular interaction is particularly characteristic of lesions of the flocculonodular lobe (Baloh et al. 1986). The normal saccade velocity, accuracy, and latency measurements, along with normal VOR function and the absence of any long-tract signs, determined by neurological examination, indicate that the brain stem was not involved. MRI confirmed severe cerebellar at-

rophy with a normal-appearing brain stem. Although CACNA1A is expressed in neurons throughout the brain, it is expressed heavily in the cerebellum (Mori et al. 1991). Hybridization with CACNA1A-specific riboprobes in rat found heavy labeling of Purkinje neurons, granule cells, and neurons in the molecular layer (Ludwig et al. 1997).

Functional Implications of the Mutation in CACNA1A

The four homologous domains in the α 1A calcium-channel subunit each have six putative α -helical membrane-spanning segments (S1–S6) (Catterall 1995). The central pore is lined by four P regions (pore loops) interconnecting putative membrane-spanning segments S5 and S6 of each domain (fig. 3A). Calcium selectivity is achieved by interaction between calcium ions and high-affinity binding sites in the P regions. Studies using mutagenesis to change specific amino acids in the pore region have shown that the selectivity filter is formed by the pore loops, which are relatively short polypeptide segments that extend into the aqueous pore from one side of the membrane (fig. 3B) (Heinemann et al. 1992; Yang et al. 1993). A large portion of the pore loop is near the extracellular face of the channel, and only a small segment extends into the membrane, to produce the selectivity filter. The loops extending into the pore allow the ion channel to place the proper functional groups at the correct positions in space, to achieve selective ion binding. For example, with calcium channels, glutamate residues from each of the four pore loops determine the calcium selectivity (Heinemann et al. 1992). The calcium channel is believed to be composed of the integral membrane subunits (S1–S6) arranged in a ring, like the staves of a barrel, around a central pore (MacKinnon 1995). The four pore loops reach into the barrel and confer the ion-conduction properties. Channel gating arises from conformational changes in the transmembrane barrel staves of the subunits.

The missense mutation causing the clinical syndrome in this family resulted in a glycine-to-arginine change in the putative P region in domain I (fig. 3A). Phylogenetically, in all organisms, from the fly (*Drosophila*) to humans, glycine at codon 293 is a highly conserved amino acid in all sequenced α 1 units (fig. 3C) (Stea et al. 1995; Peixoto et al. 1997). The mutation results in a major change in this key pore region, since glycine is an uncharged amino acid with a small side chain, whereas arginine is a basic hydrophilic amino acid with a large, positive side chain. Furthermore, position 293 is only a few amino acids away from the key conserved glutamate position, the central pore amino acid critical for the determination of calcium selectivity (fig. 3C).

Comparison with Other CACNA1A Mutations

A range of phenotypes has been associated with different mutations in CACNA1A (table 2). Four different missense mutations were found in families with hemiplegic migraine (Ophoff et al. 1996). All four point mutations were in conserved functional domains, one, a missense mutation resulting in a shift from threonine to methionine at position 666, being in the pore region of the S2 domain. The latter family had a mild interictal ataxia in addition to episodes of hemiplegic migraine. Two families with EA-2 were found to have mutations that disrupted the reading frame in S1 and S2 of domain 3 and that, thus, were predicted to produce truncated α 1A subunits (Ophoff et al. 1996). We recently identified a spontaneous mutation, in a patient with EA-2, that resulted in a premature stop codon and a putative truncated α 1A subunit (authors' unpublished data). Expanded CAG repeats in the open reading frame of the carboxyl terminus of CACNA1A result in a progressive ataxia beginning later in life, usually after the age of 40 years (Zhuchenko et al. 1997). Some members of families with these expanded CAG repeats can have episodic features, although these features are not as prominent as those with EA-2. The ataxia with SCA-6 is more progressive than that with hemiplegic migraine or with EA-2, although it does not appear to shorten the life span. The family in this study exhibited a severe progressive ataxia involving the trunk and the extremities, with two members also having prominent episodes of vertigo and ataxia. The early onset and the progression to a condition requiring a wheelchair, in the 40s, is unique.

