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Calcium Channels and Channelopathies of the Central Nervous System

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Abstract

Several inherited human neurological disorders can be caused by mutations in genes encoding Ca^{2+} channel subunits. This review deals with known human and mouse calcium channelopathies of the central nervous system (CNS). The human diseases comprise: 1) a recessive retinal disorder, X-linked congenital stationary night blindness, associated with mutations in the CACNA1F gene, encoding $\alpha_1$1.4 subunits of L-type channels; and 2) a group of rare allelic autosomal dominant human neurological disorders including familial hemiplegic migraine, episodic ataxia type 2, and spinocerebellar ataxia type 6, all associated with mutations in the CACNA1A gene, encoding $\alpha_1$2.1 subunits of P/Q-type calcium channels. Mutations at the mouse orthologue of the CACNA1A gene cause a group of recessive neurological disorders, including the *tottering*, *leaner,* and *rocker* phenotypes with ataxia and absence epilepsy, and the *rolling Nagoya* phenotype with ataxia without seizures. Two other spontaneous mouse mutants with ataxia and absence epilepsy, *lethargic* and *stargazer,* have mutations in genes encoding a calcium channel auxiliary β subunit and a putative calcium channel auxiliary γ subunit. For each channelopathy, the review describes disease phenotype, channel genotype, and known functional consequences of the pathological mutations; in some cases, it also describes working hypothesis and/or speculations addressing the challenging question of how the alterations in channel function lead to selective cellular dysfunction and disease.

Index Entries: Calcium channel; channelopathy; migraine; ataxia; epilepsy; neurodegeneration; genotypephenotype; mutation; mouse mutants; cerebellum.

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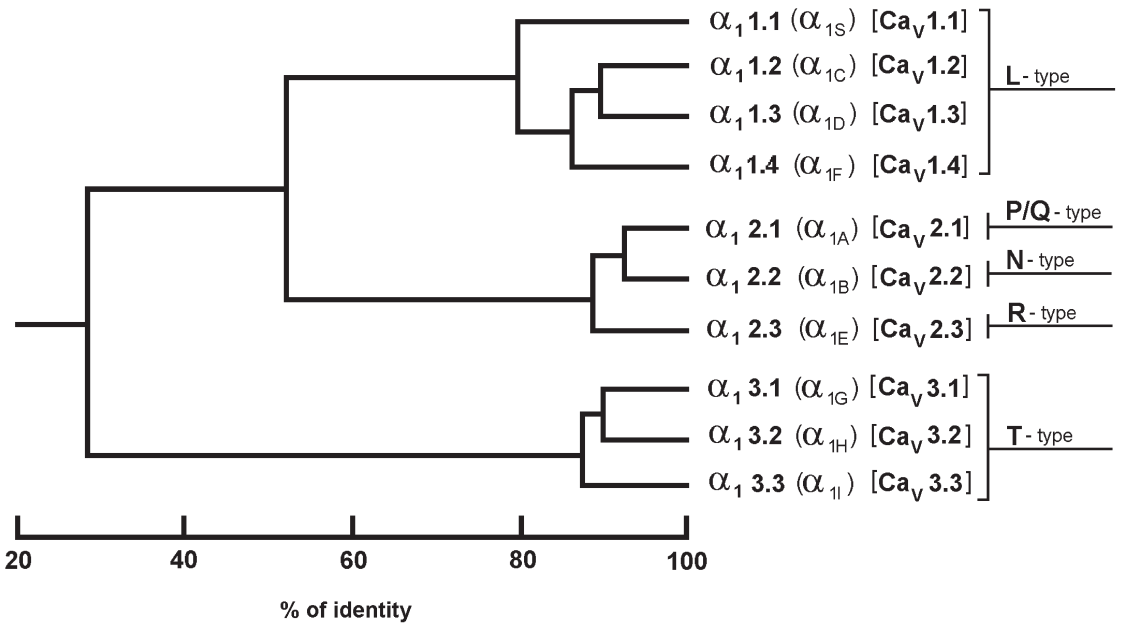

Fig. 1. Nomenclature of voltage-dependent Ca^{2+} channels and matching percentage in membrane-spanning segments and pore loops (*see [3]* for details). For α_1 subunits, both the new numerical and, in parenthesis, the old alphabetical nomenclature are shown. Ca^{2+} channels are named using the chemical symbol of the principal permeating ion (Ca) with the principal regulator (V) as subscript. Also shown are the corresponding classes of native high-voltage-activated (L-, N-, P/Q- and R-types) and low-voltage-activated (T-type) Ca^{2+} channels.

Neuronal Voltage-Dependent Calcium Channels

Since Ca^{2+} ions (unlike K^+, Na^+, Cl^- ions) are intracellular second messengers, calcium channels are unique among ion channels because they regulate a diversity of additional cellular functions, besides cellular excitability. Voltage-dependent calcium channels mediate Ca^{2+} entry into cells in response to membrane depolarization, and thus, transduce electrical signals into chemical signals. In the nervous system they control a broad array of functions including neurotransmitter release, neurite outgrowth, synaptogenesis, neuronal excitability, activity-dependent gene expression, as well as neuronal survival, differentiation, and plasticity.

Voltage-dependent Ca^{2+} channels are multisubunit complexes composed of a pore-forming and voltage-sensing α_1 subunit and several auxiliary subunits, including $\alpha_2\delta$ and β subunits (and in some cases γ subunits). They constitute a complex family of channels comprising a large number of different subtypes, which have in common a steep voltage dependence of the open probability and a very high selectivity for Ca^{2+} over Na^+ and K^+ ions in physiological solutions. The structure of the α_1 subunit shares a basic design with other voltage-gated ion channels, consisting of six membrane-spanning segments (S1 to S6) flanked by cytoplasmic and extracellular loops, with the loop between S5 and S6 folded into the membrane to form part of the pore. This basic design is repeated in four homologous domains (Fig. 2). The S4 segment has been

shown to play a major role in voltage-sensing. High selectivity for Ca^{2+} ions is achieved through a high-affinity binding site formed by a ring of four glutamates, located within the pore close to the external mouth *(1,2).*

At least 10 genes (CACNA1S, C, D, F, A, B, E, G, H, I) encode Ca^{2+} channels α_1 subunits. According to aminoacid sequence homology, the α_1 subunits can be grouped into three families (identity in sequence is more than 70% within a family and less than 40% among families; *see* Fig. 1). Since the alphabetical nomenclature in use up to now does not reveal these structural relationships, a new nomenclature has been recently proposed, where the first number refer to the family and the second number to the order of discovery of the α_1 subunit within that family *(3)* (Fig. 1).

According to pharmacological criteria, native high-voltage-activated Ca^{2+} channels have been classified as dihydropyridine-sensitive channels (L-type), ω-conotoxin-GVIA-sensitive channels (N-type), and ω-agatoxin-IVA-sensitive channels (P/Q-type). An additional component of current (R-type) has been identified as the calcium current resistant to the specific inhibitors of L-, N-, and P/Q-type channels *(4,5).* The following correlation between native neuronal calcium channels and cloned α_1 subunits has been established (Fig. 1): $\alpha_1$2.1, $\alpha_1$2.2 and $\alpha_1$2.3 subunits are the pore-forming subunits of P/Q- (Ca_v2.1), N- (Ca_v2.2), and R-type (Ca_v2.3) calcium channels, respectively: these channels are expressed primarily in neurons, where they initiate neurotransmitter release at most fast synapses. $\alpha_1$1.1, $\alpha_1$1.2, $\alpha_1$1.3, and $\alpha_1$1.4 subunits are pore-forming subunits of different L-type calcium channels: Ca_v1.1 and Ca_v1.2 are the main Ca^{2+} channels of muscle, where they initiate contraction; Ca_v1.3 channels are highly expressed in endocrine cells, where they initiate secretion; Ca_v1.2 and Ca_v1.3 are also widely expressed in neurons, where they play an important role in controlling gene expression; Ca_v1.4 channels are expressed only in the retina. $\alpha_1$3.1, $\alpha_1$3.2, and $\alpha_1$3.3 subunits are pore-forming subunits of different low-voltage-activated T-type Ca^{2+} channels: Ca_v3 channels are expressed in a wide variety of cell types, and in neurons they are involved in shaping the action potential, in controlling patterns of repetitive firing, and in neuronal integration.

