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Coding and Noncoding Variation of the Human Calcium-Channel β_4-Subunit Gene *CACNB4* in Patients with Idiopathic Generalized Epilepsy and Episodic Ataxia

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Inactivation of the β_4 subunit of the calcium channel in the mouse neurological mutant *lethargic* results in a complex neurological disorder that includes absence epilepsy and ataxia. To determine the role of the calcium-channel β_4-subunit gene *CACNB4* on chromosome 2q22-23 in related human disorders, we screened for mutations in small pedigrees with familial epilepsy and ataxia. The premature-termination mutation R482X was identified in a patient with juvenile myoclonic epilepsy. The R482X protein lacks the 38 C-terminal amino acids containing part of an interaction domain for the α_1 subunit. The missense mutation C104F was identified both in a German family with generalized epilepsy and praxis-induced seizures and in a French Canadian family with episodic ataxia. These coding mutations were not detected in 255 unaffected control individuals (510 chromosomes), and they may be considered candidate disease mutations. The results of functional tests of the truncated protein R482X in *Xenopus laevis* oocytes demonstrated a small decrease in the fast time constant for inactivation of the cotransfected α_1 subunit. Further studies will be required to evaluate the in vivo consequences of these mutations. We also describe eight noncoding single-nucleotide substitutions, two of which are present at polymorphic frequency, and a previously unrecognized first intron of *CACNB4* that interrupts exon 1 at codon 21.

Introduction

Inherited movement and seizure disorders are a major clinical burden, the molecular etiology of which remains, for the most part, undefined. The results of recent studies have indicated that mutations in the neuronal voltage-gated ion channels are a major contributor to these conditions (Cooper and Jan 1999; McNamara 1999). Mutations in five neuronal ion-channel genes have been associated with idiopathic epilepsies. These mutations are found in the α and β_1 subunits of the voltage-gated sodium channel in generalized epilepsy with febrile seizures plus (GEFS+) (Wallace et al. 1998; Escayg et al. 2000), in the potassium-channel genes *KCNQ2* and *KCNQ3* in benign familial neonatal convulsions (Biervert et al. 1998; Charlier et al. 1998; Singh et al. 1998), and in the α_4 subunit of the neuronal nicotinic acetylcholine receptor in autosomal dominant nocturnal frontal-lobe epilepsy (Steinlein et al. 1995). The voltage-gated sodium-channel gene *SCN5A* has also been implicated in the seizure activity of individuals with LQT3 (Hartmann et al. 1999). With regard to ion channels and ataxia, the potassium-channel gene *KCNA1* and the calcium-channel gene *CACNA1A* are associated with episodic ataxia type 1 and type 2, respectively. Other alleles of *CACNA1A* cause spinocerebellar ataxia type 6 and familial hemiplegic migraine, demonstrating that different mutations in the same ion-channel gene can produce different neurological phenotypes. In the mouse, allelic mutations in the sodium-channel gene *SCN8A* result in ataxia, dystonia, and paralysis (Meisler et al. 1997; Sprunger et al. 1999), and several other mouse neurological mutants have been associated with ion channels (Burgess and Noebels 1999).

The voltage-gated calcium channels play a critical role in neurotransmitter release in neurons. Calcium channels are multimeric proteins that contain an α_1 subunit with pore-forming domains and the auxiliary subunits β, γ, and $\alpha 2\delta$ (Perez-Reyes and Schneider 1994). At least seven distinct mammalian genes encoding calcium-channel α_1 subunits have been identified. Four paralogous genes encoding the β subunits β_1–β_4 have been

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mapped to conserved chromosome segments in human and mouse (Plummer and Meisler 1999). Interaction of α_1 and β subunits contributes to the diversity of calcium currents by modulation of current amplitude, voltage dependence, and kinetics of activation and inactivation (De Waard and Campbell 1995). The β subunits also appear to participate in the membrane trafficking of the α_1 subunit (Yamaguchi et al. 1998; Gerster et al. 1999). The amino acid sequences of the four mammalian β subunits differ by ~20%. All β subunits are expressed in the brain, with regional differences in the levels of expression. The β_4 subunit is the most highly expressed subunit in the cerebellum (Burgess et al. 1999).