How do mutations in the same gene lead to such varied clinical syndromes? The location and type of mutation are important but probably not the only determining factors. Features of the normal allele and features of other similar and modifying genes could alter the phenotypic expression. Environmental factors also may be important. Migraine headaches, which occur with hemiplegic migraine and EA-2 (Baloh et al. 1997) and which occurred in the family reported here, may be a nonspecific secondary phenomenon due to the underlying neuronal metabolic defect (Moskowitz 1990).

Comparison with Other Channelopathies

A range of human ion channelopathies has been described elsewhere (Ptacek 1994). Most involve muscle, presenting with episodic weakness or paralysis that usually is responsive to acetazolamide. Hypokalemic periodic paralysis is caused by mutations in CACNA1S, which codes for the α 1S transmembrane subunit of the skeletal muscle L-type voltage-gated calcium channel (Ptacek et al. 1994). Mutations in the sodium-channel gene SCN4A, resulting in hyperkalemic periodic paral-

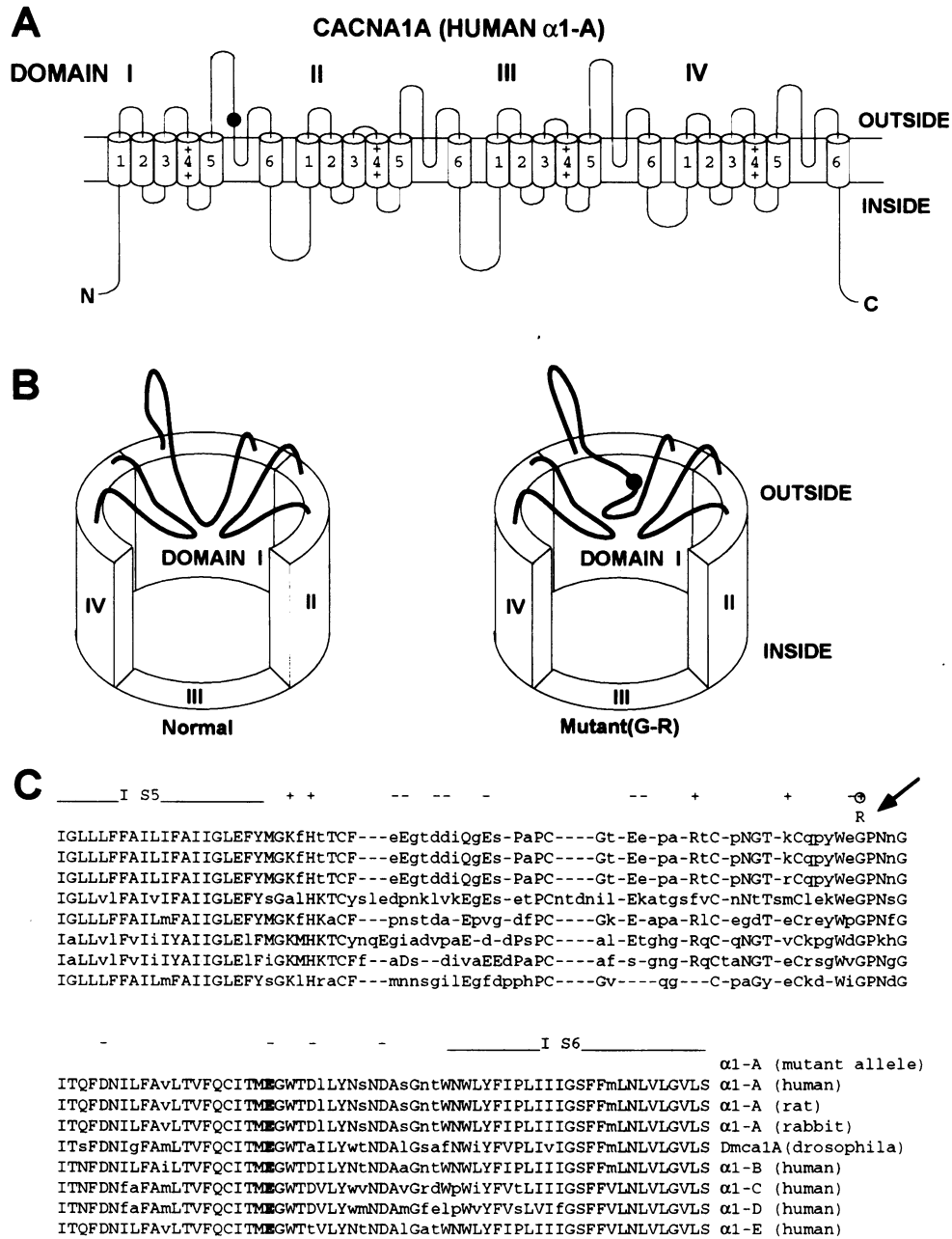


Figure 3 A, Schematic drawing of the α 1A subunit of a calcium channel coded for by CACNA1A. Each of the four domains (I-IV) has six transmembrane segments (1-6). The pore-forming regions (pore loops) are between segments 5 and 6. The blackened circle indicates the location of the predicted glycine-to-arginine substitution in the family in this study. B, Illustration of how the central pore of the channel is thought to be formed by pore loops from the four domains (left) and of how the pore might be distorted by the glycine-to-arginine substitution in the pore loop of domain I (right) (domain III was removed, for clarity). C, Sequence of the pore region of domain I, in different α 1 subunits, showing conserved amino acids (Stea et al 1995; Peixoto et al. 1997). The arrow indicates the glycine (G)-to-arginine (R) substitution in the family in this study. The boldface "E" indicates the position of the key central-pore glutamate.

ysis, are probably the best characterized, in terms of phenotype-genotype correlation (Ptacek et al. 1991; Rojas et al. 1991). Hyperkalemic periodic paralysis is an autosomal dominant disorder in which elevations in se-

rum potassium concentration trigger attacks of muscle weakness. During the attacks, the muscle fiber's resting potential is abnormally depolarized, thus making the sarcolemma electrically inexcitable. Cannon (1996) ob-

Table 2
Phenotypes Associated with Different Mutations in CACNA1A