The Ca^{2+} channel auxiliary α_2-δ (α_2δ-1, –2, –3), β ($\beta_{1,2,3,4}$) and γ ($\gamma_{1,2,3,4,5,6,7,8}$) subunits are encoded by three, four, and eight different genes, respectively *(3–7).* Whereas the skeletal muscle Ca^{2+} channel (Ca_v1.1) certainly contains the γ_1 subunit, there is still considerable debate as to whether all the different types of Ca^{2+} channels contain γ subunits *(5).* Further molecular diversity of Ca^{2+} channels is created by the existence of multiple splice variants for each pore-forming and auxiliary subunit gene. The β subunits have major functional effects on both membrane targeting and modulation of calcium channels containing $\alpha_1$1 and $\alpha_1$2 subunits *(8,9).* In heterologous expression systems different β and α_2-δ subunits in combination with a given α_1 subunit give rise to calcium channels with different biophysical properties *(8,10).* Moreover, functionally different calcium channels can be formed by different splice variants of a given α_1 subunit *(11–13).* All the different Ca^{2+} channel subunits, except $\alpha_1$1.1, are expressed in the brain, with a differential distribution in different neuronal populations and different localization within the same neuron. Different splice variants of a given subunit are also differentially distributed and localized in the brain. Therefore, the potential for combinatorial structural and functional heterogeneity of brain calcium channels is enormous. Indeed, electrophysiological studies have revealed functional diversity of native L-, P/Q-, N-, R-, and T-type Ca^{2+} channels *(14–18).* Different combinations with auxiliary subunits and/or alternative splicing of α_1 subunits most likely account for the large functional diversity of native calcium channels.

As might be expected from their central role in signal transduction, Ca^{2+} channels are

tightly regulated by second-messenger pathways and protein-protein interactions. The different families of Ca^{2+} channels have different primary modes of regulation: e.g., the Ca_v1 family is regulated primarily by protein phosphorylation through second messenger-activated kinase pathways; in contrast, the Ca_v2 family is regulated primarily by direct binding of G proteins and SNARE proteins, and that mode of regulation is itself regulated by protein phosphorylation pathways (*see [19]* for a recent review on regulation of calcium channels, which is outside the scope of the present review).

Calcium Channelopathies

Several inherited human neurological disorders can be caused by mutations in genes encoding Ca^{2+} channel subunits: we refer to them as calcium channelopathies. This review deals with known human and mouse calcium channelopathies of the central nervous system (CNS). The human diseases comprise: 1) a recessive retinal disorder, X-linked congenital stationary night blindness (xlCSNB), associated with mutations in the CACNA1F gene, encoding $\alpha_1$1.4 subunits of L-type channels; and 2) a group of rare allelic autosomal dominant human neurological disorders including familial hemiplegic migraine (FHM), episodic ataxia type 2 (EA-2), and spinocerebellar ataxia type 6 (SCA6), all associated with mutations in the CACNA1A gene, encoding $\alpha_1$2.1 subunits of P/Q-type channels. Mutations at the mouse orthologue of the CACNA1A gene cause a group of recessive neurological disorders, including the *tottering* (*cacna1a*^{tg}), leaner (*cacna1a*$^{tg-la}$), and *rocker* (*cacna1a*^{rkr}) phenotypes with ataxia and absence epilepsy, and the *rolling Nagoya* (*cacna1a*$^{tg\text{-}rol}$) phenotype with ataxia without seizures. Two other spontaneous mouse mutants with ataxia and absence epilepsy, *lethargic* and *stargazer,* have mutations in genes encoding a calcium-channel auxiliary β subunit and a putative calcium-channel auxiliary γ subunit.

X-Linked Congenital Stationary Night Blindness: A Human L-Type ($Ca_v1.4$) Calcium Channelopathy

xlCSNB is a recessive nonprogressive human eye disease characterized by night blindness, variable reduced day vision, decreased visual acuity, myopia, nystagmus, and strabismus. xlCSNB is thought to result from decreased effectiveness of synaptic transmission between photoreceptors and second-order neurons in the retina. Two distinct clinical entities have been proposed, complete and incomplete xlCSNB, whose loci have been mapped to chromosome Xp11.4 and Xp11.23, respectively. A gene, called CACNA1F, which encodes the calcium channel $\alpha_1$1.4 subunit, has been mapped to Xp11.23, and mutations in this gene have been found in the majority of screened families with incomplete xlCSNB *(20–22).* $Ca_v1.4$ channels are L-type Ca^{2+} channels expressed only in the retina, in both the outer and inner nuclear cell layers. Of the 20 mutations associated to xlCSNB identified so far, 14 are nonsense mutations or deletion/insertions causing truncated and deleted proteins, one is a splice donor site mutation, and five are missense mutations leading to substitution of conserved aminoacids and a change in net charge (Fig. 2). Three of the four aminoacid substitutions are in regions—the P loops of domains III and IV and the S6 segment of domain I—that are thought to form part of the pore. Although the consequences of the mutations on channel function have not been investigated, the truncated mutant proteins are unlikely to form functional channels, and therefore the association of xlCSNB with loss-of-function mutations appears most likely. Since L-type channels control neurotransmitter release from photoreceptor presynaptic terminals, loss-of-function mutations in presynaptic L-type channels would decrease presynaptic calcium influx and tonic glutamate release in darkness, with consequent relative depolarization of bipolar cells. The

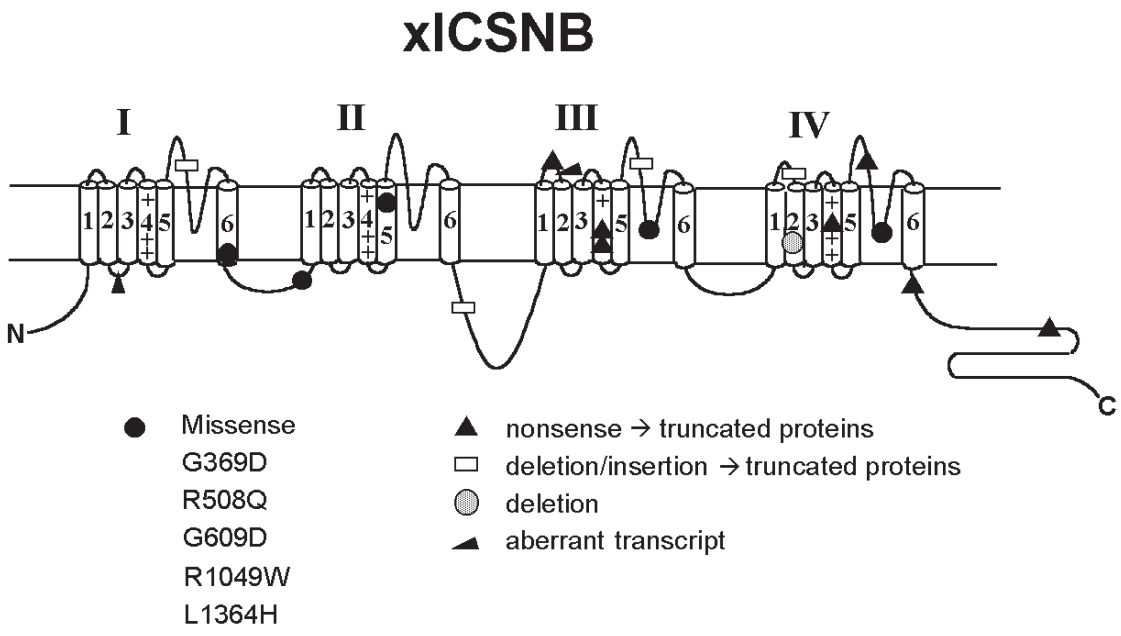

Fig. 2. Mutations of the CACNA1F gene associated with X-linked congenital stationary night blindness (xlCSNB): location in the secondary structure of the calcium channel $\alpha_1$1.4 subunit.

expression of $\alpha_1$1.4 subunits, not only in photoreceptors, but also in other neurons of the retina suggests that additional mechanisms are probably involved in the disease.