We demonstrated that a null mutation in the β_4 subunit is responsible for an autosomal recessive neurological disorder in the mouse mutant *lethargic* (Burgess et al. 1997). A 4-bp insertion into a splice-donor site results in exon skipping and premature termination of the protein upstream of the major site for interaction with the α_1 subunit. Absence of β_4-subunit protein in *lethargic* brain has been confirmed with the use of specific antisera (McEnery et al. 1998). The lack of β_4 subunits results in severe ataxia, focal motor abnormalities, and brief periods of behavioral immobility that are accompanied by bilaterally synchronous, generalized cortical spike-wave discharges with a mean duration of 1.5 s and a frequency of 100/h (Hosford et al. 1992). These spontaneous absence seizures share behavioral, electrographic, and pharmacological responses with absence seizures in patients (Hosford et al. 1999). The β_4-subunit deficiency results in a selective decrease in excitatory synaptic transmission in somatosensory thalamic neurons (Caddick et al. 1999). Despite the compensatory association of α_1-subunit proteins with other β subunits (Burgess et al. 1999), only 20% of *lethargic* homozygotes survive beyond weaning.

To determine the effects of mutations in the human orthologue of *lethargic,* we characterized the exon structure of the human calcium-channel β_4-subunit gene (*CACNB4* [MIM 601949]) and mapped the gene to chromosome 2q22-23 (Escayg et al. 1998). Genes for two epilepsy syndromes—generalized epilepsy with febrile seizures plus (GEFS+) and febrile seizures (FEB3)—have recently been mapped to this chromosome region (Baulac et al. 1999; Moulard et al. 1999; Peiffer et al. 1999; Lopez-Cendes et al. 2000). In each family, one or two recombinants placed the disease gene distal to markers D2S141 and D2S284. Since we mapped *CACNB4* proximal to these markers on the GB4 radiation-hybrid panel, it is apparently excluded as a candidate gene (Escayg et al. 1998). We now report the results of screening for *CACNB4* mutations in affected individuals from small pedigrees with common subtypes of idiopathic generalized epilepsy and hereditary episodic ataxia. The results of exon amplification and conformation-sensitive gel electrophoresis (CSGE) revealed several noncoding polymorphic variants and two coding mutations in affected individuals.

Material and Methods

Mutation Detection

The 14 exons of *CACNB4* were individually amplified from genomic DNA, by use of primers designed from intron sequences. PCR amplification was performed for 32 cycles of 1 min at 94°C, 45 s at 60°C, and 1 min at 72°C. PCR products were incubated for 10 min at 99°C, followed by 30 min at 68°C to facilitate heteroduplex formation, and they were analyzed by CSGE, as described elsewhere (Raman et al. 1997). Exons with electrophoretic variants were reamplified, PCR products were purified from agarose gels by use of the Qiaex II gel extraction kit (Qiagen), and manual sequencing was performed with use of the Thermo Sequenase sequencing kit (USB).

Patients

DNA was obtained from index patients from 90 families with idiopathic generalized epilepsy (Sander et al. 1999) and from 71 families with episodic ataxia. The families with epilepsy included 19 families with childhood absence epilepsy, 22 with juvenile absence epilepsy, and 49 with juvenile myoclonic epilepsy (JME). More than 250 unrelated controls were tested for variants detected in the affected individuals. The clinical features of pedigrees with coding variants of *CACNB4* are described in the Appendix. Informed consent was obtained from all participants.

Functional Tests in Xenopus laevis *Oocytes*

The mutations R482X and C104F were introduced into the previously described rat β_4-subunit cDNA (Walker et al. 1998). The rat and human *CACNB4* proteins exhibit 99% sequence identity (512/519 amino acids; Escayg et al. 1998), and human residue C104 corresponds to rat C103. R482X was constructed by means of PCR amplification of the corresponding region of the wild-type cDNA with the addition of a Kozak sequence and a termination codon. The PCR product was subcloned into pcDNA3, with the use of *Hin*dIII and *Bam*HI sites added to the PCR primers. The C104F mutation was introduced by use of the QuickChange site-directed mutagenesis kit (Stratagene) with the following mutagenic primers: 5′-AAA ACG AAT GTG AGC TAC TTT GGT GCC CTG GAT GAG GAT GTG-3′ (forward) and 5′-CAC ATC CTC ATC CAG GGC ACC AAA GTA GCT CAC ATT CGT TTT CAC-3′ (reverse). Oocytes

were injected with each β_4-subunit mRNA (0.1 μg/μl), in combination with the α_1-subunit gene *CACNA1A* (0.4 μg/μl). Electrophysiological recordings were performed 4–5 d after injection, as described elsewhere (Walker et al.1998).

Results

An Additional Intron in CACNB4

We have previously reported that the *CACNB4* coding sequence is interrupted by 12 introns at positions corresponding to the introns of the human *CACNB3* gene (Escayg et al. 1998). Amplification of genomic DNA, by use of primers from the 5′ untranslated region and intron 1, generated a product that was 0.5 kb longer than that which was predicted by the cDNA. Sequence analysis identified a small intron (535 bp) that interrupts exon 1 (GenBank accession number AF216867). The first two exons of *CACNB4* are now designated as "1A" and "1B" (fig. 1*A*).