Phenotype	Mutation	Age at Onset (years)	Ataxia Course
Hemiplegic migraine/ basilar migraine	Missense	2–30	Stable, exam often normal
EA-2	Interrupts reading frame	3–30	Mildly progressive
SCA-6	CAG repeats in open reading frame	>30	Moderately progressive
Episodic and progressive ataxia	Missense	5–15	Severely progressive

served that, at the single-channel level, 5%–10% of mutant sodium channels from biopsied muscle failed to inactivate and that there was an increased open time and open probability in the setting of high extracellular potassium concentration. Impaired fast inactivation of this seemingly small fraction of sodium channels was shown to be empirically and theoretically sufficient to cause muscle paralysis (Cannon and Corey 1993; Cannon and Stritmatter 1993).

The only other human channelopathy involving neurons is episodic ataxia type 1 (EA-1), resulting from missense mutations in a brain potassium channel, KCNA1 (Browne et al. 1994). Patients with EA-1 typically present in early childhood, with brief attacks of ataxia lasting minutes and with interictal myokymia but no interictal nystagmus or ataxia. So far, in families with EA-1, six different missense mutations have been identified; these mutations result in changes in amino acids in the transmembrane or the intracellular segments but do not involve the pore region (Browne et al. 1994, 1995). Studies of the mutated channels expressed in *Xenopus* oocytes suggested that the cellular mechanism underlying EA-1 is an inability of neurons to repolarize after an action potential, because of altered delayed rectifier function (Adelman et al. 1995). The abnormal subunits affected potassium-channel function, either by formation of homomeric channels with altered functions or by coassembly with wild-type subunits.

Mutations in the mouse $\alpha 1A$ voltage-gated calcium-channel gene cause two recessively inherited neurological disorders, “tottering” and “leaner.” Tottering mice have motor seizures and slowly progressive ataxia, beginning around the third postnatal week, whereas leaner mice have absence spells (i.e., brief seizures) and are severely ataxic, often not surviving past weaning. With regard to tottering, a missense mutation results in a non-conservative amino acid change near the second P domain, and, with regard to leaner, mutations of a splice donor consensus result in expression of novel carboxy-terminal sequences (Fletcher et al. 1996). The finding of epileptic seizures in the mouse models raises the likely

possibility that even more phenotypes will be associated with mutations in CACNA1A in humans.