Human Ca_V2.1 Channelopathies

$\alpha_1$2.1 subunits are expressed throughout the human and murine brain in most presynaptic terminals and also in the cell body and dendrites of most central neurons *(23,24).* Ca_V2.1 channels play a prominent role in controlling neurotransmitter release at many synapses *(4).* Their localization in somatodendritic membranes points to additional postsynaptic roles, e.g., in neural excitability and integration. In both humans and mice the expression of $\alpha_1$2.1 subunits is particularly high in the cerebellum, in both Purkinje and granule cells and also in the molecular layer *(23,25,26).* Most of the Ca^{2+} current of Purkinje cells and a large fraction of the Ca^{2+} current of cerebellar granule cells is inhibited by ω-AgaIVA, the spider toxin that specifically inhibits P/Q-type Ca^{2+} channels *(15,27,28).* In the rat the same toxin inhibits most of the excitatory synaptic transmission onto Purkinje cells, at both parallel fibers and climbing fibers synapses *(29).* Moreover, it inhibits also most of the inhibitory synaptic transmission between Purkinje cells and deep cerebellar nuclei *(30).*

Migraine is a frequent and clinically heterogeneous neurological disorder, affecting up to 15% of females and 6% of males in Caucasian populations. Migraine attacks, typically lasting 1–3 d, are characterized by severe, unilateral pounding head pain associated with nausea, vomiting, and sensitivity to light and sound (migraine without aura). In about 15% of patients the attacks are preceded by transient neurologic abnormalities, such as visual, sensory, motor, or cognitive impairment (migraine with aura). Family-, twin-, and population-based studies suggest that genetic factors are involved, most likely as part of a multifactorial

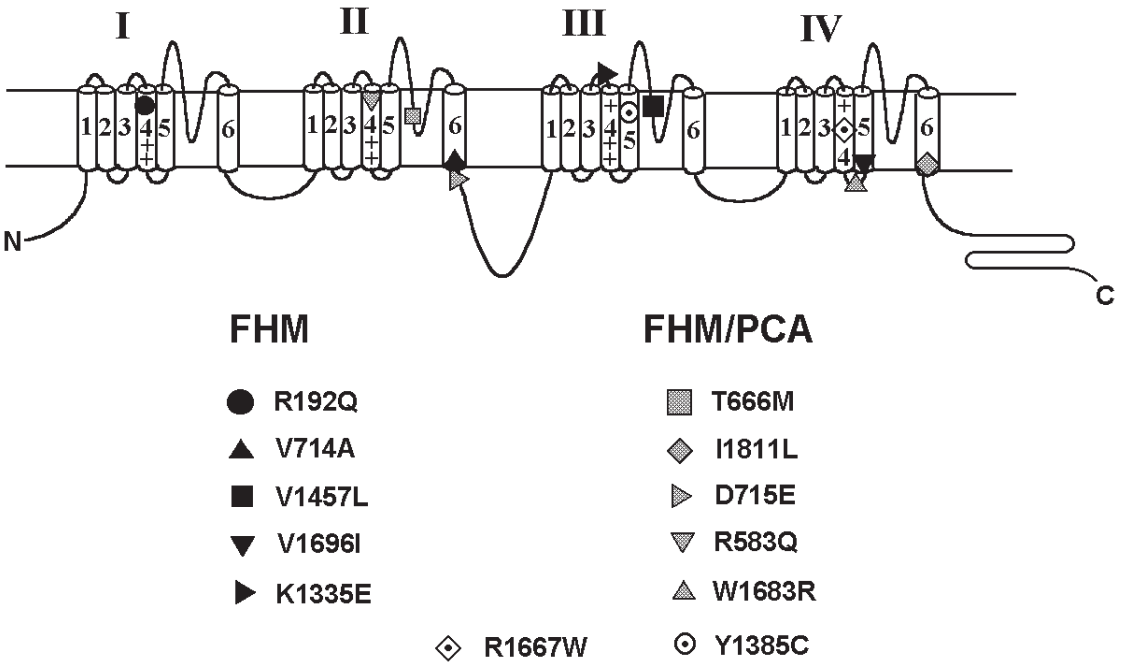

Fig. 3. Mutations of the CACNA1A gene associated with pure familial hemiplegic migraine (FHM) and FHM with progressive ataxia (FHM/PCA): location in the secondary structure of the calcium channel $\alpha_1$2.1 subunit. Mutation R1667W could be associated or not to progressive ataxia.

mechanism. FHM is a rare autosomal dominant subtype of migraine with aura of childhood onset, characterized by intermittent unilateral weakness or paralysis lasting for hours to days. Some patients show nystagmus and develop a slowly progressive ataxia with evidence of cerebellar atrophy later in life. Unilateral weakness is suggestive of brainstem, subcortical, or possibly cortical involvement, whereas ataxia points to cerebellar involvement. A gene for FHM has been assigned to chromosome 19p13 in about 50% of families tested, and identified as being CACNA1A in 1996 *(31,32).* Evidence from sibling pairs analysis points to an important involvement of the CACNA1A gene containing region in the more common types of migraine, especially in migraine with aura *(33).*

Twelve different missense mutations in the CACNA1A gene in 28 unrelated FHM families and one *de novo* FHM mutation have been reported, all resulting in substitutions of conserved aminoacids in important functional regions of the $\alpha_1$2.1 subunit *(31,34–38)* (Fig. 3). Three mutations are substitutions of a positively charged argininine with a neutral aminoacid in S4 segments, that form part of the voltage sensor; two mutations are in pore-lining segments (P-loops) in close proximity to one of the key glutamates that form the high-affinity binding site for divalent ions in the selectivity filter; three mutations are at the intracellular end of transmembrane segments S6: these segments are thought to contribute to the lining of the pore internal to the selectivity filter; two mutations are in transmembrane segments S5 and the remaining three are in linkers of transmembrane segments S3–S4 and S4–S5. As shown in Fig. 3, half of the mutations cause FHM associated with progressive cerebellar ataxia and atrophy (FHM/PCA). By far the most recurrent mutation is T666M, in the P-

loop of domain II, found in 12 families with FHM/PCA. There is a strong correlation between the T666M genotype and the FHM/PCA phenotype *(36).*

The functional consequences of the four FHM mutations described by Ophoff et al. *(30)* have been investigated in heterologous expression systems expressing either rabbit *(39)* or human *(40)* $Ca_v2.1$ channels. The mutations altered in a complex manner both the density of functional channels in the membrane and the biophysical properties of recombinant $Ca_v2.1$ channels. The three mutations located in pore regions (T666M, V714A, and I1811L) decreased the density of functional human P/Q channels in the membrane *(40).* Strikingly, mutation R192Q, located in the voltage-sensor region, had the opposite effect. Two of the mutations located in pore regions (T666M and V714A) reduced the single-channel conductance of human $Ca_v2.1$ channels. Surprisingly, a minor fraction of mutant channels had the wild-type conductance, suggesting that the abnormal channel may switch on and off, perhaps depending on some unknown factor *(40).* Two mutations located in S6 segments (V714A and I1811L) increased the single-channel open probability at all voltages and shifted the voltage-range of channel activation towards more negative voltages. The single-channel open probability was increased also by mutation R192Q. The rate of recovery from inactivation of both human and rabbit $Ca_v2.1$ channels was increased by both mutations V714A and I811L, and decreased by mutation T666M, with consequent reduced inactivation during train of pulses with the first two mutations and increased inactivation with the latter *(39,40).* The rate of recovery from inactivation of rabbit $Ca_v2.1$ channels was decreased also by mutations V1457L in the P loop of domain III and R583Q in the S4 segment of domain II *(41).* Inactivation during train of pulses was larger than wt for mutant R583Q, but was similar to wt for mutant V1457L, because the slower recovery from inactivation was counteracted by the slower inactivation produced by mutation V1457L. The rate of inactivation was increased by mutation D715E, adjacent to V714A, with consequent increased inactivation during train of pulses, despite the absence of effect on the rate of recovery from inactivation. Judging from the macroscopic current-voltage relationship, all three new FHM mutations studied by Kraus et al. *(41)* shifted the voltage dependence of activation of rabbit $Ca_v2.1$ channels towards more negative voltages. Their effect on human $Ca_v2.1$ channels and, in general, on the density of functional channels in the membrane and on the single-channel current and open probability remains unknown.

Tables 1 and 2 show how the changes in functional properties of recombinant human and rabbit $Ca_v2.1$ channels produced by the FHM mutations are predicted to affect calcium influx into neurons. The question of whether the FHM mutations lead to gain- or loss-of-function in terms of Ca^{2+} influx does not have a simple and univocal answer. Mutation T666M should lead to a reduction of Ca^{2+} influx (loss-of-function) and mutation R192Q to an increase of Ca^{2+} influx (gain-of-function), both at the single-channel level and at the level of the whole-cell calcium current. On the other hand, mutations V714A and I1811L would lead to an overall gain-of-function at the single-channel level, given the higher open probability and the faster rate of recovery from inactivation, while they may lead to an overall loss-of-function at the level of the whole-cell calcium current, given the decreased density of functional channels (particularly large for I1811L). Considering the possibility that the FHM mutations may differentially affect the expression of $Ca_v2.1$ channels in different neurons, then one can predict either an increased or a decreased Ca^{2+} influx through mutant channels (especially V714A and I1811L) depending on the type of neuron. Moreover, the FHM mutations might affect differently P/Q-type calcium influx in different neurons also as a consequence of expression of different $\alpha_1 2.1$ splice variants and/or different auxiliary subunits. For example, the predicted effect of the increased rate of recovery from inactivation

Table 1
Effect of FHM Mutations on Ca^{2+} Influx

	Functional channel density	Single channel current	Open probability	Inactivation during train of pulses	Recovery from inactivation
T666M	↓↓	↓↓ (–)	– ?	–	↓
V714A	↓↓	↓ (–)	↑↑	↑↑	↑↑
I1811L	↓↓↓	– (↓)	↑	↑↑	↑↑
R192Q	↑↑	–	↑	–	–

The table shows how the changes in functional properties of human recombinant Ca^{2+} channels produced by FHM mutations *(40)* are predicted to affect Ca^{2+} influx into neurons. An increased, a decreased or an unchanged Ca^{2+} influx with respect to wt are indicated with ↑, ↓, and –, respectively. The smaller symbols in parenthesis refer to the function of a minority of mutants.