Noncoding Variation in CACNB4

Eight noncoding variants were identified by CSGE analysis of exons amplified from genomic DNA (table 1). Two of these noncoding variants are present at polymorphic frequencies ⩾1%, whereas the others are rare variants.

Premature-Termination Mutation in a Family with JME

The proband in pedigree A (individual I-1; fig. 2*A*) exhibited JME. Her symptoms include characteristic bilateral myoclonic jerks of the shoulders and arms without loss of consciousness, sporadic absence seizures, and generalized tonic-clonic seizures (GTCS). A unique conformer of exon 13 was observed in individual I-1, by means of CSGE (fig. 2*B*, lane 1). Sequencing demonstrated heterozygosity for a C→T transition (fig. 2*C*). This mutation generates a premature stop codon (R482X) and eliminates 38 amino acids comprising the C-terminal 10% of the protein. This mutation can also be detected by generation of a novel *Alu*I site (fig. 2*D*).

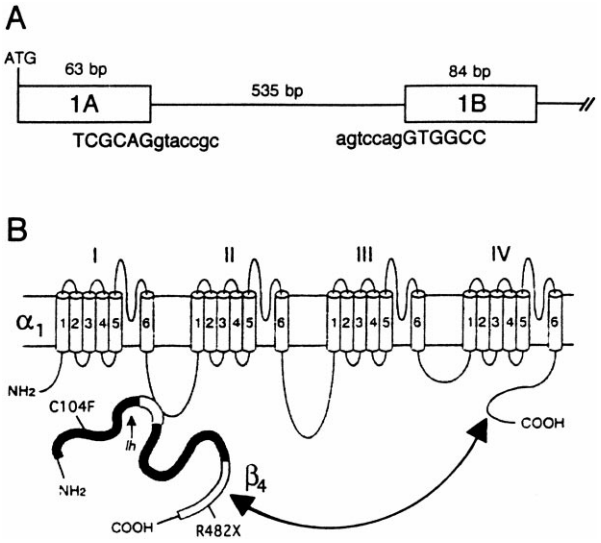

Figure 1 Structure and interactions of *CACNB4*. *A*, Intron/exon junctions for exons 1A and 1B. The indicated 5′ end of exon 1A corresponds to the translation-initiation site. The intron sequence is from GenBank (accession number AF216867). *B*, Locations of mutations in *CACNB4*. Two domains of *CACNB4* that interact with the α1 subunit of the voltage-gated calcium channel are indicated. In the mouse *lethargic* mutant (designated as "*lh*"), the protein is truncated upstream of the major binding site. In human mutation R482X, the protein is truncated in the middle of a domain that interacts with the C-terminus of the α_{1A} subunit.

Table 1

Single-Nucleotide Variants in Human *CACNB4*

Substitution Type and Location	Nucleotide[a]	Major/ Minor Allele	Frequency of Minor Allele[b]	Primer	
				Forward	Reverse
Noncoding:					
Exon 9	762	T/A	<.01	9F: CTG GCT TGT TCT GTT CTG TG	9R: GCA CAG CTT GAA GAA GTA CC
Exon 12	1239	G/A	.03	12F: AAC TGC CCA TAT AGC ACC TC	12R: TCC ATC CTA ATC AAG AAG TTC CC
Exon 13	1413	G/A	<.01	13F: GCA TCT TAT TGT ACA GAT CC	13R: GCT ATC TTA GCC AAG TTA AG
Exon 13	1567	T/C	<.01	13F: GCA TCT TAT TGT ACA GAT CC	13R: GCT ATC TTA GCC AAG TTA AG
Intron 2	268 −37	C/T	.01	3F: CAT TTG AGA TCC CTC TTT GG	3R: GTT TGG CTC AGA GCT GAT TC
Intron 3	390 + 22	T/C	<.01	3F: CAT TTG AGA TCC CTC TTT GG	3R: GTT TGG CTC AGA GCT GAT TC
Intron 5	599 −15	G/A	<.01	6F: AGG GAC AAA TGG GTA CAT TC	7R: GGG TGC TCG ATC AAG AAC TG
Intron 6	619 −24	C/T	<.01	6F: AGG GAC AAA TGG GTA CAT TC	7R: GGG TGC TCG ATC AAG AAC TG
Coding:					
Exon 3 (C104F)	311	G/T	<.006	3F: CAT TTG AGA TCC CTC TTT GG	3R: GTT TGG CTC AGA GCT GAT TC
Exon 13 (R482X)	1444	C/T	<.006	13F: GCA TCT TAT TGT ACA GAT CC	13R: GCT ATC TTA GCC AAG TTA AG

[a] Nucleotide positions are based on the cDNA sequence (GenBank accession number AF038852; Escayg et al. 1998).