Overview Hypothesis

A simple working hypothesis to explain the clinical syndromes associated with mutations in ion channels is that episodes are due to transient impairment of channel activation and/or deactivation, whereas progressive interictal signs are due to chronic excess entry of calcium into the cell or release of intracellular calcium, leading to abnormal activation of intracellular signals and ultimately to cell death (apoptosis). Both mechanisms seem to occur with most mutations, although some mutations are relatively specific for episodic symptoms (e.g., some families with hemiplegic migraine), whereas others produce progressive symptoms (e.g., families with SCA-6). Studies of patients with hyperkalemic periodic paralysis due to mutations in SCN4A suggest likely mechanisms for these two different processes. Increased open time and open probability of mutant channels, triggered by high extracellular potassium, lead to depolarization of the muscle cells and paralysis, whereas chronic abnormal levels of intracellular calcium lead to activation of intracellular messenger pathways that ultimately lead to cell death. Patients with hyperkalemic periodic paralysis typically develop muscle atrophy and weakness after many years of episodic weakness. Assuming analogous mechanisms with mutations in the calcium-channel gene CACNA1A, episodes such as those seen with hemiplegic migraine and EA-2 can be assumed to result from transient loss of function, leading to transient neuronal depolarization, primarily of cerebellar neurons. Chronic progressive ataxia, such as that seen in SCA-6 patients and in the family in this study, results from chronic excess entry of calcium into neurons and excess activation of calcium’s intracellular signaling pathways, ultimately leading to cell death (Koh and Cottman 1996; Rettig et al. 1996). Although interrelated, these two different pathophysiological mechanisms can be relatively independent, even among family members with the same

mutation. Two members of the family in this study had prominent episodes of ataxia, whereas two others never had episodes. All four, however, exhibited gradually progressive ataxia.

Calcium channels such as CACNA1A are exquisitely sensitive to changes in the concentration of pH and of potassium (Hille 1992). As noted above, a change in potassium levels is presumably the mechanism for acetazolamide responsiveness, in patients with hyperkalemic periodic paralysis. It has long been known that changes in pH have a prominent effect on most ion channels, but only recently has the mechanism of pH-dependent control of channel function been studied at the molecular level. A decreasing pH (increasing the number of free protons) strongly inhibits ion permeation through open calcium channels. Recently, it has been shown that the protonation site in the L-type voltage-regulated calcium channel lies within the pore (Chen et al. 1996). It is formed by a combination of conserved pore-region glutamates, the amino acids shown to be the key to calcium selectivity of these channels (fig. 3C) (Heinemann et al. 1992). This mechanism is a simple molecular explanation for the modulatory effect of H⁺ ions on open-channel flux and for the competition between H⁺ ions and divalent cations. By increasing the extracellular proton concentration in the cerebellum (Bain et al. 1992), acetazolamide presumably stabilizes mutant channels that fail to properly inactivate. Our patients were not responsive to acetazolamide, possibly because the missense mutation distorted the pore region, altering the stabilizing effect of H⁺ ions.

It is interesting to consider how the missense mutation in the pore regions found in this family led to the different clinical symptoms and signs. Replacement of a neutral amino acid, glycine, with a positively charged amino acid, arginine, near the center of the pore in domain I would be likely to lead to a distortion of the pore region (fig. 3B). The fact that two patients with this mutation had prominent episodes whereas the other two patients had no episodes indicates that other factors, such as other modifying genes or, possibly, metabolic factors such as hormone levels, are important in the determination of susceptibility to episodic dysfunction. On the other hand, all four patients exhibited gradually progressive ataxia, indicating that this pore mutation resulted in chronic increased intracellular calcium, ultimately leading to neuronal death.

Acknowledgments

This work was supported by NIH grants AG9063, PO1 DC02952, and DC00008-21.

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