Table 2
Effect of FHM Mutations on Ca^{2+} Influx

	$V_{1/2}$ activation (from I–V)	$V_{1/2}$ inactivation	Inactivation during train of pulses	Recovery from inactivation
T666M	↑	–	↓↓	↓
V714A	↑↑	–	↑	↑↑
I1811L	↑	–	↑↑	↑↑
R192Q	–	–	–	–
V1457L	↑↑	–	–	↓
D715E	↑↑↑	↓	↓↓	–
R583Q	↑↑	↓	↓↓	↓

The table shows how the changes in functional properties of rabbit recombinant Ca^{2+} current *(39,41)* produced by FHM mutations are predicted to affect Ca^{2+} influx into neurons. An increased, a decreased, or an unchanged Ca^{2+} influx with respect to wt are indicated with ↑, ↓, and –, respectively.

of the V714A and I1811L mutants would be enhancement of calcium influx during repetitive activity in neurons expressing inactivating $\alpha_1$2.1 variants (as shown in Tables 1 and 2) or no change in calcium influx during repetitive activity in neurons expressing noninactivating variants. For the same reasons, the FHM mutations might affect differently calcium influx in different compartments of the same neuron (e.g., dendrites vs synaptic terminals).

EA-2 is a rare dominantly inherited neurological disorder, characterized by interictal nystagmus and episodes (lasting hours to days) of ataxia, i.e., truncal instability, unsteady gait, loss of limb coordination, and sometimes vertigo or dizziness, that may be precipitated by stress or fatigue. Many patients develop progressive cerebellar ataxia and cerebellar atrophy predominating on the anterior vermis, and about 50% of the patients report migraine symptoms. Weakness and/or confusion are other symptoms often associated with episodic ataxia. In general, great intra- and interfamilial variability exists in the symptoms (both episodic and permanent) experienced by EA2 patients. A severe progressive ataxia in

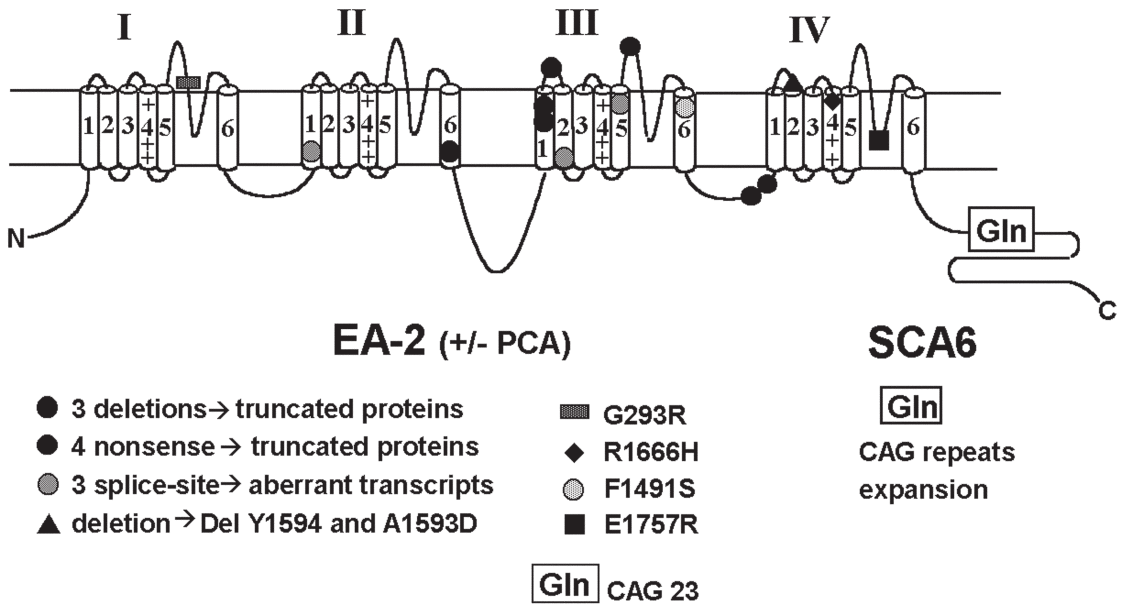

Fig. 4. Mutations of the CACNA1A gene associated with episodic ataxia type 2 (EA-2) with and without progressive ataxia (PCA) and with spinocerebellar ataxia type 6 (SCA6) location in the secondary structure of the calcium channel $\alpha_1$2.1 subunit.

the absence of paroxysmal episodes may be part of the clinical spectrum of the disease *(42,43)*. EA-2 has been mapped in the same interval as the FHM locus and mutations in the same CACNA1A gene have been found in a large fraction of familial and sporadic cases *(31,42–48)*. The majority of the 15 mutations, reported so far, disrupt the open reading frame leading to truncation, exon skipping, or intron inclusion of the gene product. However, four are missense mutations, resulting in substitutions of conserved aminoacids in important functional regions of the $\alpha_1$2.1 subunit (Fig. 4): two mutations introduce the positively charged aminoacid arginine in P loops and one of them changes one of the glutamate residues in the selectivity filter *(44,47);* one mutation substitutes an arginine with an histidine in the voltage sensor *(46)* and the fourth changes a phenylalanine with a serine in transmembrane segment S6 of domain III *(48)*. The latter is the only mutation linked to EA-2 whose functional consequences on recombinant human Ca_V2.1 channels have been investigated. The mutation, which causes EA2 with progressive ataxia and cerebellar degeneration, leads to complete loss-of-function of human Ca_V2.1 channels, without affecting channel expression *(48)*. Since it is unlikely that the truncated α1 subunits produced by other EA2 mutations form functional channels, complete loss of Ca_V2.1 channel function may be the common mechanism underlying EA2, whether due to truncating or missense mutations. It remains unknown whether a heterozygous condition for the mutation acts through a haploinsufficiency mechanism or a dominant-negative effect (e.g., produced by competition between functional and nonfunctional channels for regulatory-associated proteins).

The autosomal dominant spinocerebellar ataxias are a group of inherited neurodegenerative disorders characterized by progressive ataxia and cerebellar degeneration, caused by expansions of CAG trinucleotide repeats coding for an extended polyglutamine sequence

(35 to 135 repeats). Zhuchenko et al. *(48a)* identified small CAG expansions, ranging from 21 to 27 repeat units, in the 3′ end of CACNA1A in patients with a late-onset autosomal dominant slowly progressive ataxic syndrome that they named SCA6. Patients develop permanent balance and coordination difficulties progressively leading to impairment of gait that may cause them to become wheel-chair bound. There is evidence of marked cerebellar atrophy especially in the superior vermis, with more severe loss of Purkinje cells than granule cells, and variable mild atrophy of the brain stem. Episodic features have been reported in SCA6 patients, and a small CAG expansion was found in a family with typical EA-2 symptoms, leading to the suggestion that SCA6 and EA-2 represent a clinical continuum *(42,45).*

Alternative splicing of the CACNA1A gene results in at least six mRNA isoforms with different 3′ end, in which the CAG repeat is either part of the noncoding or part of the coding region (in 3 isoforms). In the latter isoforms, the CAG repeat encodes a polyglutamine stretch at the C-terminus. Immunohistochemistry in human brain sections showed the expression of isoforms containing a polyglutamine stretch in both control and SCA6 patients *(49).* The most notable difference with respect to control was the presence of cytoplasmic aggregations of the α_{1A} protein exclusively in the cytoplasm of SCA6 Purkinje cells, together with a mild reduction of immunoreactivity in the Purkinje and molecular layers, suggestive of reduced α_{1A} expression. There are some indirect evidences that selective neuronal degeneration in SCA6 might be associated with aggregation of α_{1A} protein. Expanded polyglutamines were found to directly alter the biophysical properties of heterologously expressed $Ca_v2.1$ channels, and to affect differently P/Q channels containing rabbit or human $\alpha_1 2.1$ subunits or different splice variants of human $\alpha_1 2.1$ *(50–52).* Particularly meaningful appear the findings that pathological expansions of 24 polyglutamines shifted the voltage dependence of steady-state inactivation of human $Ca_v2.1$ channels, but had no effect on rabbit $Ca_v2.1$ channels, and that the shift in the steady-state inactivation of channels containing different human $\alpha_1 2.1$ splice variants was in opposite directions *(51).* Thus, depending on the splice variant (–NP or +NP), either an increased or a decreased Ca^{2+} influx through mutant human $Ca_v2.1$ channels can be predicted.