[b] Frequencies are based on the results of analysis of 500–1,000 chromosomes.

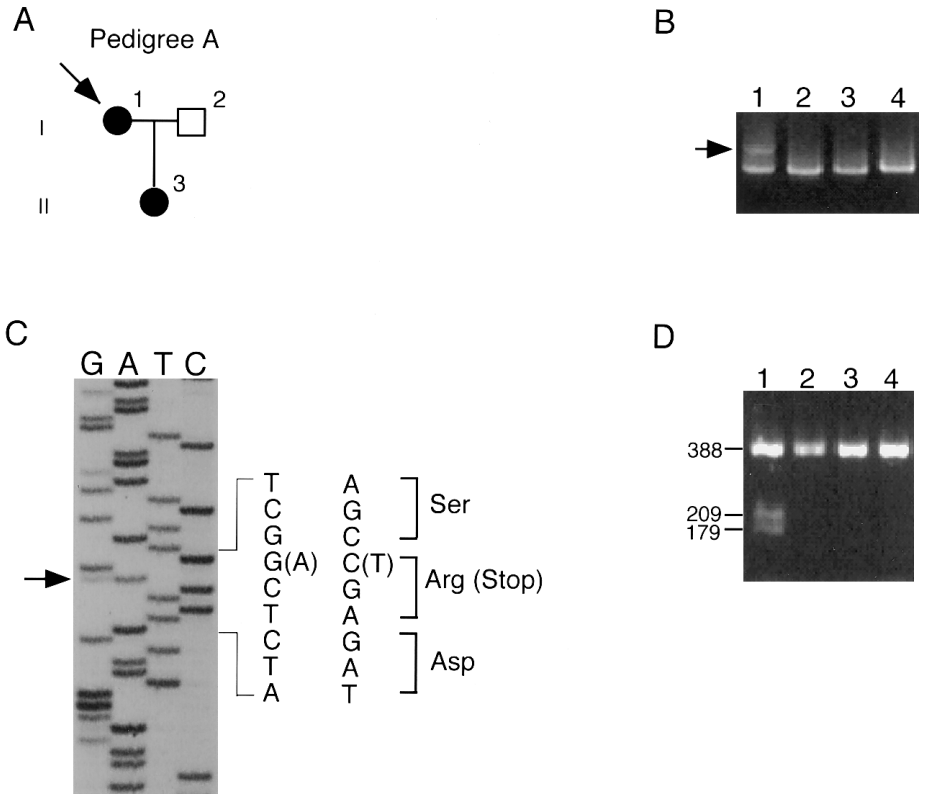

Figure 2 R482X mutation in a family with JME. *A,* Affected individuals (*blackened symbols*) are shown, with the arrow pointing to the proband. *B,* Results of CSGE of exon 13. A unique band seen in the proband (lane 1, *arrow*) is not observed in three unrelated controls (lanes 2–4). *C,* The sequence of exon 13 demonstrates that the proband is heterozygous for a C→T transition that generates the premature-termination codon R482X. *D,* Results of a restriction-site assay for R482X. A 388-bp fragment obtained by digestion of wild-type exon 13 with *Alu*I is shown (lanes 2–4). The internal *Alu*I site produced by R482X results in two fragments (209 bp and 179 bp; see lane 1).

Individual II-3 is considered to be affected, on the basis of an abnormal electroencephalogram (EEG) with generalized 3/s spike-wave discharges (for clinical details, see the Appendix). To date, efforts to obtain DNA samples from individuals I-2 and II-1 have not been successful.

Segregation of C104F in Two Pedigrees with Different Neurological Phenotypes

The proband in pedigree B (individual I-1; fig. 3*A*) and his son (individual II-3) exhibit idiopathic generalized epilepsy with rare GTCS, atypical absence seizures, and evidence of an unusual cognitive trigger of seizure initiation. An exon 3 variant was detected, by means of CSGE, in both affected individuals (fig 3*C, left*). The results of sequence analysis demonstrated heterozygosity for a G→T transversion (fig. 3*D*) resulting in the replacement of cysteine residue 104 by phenylalanine (C104F). The unaffected members of family B do not carry the mutation.

C104F was also observed in an unrelated French Canadian family with episodic ataxia (pedigree C; fig. 3*B* and *C*). In this family, five affected and two asymptomatic individuals carry the C104F mutation, consistent with incomplete penetrance (70%) if this is a causal mutation.

Analysis of R482X and C104F in Control Samples

We analyzed exon 3 and exon 13 in 255 control samples (510 chromosomes), including 133 asymptomatic individuals of northern European origin (Sander et al. 1999) and 80 CEPH parents. R482X and C104F were not present in the controls, indicating that the allele frequencies for both mutations are <.006 (95% confidence level).