Mouse $Ca_v2.1$ Channelopathies

Spontaneously arising mutations in the mouse orthologue of CACNA1A were identified in four recessive neurological mouse mutants: *tottering (cacna1a^{tg}), leaner (cacna1a$^{tg\text{-}1a}$), rolling Nagoya (cacna1a$^{tg\text{-}rol}$)* and *rocker (cacna1a^{rkr}) (25,53–55).* The *tottering*, *rolling*, and *rocker* phenotypes are caused by three different missense mutations in the cacna1a gene: a substitution of proline with leucine in the S5–S6 linker of domain II (located about 20 aminoacids from the T666M mutation causing FHM/PCA) in *tottering;* a threonine to lysine substitution in a similar position in domain III in *rocker;* an arginine to glycine substitution in the voltage sensor IIIS4 in *rolling.* The *leaner* phenotype is caused by a splice-site mutation producing a frameshift in the reading frame and two aberrant splice products with altered C-terminal sequences (Fig. 5).

Homozygous *tottering, leaner,* and *rocker* mice exhibit intermittent seizures very similar to human absence epilepsy, a generalized, nonconvulsive epileptic disorder that is most common among children and is characterized by cortical spike-wave discharges recorded by EEG concomitant with behavioral immobility. The neurological phenotype of *tottering* mice is in addition characterized by mild ataxia (wobbly gait) and episodic dyskinesia, a form of dystonia involving only specific muscles. *Rocker* mice exhibit mild ataxia similar to that of *tottering*, but without episodic dyskinesia. The *leaner* phenotype is usually described as being characterized by much more severe ataxia than *tottering*, without episodic dyskinesia; but the typical stiff posture with extended

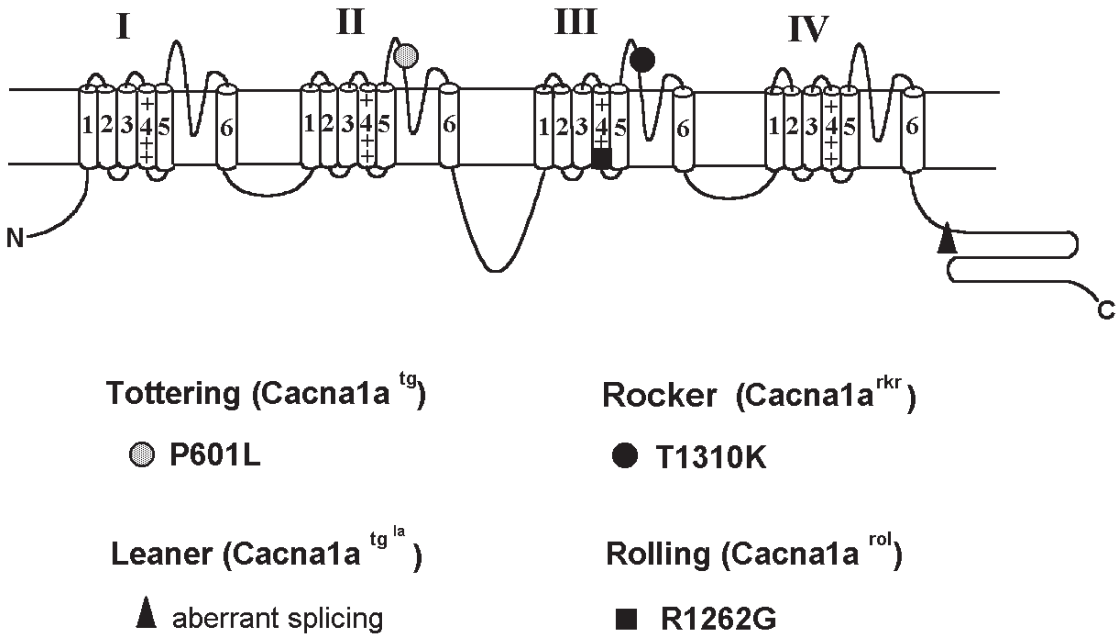

Fig. 5. Spontaneous mutations in the cacna1a gene causing the *tottering, leaner, rocker,* and *rolling Nagoya* phenotypes: location in the secondary structure of the calcium-channel $\alpha_1$2.1 subunit.

limbs and severe impairment in the ability to walk can be more appropriately described as dystonia. The ataxic phenotype of *rolling* mice is intermediate between that of *tottering* and *leaner.* However, *rolling* mice do not have seizures. Neurological symptoms appear in the second week after birth in *leaner* and *rolling* and later, at 3–4 wk after birth, in *tottering* and *rocker.* In the *leaner* cerebellum there is extensive degeneration of granule, Golgi and Purkinje cells. Granule cell loss begins in the second week after birth, and progresses slowly over a period of months, showing an anterior-posterior gradient, with more severe loss in the anterior lobe. Purkinje cell loss begins later at about 4 wk after birth, and occurs in parasagittal stripes separated by areas of normal cells. Interestingly the pattern of surviving Purkinje cells is essentially coextensive with the striped pattern of zebrin staining and tyrosine hydroxilase expression. In *leaner, rolling,* and *tottering* mice the normally transient expression of tyrosine hydroxilase is not suppressed, resulting in expression in the adult. However, in contrast with *leaner,* no Purkinje cell loss was found in *tottering* and *rolling;* cerebellar atrophy has been reported only in some old *tottering* mice and there are conflicting reports on granule cell loss in *rolling.* In *rocker* mice tyrosine hydroxilase is not expressed in the adult, and there is no evidence of cell loss in the cerebellum of old animals. At the ultrastructural level *leaner, rolling,* and *tottering* mice show an altered cerebellar phenotype consisting in enlarged parallel fibers varicosities synapsing on several Purkinje cell dendritic spines, in addition to the common monosynaptic contacts *(56,57).* Ectopic spines and axonal swellings are present in the Purkinje cells of the three allelic mouse mutants. They are absent in *rocker,* whose Purkinje cells, on the other hand, show a characteristic reduction of branching of the dendritic arbor and a "weeping willow" appearance of the secondary branches in aged animals *(55).* A characteristic feature of *tottering* and *rolling* brain is the aberrant synaptoge-

nesis of locus ceruleus neurons, with consequent hyperinnervation by noradrenergic terminals of all major locus ceruleus targets, including the cerebellum and the hippocampus *(53).*

Immnunocytochemistry and in situ hybridization in *leaner* cerebellar slices showed no difference in $\alpha_1$2.1 protein and mRNA level with respect to wild-type *(58).* However, Northern-blot analysis revealed selective reduced expression of one of the two major $\alpha_1$2.1 transcripts already at 9 d after birth (P9) *(59).* No differences in $\alpha_1$2.1 mRNA levels were detected in adult *tottering* cerebellum, but an interesting upregulation of $\alpha_1$1.2 mRNA level in Purkinje cells was observed *(59,60).* The functional consequences of the *tottering, leaner,* and *rolling* mutations have been investigated in both heterologous expression systems *(54,61)* and native Purkinje cells *(54,61–63),* whereas the functional consequences of the *rocker* mutation have not been reported. In freshly dissociated Purkinje cells of *leaner, rolling,* and *tottering* mice (10–35 d in age), the mutations caused a reduction of peak P/Q-type calcium current density of 70–80, 25–30, and 15–50%, respectively, with no change in the non-P/Q-type current. A 50–70% decrease in current density was also measured after transient expression of rabbit $\alpha_1$2.1 subunits containing the *tottering* or *rolling* mutations, or one of the two possible abnormal *leaner* C-termini (whereas the same current density was found for the other abnormal splicing product). Neither in the Purkinje cells nor in the heterologous expression system was there evidence of changes in the kinetics or voltage-dependent properties of mutant *tottering* Ca_v2.1 channels. A small shift of the voltage range of both activation and inactivation towards more positive voltages has been reported for *leaner* by some authors *(61)* but not by others *(62,63).* A similar shift of activation (but not inactivation) was produced by the *rolling* mutation *(54).* Neither the *tottering* nor the *leaner* mutation apparently changed the single-channel conductance.

Neurotransmitter release appears to be differentially affected by the *tottering* mutation depending on the synapse. A strong reduction of the contribution of P/Q-type channels to calcium influx into presynaptic terminals of CA3 pyramidal cells was measured in hippocampal slices of adult *tottering (64).* The reduced presynaptic P/Q-type calcium influx was at least partially compensated by an increased influx through presynaptic N-type calcium channels, with a consequent much lower reduction of synaptic transmission than expected. Evoked neurotransmitter release at the neuromuscular junctions of *tottering* and wt mice was similar *(65).* Quite interestingly, a reduced excitatory but not inhibitory synaptic transmission has been measured at synapses on neurons of the ventrobasal nucleus in the *tottering* thalamus *(66).* A decrease of both glutamate and GABA release was measured in neocortex by in vivo microdyalisis in *tottering (67).* In contrast, the same method revealed a decrease of glutamate but not GABA release in *leaner.*

Recently, a cacna1a null mouse ($\alpha_1$2.1$^{-/-}$) was generated in two laboratories *(68,69).* Interestingly, it exhibits a neurological phenotype very similar to that of *leaner.* Moreover, histological examination at 15 wk of age revealed selective degeneration of the cerebellum in a specific pattern, with loss of Purkinje cells in parasagittal stripes and graded loss of granule cells more severe in the anterior lobe, like in *leaner (69).* A selective histopathological involvement of the cerebellum might be predicted from the known high level of α_{1A} expression in the cerebellum. However, the highly specific pattern of neurodegeneration is surprising; it implies the existence of two types of cerebellar neurons: one with absolute dependence of Ca_v2.1 channel function for survival, and another that can tolerate lack of Ca_v2.1 channels. In $\alpha_1$2.1$^{-/-}$ Purkinje cells the ablation of the P/Q-type current was partially compensated by an increase of the L- and N-type calcium currents (unlike *leaner*) *(68).* A partially compensating increase of L- and N-type calcium current, with no change of the

R-type component, can also be observed in $\alpha_1 2.1^{-/-}$ cerebellar granule cells *(69)*, but cf *(68)*. Interestingly, heterozygous $\alpha_1 2.1^{-/+}$ mice have no phenotype in terms of motor performance, cerebellar neuroanatomy or EEG, despite having a 50% reduction of P/Q-type calcium current *(69)*. No compensatory increase of other Ca^{2+} channel types was measured in cerebellar granule cells of heterozygous $\alpha_1 2.1^{-/+}$ mice, and the reduction of total calcium current was similar in cacna1a null homozygous and heterozygous mice.

Lethargic mice have a clinical phenotype almost identical to that of *tottering*, except that they exhibit earlier (onset at P15) and more pronounced ataxia, and also suffer from reduced body weight and transient defects in the immune system *(53)*. The mutation causing the *lethargic* phenotype is in the Cacnb4 gene encoding the auxiliary calcium-channel subunit β_4, and is equivalent to a null mutation *(70)*. The level of expression of different β subunits vary considerably in different regions of mouse brain and in different cells within the same region. In both humans and mice the expression of β4 subunits, like that of $\alpha_1 2.1$ subunits, is particularly high in the cerebellum in both Purkinje and granule cells *(26,71)*. In *lethargic* mutants there is evidence for an increased level of expression of β_{1b} subunits in both forebrain and cerebellum, which partially compensates for the lack of β_4 subunit *(72)*. Moreover, coimmunoprecipitation experiments show an increase in the fraction of $\alpha_1 2.1$ and $\alpha_1 2.2$ subunits associated with β_{1b} and β_3 in the brain, and suggest that there are no channels lacking an auxiliary β subunit in the mutant *(26)*. Thus, the β_4 subunit can be replaced by the remaining coexpressed β subunits in the lethargic brain, a process termed "subunit reshuffling." There are conflicting data on the effect of the *lethargic* mutation on the level of expression of α_1 subunits (no change in either $\alpha_1 2.1$ or $\alpha_1 2.2$ subunits in *[25]*, but decreased expression of $\alpha_1 2.2$ in *[70]*). The kinetics and voltage-dependent properties of the P/Q-type calcium current in wild-type and *lethargic* Purkinje cells were very similar, and although quantitative values were not provided, there was no evidence for significantly different current densities *(26)*. Synaptic transmission and the pharmacological profile of presynaptic calcium influx at CA3-CA1 synapses in wild-type and *lethargic* hippocampal slices were also very similar *(64)*. In contrast, as in *tottering*, excitatory but not inhibitory synaptic transmission was reduced at synapses on the ventrobasal nucleus in the *lethargic* thalamus *(66)*.

A mutation in a gene encoding a brain specific protein called stargazin, that shares a modest partial sequence similarity and predicted secondary structure with the auxiliary γ subunit of the skeletal muscle calcium channel, is the genetic defect in *stargazer* and allelic *waggler* mice. These mouse mutants exhibit recurrent seizures characteristic of absence epilepsy, distinctive head-tossing, and severe ataxia beginning at P15 *(53,73)*. Complex alterations are present in the *stargazer* brain, including remarkable outgrowth of dentate granule axons in the hippocampus. The adult cerebellum is morphologically normal, but there is evidence for delayed granule-cell migration and maturation, most likely as a consequence of striking downregulation of BDNF expression in cerebellar granule cells. Cerebellar granule cells of adult *stargazer* and *waggler* lack functional AMPA receptors, which implies a virtual absence of massive afferent information from mossy fibers, since at the mossy fiber-granule cell synapse usual excitatory synaptic transmission at low frequency is mediated mainly by AMPA receptors *(74,75)*. Moreover, synaptic transmission was reduced also at the parallel-fiber-Purkinje cell synapse, most likely as a consequence of lower transmitter release. Recently, it has been shown that stargazin is critical for bringing AMPA receptors to the cell membrane and for targeting them specifically to postsynaptic sites, and it has been hypothesized that in *stargazer* the defect is restricted to cerebellar granule cells because in other types of neurons the loss of stargazin is compensated by other members of the stargazin/γ-subunit family *(76)*.

The only reported evidence linking stargazin to calcium-channel function is a small shift towards more negative voltages of the steady state inactivation curve of recombinant $Ca_v2.1$ channels (and small shifts towards more positive voltages of both activation and inactivation of $Ca_v1.2$ channels) upon coexpression with stargazin *(7,73).* The mutation in *stargazer* leads to premature termination of the transcript and therefore most likely to a nonfunctional protein. Assuming stargazin is a neuronal γ subunit affecting steady-state inactivation of calcium channels, then one would predict enhanced P/Q calcium influx in *stargazer* neuronal cells as a consequence of increased $Ca_v2.1$ channel availability in the absence of the γ subunit. However, the predicted increased calcium entry cannot account for the functional deficits measured in *stargazer* cerebellar synapses, showing a decreased (not increased) excitatory transmission. As a matter of fact, no difference in steady-state inactivation (nor activation or peak calcium current amplitude) was found in cerebellar granule cells of *stargazer* and wt mice *(76)*(even though, in our experience, expression of $Ca_v2.1$ channels in cerebellar granule cells is very low at 2 d in culture, when the recordings were performed: Tottene and Pietrobon, unpublished data). Thus, the connection between stargazin and neuronal calcium channels remains unclear, and there is still considerable uncertainty as to whether stargazin and its human ortholog γ2 subunit (and other γ isoforms) really form part of neuronal voltage-dependent calcium channels.

How Do Calcium-Channel Mutations Produce Disease?