Functional Analysis of R482X and C104F in Xenopus *Oocytes*

To evaluate their effects on channel properties, the mutations R482X and C104F were introduced into the rat β_4-subunit cDNA and were coexpressed with the α_{1A}-subunit in *Xenopus* oocytes. Expression of the wild-type β_4 subunit together with the α_{1A} subunit produces a slowly inactivating inward Ba^{2+} current (fig. 4*a*). This current is not seen in the absence of the β_4 subunit. Both

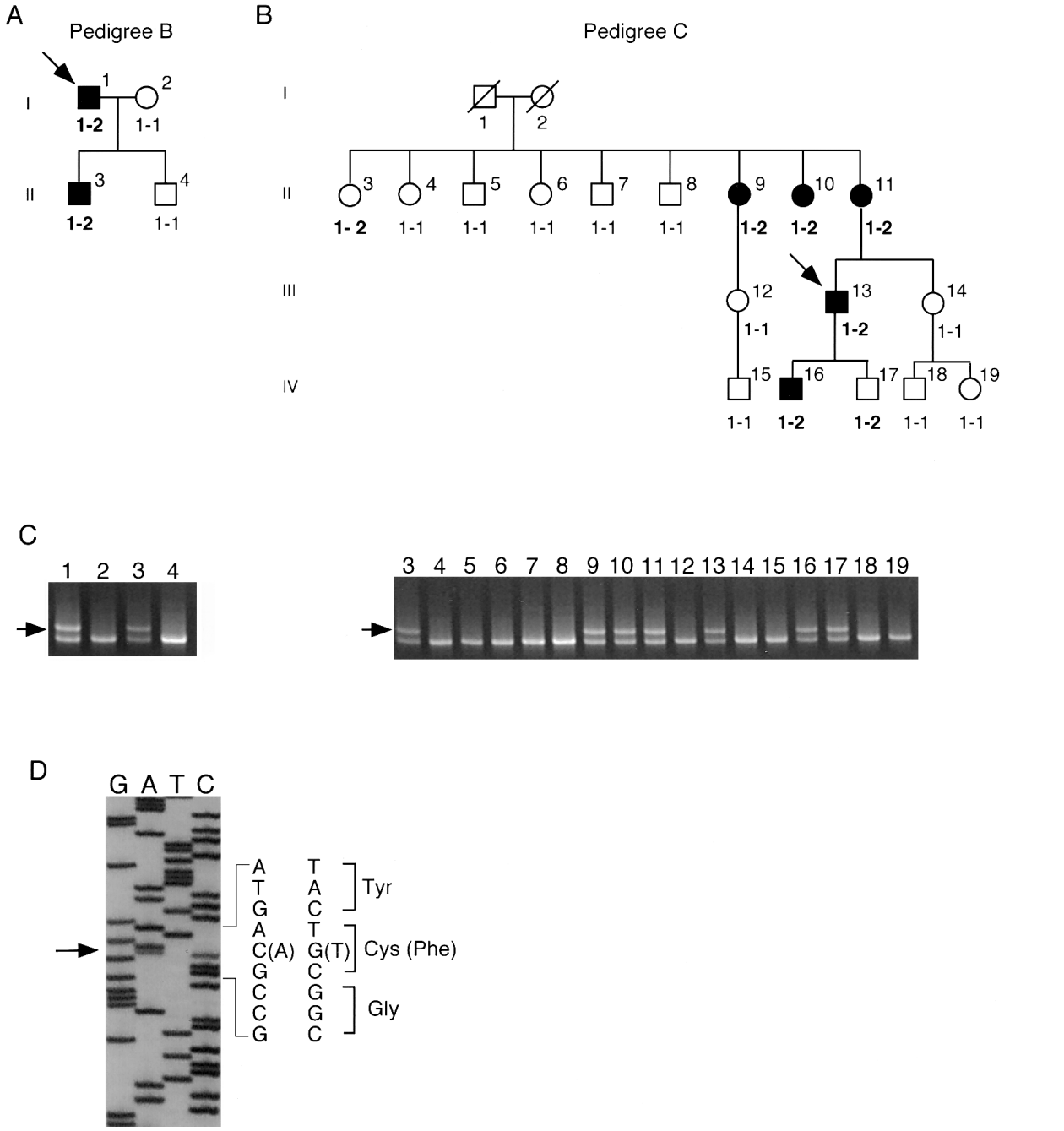

Figure 3 C104F mutation cosegregating with neurological disease in two unrelated pedigrees. *A*, Pedigree B, which is of German origin and exhibits idiopathic generalized epilepsy. *B*, Pedigree C, which is of French Canadian origin and exhibits episodic ataxia. *C*, CSGE analysis of exon 3 from pedigree B (*left*) and from pedigree C (*right*). The variant conformer is indicated (*arrow*). Numbers above the lanes correspond to individuals in the pedigrees. *D*, Sequence of exon 3 that demonstrates a G→T transversion that changes the wild-type cysteine 104 codon to phenylalanine. *CACNB4* genotypes are indicated below the symbols. G = allele 1; T = allele 2.

mutants were able to associate with the α_{1A} subunit, to produce inward currents (fig. 4*a*). Increases in current density of 1.4-fold and 1.3-fold were observed for R482X and C104F, respectively (fig. 4*b*). There was no change in the voltage dependence of activation.