Researchers are beginning to answer the question of how specific calcium-channel mutations alter channel function. The more challenging question of how the alterations in channel function lead to selective cellular dysfunction and to the episodic as well as persistent neurological symptoms of calcium channelopathies remains unanswered. To answer this question, parallel studies at the molecular, cellular, neuronal network, and behavioral levels in mouse genetic models will be extremely important. Since Ca^{2+} ions regulate numerous intracellular signaling pathways, including those regulating gene expression, the activity of calcium channels is inextricably linked to a broad array of functions in the developing and mature nervous system, including cell proliferation, differentiation, survival, neurite outgrowth, synaptogenesis, neuronal excitability, transmitter release, and plasticity. Although the primary functional defect may be abnormal Ca^{2+} entry into neurones, any of these secondary processes could contribute substantially to the disease phenotype. It is tempting to speculate that abnormal neuronal excitability due to dysfunctional calcium channels may trigger the episodic neurologic symptoms (e.g., attacks of migraine, ataxia in humans, absence epilepsy in mouse mutants), whereas chronic abnormal calcium homeostasis may lead to progressive neuronal degeneration and to progressive fixed dysfunction of the involved tissue *(42).*

The human $Ca_v2.1$ channelopathies (FHM, EA-2, and SCA6) show a number of overlapping symptoms, including progressive ataxia and cerebellar degeneration. The available data from channel genotype-phenotype studies suggest that mutations in $Ca_v2.1$ channels cause progressive ataxia and cerebellar atrophy through a loss of function mechanism. An interictal cerebellar syndrome of variable severity, ranging from nystagmus to severe progressive ataxia and cerebellar atrophy predominating in the anterior vermis, is typical of EA-2. The only missense mutation linked to EA-2 (with interictal ataxia and cerebellar vermis atrophy), investigated so far, leads to complete loss of P/Q channel activity. Most likely, nonfunctional proteins are produced also by nonsense truncating EA2 mutations. Expanded polyglutamines reduce the availability of human $Ca_v2.1$ channels containing the $\alpha_1 2.1$ (-NP) splice variant, the isoform largely predominant in Purkinje cells. It has been pro-

posed that reduction of Ca^{2+} influx, due to reduced availability of P-type channels, may contribute to the severe Purkinje cell loss typical of SCA6, whereas compensation, due to the additional presence of the +NP splice variant in granule cells, might explain the milder loss of these neurons (cf shift in opposite directions of steady-state inactivation for the two isoforms). Progressive ataxia and cerebellar atrophy are present only in a fraction of FHM families linked to CACNA1A and in none of the unlinked families, and are strongly correlated with the T666M genotype. Mutation T666M is the only FHM mutation, of the four analyzed, that leads to a predicted decrease of P/Q-type Ca^{2+} influx into cerebellar neurons at both the single channel (cf. lower single channel conductance and slower recovery from inactivation) and whole-cell level (cf. lower density of functional channels).

The hypothesis that mutations in $Ca_v2.1$ channels cause progressive ataxia and cerebellar atrophy through a loss of function mechanism is also supported by the severe progressive ataxia (and/or dystonia) and by the cerebellar degeneration exhibited by $\alpha_1$2.1-deficient and *leaner* mice (with either absent or strongly reduced P/Q-type calcium current). Interestingly, in humans with mutations in CACNA1A cerebellar degeneration occurs predominantly in the anterior vermis *(43,77,78)*, like in $\alpha_1$2.1-deficient and *leaner* mice. The available data on mouse mutants are consistent with the hypothesis that survival of cerebellar neurons depends specifically on a critical level of P/Q channel function, that cannot be compensated for by other Ca^{2+} channel types. In fact, partial (<50%) reduction of P/Q current density in *tottering, rolling* and heterozygous knockout ($\alpha_1 2.1^{+/-}$) mice does not cause cell death, but more severe reduction, seen in *leaner* and homozygous null ($\alpha_1 2.1^{-/-}$), causes degeneration. Since the overall calcium current density is similar in heterozygous and homozygous null (due to compensation), it would appear that the degeneration is specifically related to a critical threshold of P/Q channel function. It remains unknown why, when reduction of P/Q channel function exceeds this threshold, neuronal death in the cerebellum is not homogeneous and occurs in a highly specific pattern. Moreover, the human mutations are inherited as autosomal dominants, causing cerebellar degeneration in the heterozygous state. Assuming that they are loss-of-function mutations, haploinsufficiency could be the underlying pathological mechanism, but this would be dissimilar from the recessive mouse mutations. Another possibility is that, as a consequence of the human mutations, other functions are disrupted, and a dominant negative effect could be the underlying mechanism. For example, cytoplasmic aggregations of the $\alpha_1$2.1 protein might contribute to death of Purkinje cells in SCA6, and nonfunctional mutant proteins might interfere with the wild-type channels, e.g., by competing for regulatory proteins, in EA-2.

In mice with mutations in calcium-channel subunits, ataxia is not correlated with Purkinje cell loss. Indeed, with the exception of *leaner*, all mouse mutants are ataxic but do not show Purkinje cell loss, and in both *leaner* and cacna1a null mice ataxia starts before Purkinje cell loss. Moreover, ataxia does not appear to correlate in a simple manner with the reduction of P/Q-type calcium current in Purkinje cells, since a 50% reduction of P/Q current in heterozygous $\alpha_1 2.1^{-/+}$ mice does not cause ataxia, whereas a similar or lower reduction in *tottering* and *lethargic* (with no alterations in biophysical properties of the current) is associated with ataxia. The ataxia phenotype is probably linked in a complex manner to dysfunction of cerebellar neuronal circuits that involve P/Q-type calcium channels at multiple synapses. As shown in *tottering* and *lethargic*, the same mutation in P/Q channels may affect differently neurotransmitter release at different synapses. Many mechanisms could lead to differential effect at different synapses, including different intrinsic contributions of P/Q-type channels in controlling release, different mutation-induced changes in expression of P/Q-type channels, and different compensatory changes of other calcium channel α_1 and/or

auxiliary subunits. Secondary changes in non-P/Q Ca^{2+} channels can contribute to the pathology, as shown by the role played by L-type channels in the generation of episodic dyskinesia in *tottering (60).*

The clinical distinction between the different human and mouse $Ca_v2.1$ channelopathies is based mainly on the different episodic neurological symptoms, ranging from migraine in FHM to episodic ataxia in EA-2 to absence epilepsy in the mouse mutants. It remains unclear how different mutations in the same gene can lead to such varied clinical phenotypes and, on the other hand, how mutations that are predicted to affect calcium influx in the opposite direction all lead to the same migraine phenotype. Also unclear is how in general a permanent mutation leads to episodic disorders.

Given the delicate balance between excitatory and inhibitory signals that regulates neuronal excitability, even relatively small changes in channel activity may tip this delicate balance. Thus, channelopathies may be considered as defects of cellular excitability *(79).* It has been suggested that the differential effect of the *tottering* and *lethargic* mutations on excitatory and inhibitory transmission in the thalamus may tip the balance between excitation and inhibition towards inhibition of thalamic relay neurons, that can lead to syncronization of these neurons into a burst-firing mode and generation of spike wave discharges typical of absence epilepsy *(66).* Although an established model that explains migraine attacks is still lacking, a favored hypothesis considers a persistent state of hyperexcitability of neurons in the cerebral cortex as the basis for susceptibility to migraine *(80).* This state would favor the onset of cortical spreading depression (CSD), which is believed to initiate the attacks of migraine with aura *(81).* Recently, it has been shown that P/Q channels contribute to mechanisms involved in initiation and propagation of CSD in neocortex, and that the *tottering* and *leaner* mutations raise the threshold for initiating CSD *(67).* It remains possible that FHM mutations produce cortical network excitability effects opposite to those found in *tottering* and *leaner,* and one can speculate about different mechanisms with which neuronal excitability can be increased and/or the development of CSD can be facilitated by either loss- or gain-of-function variants of presynaptic and/or postsynaptic human P/Q-type calcium channels. However, to explain the similar FHM phenotype produced by mutations that are either gain- or loss-of function in heterologous expression systems, the assumption of differential changes in calcium influx produced by a given mutation in different neurons, and/or differential up- and downregulation of inhibitory and excitatory neuronal elements appears necessary.

The episodic nature of the neurological symptoms might perhaps be explained by the mutant channels providing a continous background of neuronal instability and by the intervention of internal modulatory factors and/or external environmental factors that push the system past some boundary and precipitate an attack. The observation that anxiety or stress triggers almost instantaneously attacks of ataxia in many EA-2 patients suggests potentially important modulatory action of noradrenergic or serotonergic inputs to the cerebellum. Interestingly, lesions of central noradrenergic axons early in development obliterate seizure expression in *tottering* mutants *(53).* The finding that the functional effect of the FHM mutations on single-channel conductance was not present in some mutant channels or periods of activity, suggests the interesting possibility that some unknown factor can precipitate an attack by directly switching the abnormal channel on or off *(40).*

In human calcium channelopathies there is remarkable phenotypic variability in terms of frequency of attacks, features and severity of symptoms among affected individuals in all reported pedigrees, suggesting that environmental, metabolic, and other genetic factors contribute to the phenotypic expression of the mutations. Specific alleles or external factors that influence the expression of proteins

involved in signaling, including phosphatases, kinases, G proteins, neurotransmitter receptors, and ligand-gated or other ion-channel types may be expected to play significant modifying roles in the phenotypic expression of calcium-channel defects.