An interesting change in the kinetics of inactivation was observed for the truncated R482X mutant. The wild-type β_4 subunit produces biphasic inactivation (Walker et al. 1998) with a fast-inactivating component (F), a slower inactivating component (S), and a noninactivating component (NI). For test potentials of 0–20 mV, the F component for the R482X mutant is accel-

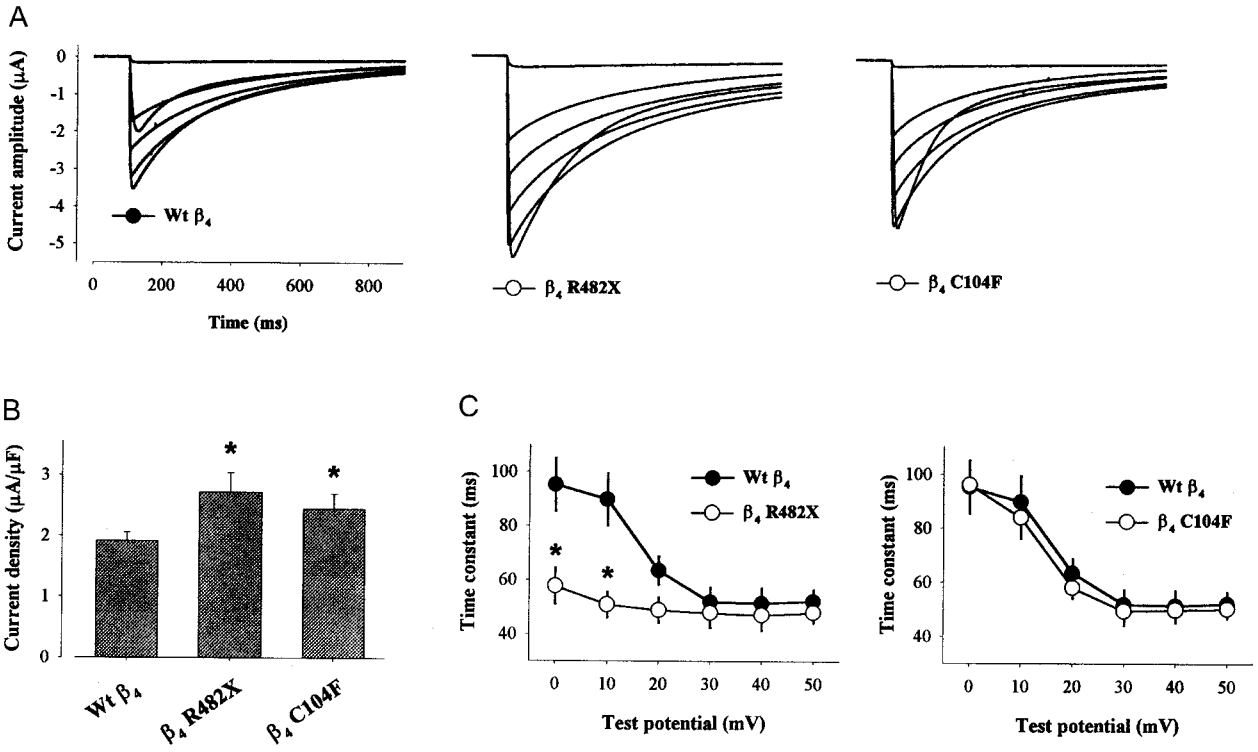

Figure 4 Electrophysiological analysis of mutant β_4 subunits. *Xenopus* oocytes were injected with mRNA from either the wild-type or mutant β_4 subunit, in combination with the *CACNA1A* α_1 subunit. *A*, Sets of current traces at test potentials of −20, −10, 0, 10, 20, and 30 mV. The potential of half-activation was not significantly modified by the mutations, with $V_{1/2}$ =−12.2 ± 0.9 mV (β_4-subunit R482X, n = 8), −11.3 ± 0.6 mV (β_4-subunit C104F; n = 8), and −10 ± 0.1 mV (wild-type β_4 subunit; n = 8). There was a small shift in the voltage dependence of inactivation of the α_{1A} β_4-subunit R482X, with half-inactivation potentials of $V_{1/2}$ = −41.1 ± 0.4 mV (β_4-subunit R482X; n = 7) and $V_{1/2}$ = −37.4 ± 0.5 (wild-type β_4 subunit; n = 6,). *B*, Average current density generated by wild-type and mutant channels at a membrane depolarization of 0 mV (peak current amplitude for each subunit combination). The average current density of the α_{1A} wild-type β_4 subunit (1.90 ± 0.15 μA/μF; n = 8) at 0 mV was increased 1.4- and 1.3-fold, to 2.72 ± 0.25 (n = 8) and 2.45 ± 0.24 (n = 8), with β_4-subunit R482X and β_4-subunit C104F, respectively. Asterisks (*) denote statistical significance (*t* test ; $P \leq .1$). *C*, Average time constants for the fast component of inactivation. At a membrane depolarization of 10 mV, the wild-type current inactivates along three components, a fast-inactivating component (F) with an average time constant of 89.6 ± 5.3 ms (n = 7), which constitutes 19.9 ± 3.4% of the total current, a slower inactivating component (S) with an average time constant of 323 ± 37.4 ms (73.9 ± 3.1%), and a noninactivating (NI) component (6.2 ± 0.9%). With β_4-subunit R482X, there was no change in the proportion of each component, but the F component demonstrated a faster rate of decrease (50.7 ± 4.7 ms; n = 6). The time constants for β_4-subunit R482X at 0 and 10 mV are statistically significantly different from those for wild type ($P < .01$). Values are mean ± SEM.

erated. For example, at 10 mV, the time constant for R482X is 50.7 ± 4.7 ms, compared with 89.6 ± 5.3 ms for the wild-type β_4 subunit (fig. 4*c*). This change is similar to that previously observed for constructs with deletions of the C-terminus (Walker et al. 1998). No kinetic alteration was observed for the C104F mutation (fig. 4*c* and data not shown).

Discussion

The β_4 calcium-channel subunit is a highly conserved protein, with 99% sequence identity between human and rat (Escayg et al. 1998). We observed a very low level of polymorphism in this initial study of human variation. In addition to several noncoding polymorphisms in *CACNB4*, we identified two coding changes that are potential disease mutations. The positions of these mutations and the mouse *lethargic* mutation are shown in figure 1*B*.

The N- and C-terminal portions of the α_1 and β subunits contain several functional domains that modulate channel kinetics (Wei et al. 1994; de Leon et al. 1995; Tareilus et al. 1997; Cens et al. 1999; Walker et al. 1999). The C-terminus of the β_4 subunit interacts directly with the C-terminus of the α_{1A} subunit (Walker et al. 1998). Experimental deletion of 109 amino acids from the C-terminus of the β_4 subunit eliminated binding to the C-terminus of the α_{1A} subunit and decreased the time constant for the fast component of inactivation (Walker et al. 1998). The R482X truncation mutant

lacking the 38 C-terminal amino acids had a similar effect on the time constant, indicating that these residues are involved in the interaction with the C-terminus of the α_{1A} subunit. The decrease in time constant observed with R482X would have the effect of increasing the rate of channel inactivation and reducing the net inward flow of calcium ions into activated neurons. This type of effect may be quite pronounced during short and rapid alterations in membrane potentials, such as those that occur during action potentials, and could be of clinical significance. The deletion of two consensus phosphorylation sites in the R482X channel could also have an effect on its activity (Hell et al. 1996; Chien et al. 1998).

Although the C104F mutation did not alter channel kinetics, other aspects of β_4-subunit function may be affected by this mutation. Replacement of cysteine with a large hydrophobic phenylalanine residue in the C104F mutation could disrupt the conformation of this domain, which is evolutionarily conserved and is thought to be involved in interaction with other proteins (Walker and De Waard 1998; Hanlon et al. 1999). Such interactions could be crucial to channel clustering or targeting without affecting the channel gating. The increase in current amplitude observed for the mutant channels could also contribute to abnormal firing properties.

The clinical features of individuals in pedigree C closely resemble those of patients with mutations in the calcium-channel α_{1A} subunit resulting in episodic ataxia type 2 (EA2). The similarities include duration of attacks, interictal imbalance and nystagmus, response to acetazolamide, and apparently incomplete penetrance. The only obvious difference is the earlier onset in infancy in patients with EA2. The proband in family 3 was screened for mutations in the α_{1A} subunit by means of SSCP, but no mutations were detected. The similarities to EA2 are consistent with a causal role for the C104F mutation in pedigree C.

The observation that the C104F mutation is associated with episodic ataxia in one family and with epilepsy in another family suggests either that it is a neutral polymorphism or that genetic and environmental factors modify the clinical effects of the mutation. The null mutation of *Cacnb4* in the *lethargic* mouse results in seizures and ataxia, indicating that *CACNB4* can contribute to both phenotypes. Another mutation conferring both episodic ataxia and susceptibility to seizures was described in a human potassium channel (Zuberi et al. 1999). We have recently mapped a genetic modifier locus that dramatically alters the phenotype associated with a sodium-channel mutation in mouse (Sprunger et al. 1999). It would not be surprising if background genetic variation in the human population could influence the phenotypic consequences of ion-channel mutations such as C104F.

Are the R482X and C104F mutations responsible for the neurological disease in these pedigrees? The mutations cosegregate with the disease in affected individuals, are associated with phenotypes similar to those in the mouse mutant, and are not present in 255 controls; however, the functional alterations in the oocyte expression system are subtle, and the pedigrees are too small to provide strong evidence for linkage. Identification of additional patients with mutations in *CACNB4* and demonstration of functional consequences in a mammalian assay system could provide additional support for a causal role for these mutations. Because ataxia and epilepsy can result from mutations in several different ion-channel genes, continued identification of disease-causing mutations will be a necessary step toward the goal of developing therapies that are tailored to each specific genetic disorder.

Acknowledgments

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Appendix

Clinical Features of Three Pedigrees with Coding Variants of *CACNB4*

Beginning at age 9 years, the proband in pedigree A experienced sporadic typical absences (brief spells of loss of consciousness) and repetitive bilateral myoclonic jerks in the shoulders and arms after awakening, without loss of consciousness. She later experienced several GTCS, usually within 1 h of awakening. Her epilepsy syndrome was classified as JME (Commission on Classification and Terminology of the International League Against Epilepsy 1989). EEGs of the proband's daughter showed generalized 3/s spike-wave discharges without epileptic seizures.

The affected father and son in pedigree B presented with an atypical but similar clinical syndrome of idiopathic generalized epilepsy with rare juvenile atypical prolonged absences and occasional GTCS. The proband (individual I-1; fig. 3*A*) had normal intellectual and psychomotor development. One febrile convulsion occurred at age 3 years. After age 6 years, he had occasional GTCS, predominantly on awakening, and, after age 12 years, occasional episodes of absence were described. The interictal EEGs showed atypical generalized spike-

wave discharges in the resting EEG. The ictal EEG was highly unusual, with initial generalized desynchronization and flattening, followed by irregular generalized high-voltage delta activity interspersed with spikes and wave. The syndrome was classified as idiopathic generalized epilepsy with GTCS on awakening, probable absence seizures, and praxis-induced seizures. A prolonged atypical absence occurred in the the eldest son (individual II-3) at age 14 years, while he was playing cards. At age 17 years, he experienced GTCS shortly after awakening. Two years later, he experienced a generalized tonic seizure while playing a complex strategic game after sleep withdrawal. He experiences occasional prolonged staring spells when lacking sleep. His interictal EEG presents some fast generalized spike-wave activity (4–5 Hz) that increases with hyperventilation. Intellectual and psychomotor development was normal. Both affected individuals report that seizures are precipitated by playing complex strategic games (praxis induction), suggesting an unusual cognitive trigger of seizure initiation (Inoue et al. 1992; Ritaccio 1994). Clinical exploration revealed no evidence for epileptic seizures in either the 51-year-old mother (individual II-2) or the 17-year-old son (individual II-4). Individual II-4 had normal EEG results.

After age 20 years, the proband in pedigree C (individual III-13; fig. 3*B*) experienced recurrent episodes of vertigo and ataxia that lasted for several hours. Interictal examination revealed spontaneous downbeat and gaze-evoked nystagmus and mild dysarthria and truncal ataxia. The proband's mother had identical episodes of vertigo and ataxia after age 30 years as well as long-standing dysarthria and imbalance. Acetazolamide prevented the attacks in both the proband and mother, and the attacks recurred when acetazolamide was briefly discontinued. A maternal aunt (individual II-10) has reported mild episodes of ataxia for the past 5 years. Seven years ago, another maternal aunt (individual II-9) experienced one prolonged episode of vertigo and imbalance that lasted for several weeks. Although the proband's 12-year-old son (individual IV-16) had febrile seizures as a child and exhibits dysarthria and imbalance, he has not had episodes of vertigo or ataxia. The other family members are asymptomatic, including individuals II-3 and IV-17, both of whom carry the C104F mutation.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

CEPH, http:/www.cephb.fr/

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/index.html (for the small intron of 535 bp that interrupts exon 1 [accession number AF216867])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *CACNB4* [MIM 601949])

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