References

1. Sather W. A., Yang J., and Tsien R. W. (1994) Structural basis of ion channel permeation and selectivity. *Curr. Opin. Neurobiol.* **4,** 313–323.
2. Armstrong C. M. and Hille B. (1998) Voltage-gated ion channels and electrical excitability. *Neuron* **20,** 371–380.
3. Ertel A. E., Campbell K. P., Harpold M. M., Hofmann F., Mori Y., Perez-Reyes E., et al. (2000) Nomenclature of voltage-gated calcium channels. *Neuron* **25,** 533–535.
4. Dunlap K., Luebke J. I., and Turner T. J. (1995) Exocytotic Ca^{2+} channels in mammalian central neurons. *Trends Neurosci.* **18,** 89–98.
5. Randall A. and Benham C. D. (1999) Recent advances in the molecular understanding of voltage-gated Ca^{2+} channels. *Mol. Cell. Neurosci.* **14,** 255–272.
6. Burgess D. L., Gefrides L. A., Foreman P. J., and Noebels J. L. (2001) A cluster of three novel Ca^{2+} channel gamma subunit genes on chromosome 19q13.4: evolution and expression profile of the gamma subunit gene family. *Genomics* **71,** 339–350.
7. Klugbauer N., Dai S., Specht V., Lacinova L., Marais E., Bohn G., and Hofmann F. (2000) A family of γ-like calcium channel subunits. *FEBS Lett.* **470,** 189–197.
8. Walker D. and De Waard M. (1998) Subunit interaction sites in voltage-dependent Ca^{2+} channels: role in channel function. *Trends Neurosci.* **21,** 148–154.
9. Bichet D., Cornet V., Geib S., Carlier E., Volsen S., Hoshi T., Mori Y., and De Waard M. (2000) The I–II loop of the Ca^{2+} channels α_1 subunit contains an endoplasmic reticulum retention signal antagonized by the β subunit. *Neuron* **25,** 177–190.
10. Hobom M., Dai S., Marais E., Lacinova L., Hofmann F., and Klugbauer N. (2000) Neuronal distribution and functional characterization of the calcium channel $\alpha_2\delta$-2 subunit. *Eur. J. Neurosci.* **12,** 1217–1226.
11. Lin Z., Haus S., Edgerton J., and Lipscombe D. (1997) Identification of functionally distinct isoforms of the N-type Ca^{2+} channel in rat sympathetic ganglia and brain. *Neuron* **18,** 153–166.
12. Bourinet E., Soong T. W., Sutton K., Slaymaker S., Mathews E., Monteil A., et al. (1999) Splicing of α_{1A} subunit gene generates phenotypic variants of P- and Q-type calcium channels. *Nature Neuroscience* **2,** 407–415.
13. Tottene A., Volsen S., and Pietrobon D. (2000) α_{1E} subunits form the pore of three cerebellar R-type calcium channels with different pharmacological and permeation properties. *J. Neurosci.* **1,** 171–178.
14. Forti L. and Pietrobon D. (1993) Functional diversity of L-type calcium channels in rat cerebellar neurons. *Neuron* **10,** 437–450.
15. Tottene A., Moretti A., and Pietrobon D. (1996) Functional diversity of P-type and R-type calcium channels in rat cerebellar neurons. *J. Neurosci.* **16,** 6353–6363.
16. Huguenard J. R. and Prince D. A. (1992) A novel T-type current underlies prolonged Ca^{2+}-dependent burst firing in GABAergic neurons of rat thalamic reticular nucleus. *J. Neurosci.* **12,** 3804–3817.
17. Kasai H. and Neher E. (1992) Dihydropyridine-sensitive and ω-conotoxin-sensitive calcium channels in a mammalian neuroblastoma-glioma cell line. *J. Physiol.* **448,** 161–188.
18. Cahill A. L., Hurley J. H., and Fox A. P. (2000) Coexpression of cloned α_{1B}, β_{2A}, and α_2/δ subunits produces non-inactivating calcium currents similar to those found in bovine chromaffin cells. *J. Neurosci.* **20,** 1685–1693.
19. Catterall W. A. (2000) Structure and regulation of voltage-gated Ca^{2+} channels *Annu. Rev. Cell Dev. Biol.* **16,** 521–555.
20. Torben Bech-Hansen N., Naylor M., Maybaum T. A., Pearce W. G., Koop B., Fishman G. A., et al. (1998) Loss-of-function mutations in a calcium-channel α_1-subunit gene in Xp11.23 cause X-linked congenital stationary night blindness. *Nature Genet.* **19,** 264–267.
21. Strom T. M., Nyakatura G., Apfelstedt-Sylla E., Hellebrand H., Lorenz B., Weber B. H. F., et al. (1998) An L-type calcium-channel gene mutated in incomplete X-linked congenital stationary night blindness. *Nature Genet.* **19,** 260–263.
22. Nakamura M., Ito S., Terasaki H., and Miyake Y. (2001) Novel CACNA1F mutations in japanese patients with incomplete congenital station-

ary night blindness. *Invest. Ophthalmol. Vis. Sci.* **42,** 1610–6.
23. Volsen S. G., Day N. C., McCormack A. L., Smith W., Craig P. J., Beattie R., et al. (1995) The expression of neuronal voltage-dependent calcium channels in human cerebellum. *Mol. Brain Res.* **34,** 271–282.
24. Westenbroek R. E., Sakurai T., Elliott E. M., Hell J. W., Starr T. V. B., Snutch T. P., and Catterall W. A. (1995) Immunochemical identification and subcellular distribution of the α_{1A} subunits of brain calcium channels. *J. Neurosci.* **15,** 6403–6418.
25. Fletcher C. F., Lutz C. M., O'Sullivan T. N., Shaughnessy J. D. J., Hawkes R. H., Frankel W. N., et al. (1996) Absence epilepsy in Tottering mutant mice is associated with calcium channel defects. *Cell* **87,** 607–617.
26. Burgess D. L., Biddlecome G. H., McDonough S. I., Diaz M. E., Zilinksi C. A., Bean B. P., et al. (1999) b reshuffling modifies N- and P/Q-type Ca^{2+} channel subunit compositions in lethargic mouse brain. *Mol. Cell. Neurosci.* **13,** 293–311.
27. Mintz I. M., Venema V. J., Swiderek K. M., Lee T. D., Bean B. P., and Adams M. E. (1992) P-type calcium channels blocked by the spider toxin ω-Aga-IVA. *Nature* **355,** 827–829.
28. Randall A. and Tsien R. W. (1995) Pharmacological dissection of multiple types of Ca^{2+} channel currents in rat cerebellar granule neurons. *J. Neurosci.* **15,** 2995–3012.
29. Mintz I. M., Sabatini B. L., and Regehr W. G. (1995) Calcium control of transmitter release at a cerebellar synapse. *Neuron* **15,** 675–688.
30. Iwasaki S., Momiyama A., Uchitel O. D., and Takahashi T. (2000) Developmental changes in calcium channel types mediating central synaptic transmission. *J. Neurosci.* **20,** 59–65.
31. Ophoff R. A., Terwindt G. M., Vergouwe M. N., van Eijk R., Oefner P. J., Hoffman S. M. G., et al. (1996) Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca^{2+} channel gene CACNL1A4. *Cell* **87,** 543–552.
32. Ophoff R. A., Terwindt G. M., Frants R. R., and Ferrari M. D. (1998) P/Q-type Ca^{2+} channel defects in migraine, ataxia and epilepsy. *Trends Pharmacol. Sci.* **19,** 121–127.
33. Terwindt G. M., Ophoff R. A., van Eijk R., Vergouwe M. N., Haan J., Frants R. R., et al. (2001) Involvement of the CACNA1A gene containing region on 19p13 in migraine with and without aura. *Neurology* **56,** 1028–32.
34. Battistini S., Stenirri S., Piatti M., Gelfi C., Righetti P. G., Rocchi R., et al. (1999) A new CACNA1A gene mutation in acetazolamide-responsive familial hemiplegic migraine and ataxia. *Neurology* **53,** 38–43.
35. Carrera P., Piatti M., Stenirri S., Grimaldi L. M. E., Marchioni E., Curcio M., et al. (1999) Genetic heterogeneity in Italian families with familial hemiplegic migraine. *Neurology* **53,** 26–32.
36. Ducros A., Denier C., Joutel A., Vahedi K., Michel A., Darcel F., et al. (1999) Recurrence of the T666M calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia. *Am. J. Hum. Genet.* **64,** 89–98.
37. Vahedi K., Denier C., Ducros A., Bousson V., Levy C., Chabriat H., et al. (2000) CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy. *Neurology* **55,** 1040–1042.
38. Ducros A., Denier C., Joutel A., Vahedi K., Bousser M.-G., and Tournier-Lasserve E. (1999) Characterization of CACNA1A mutations in familial and sporadic migraine. *Neurology* **52,** A273.
39. Kraus R. L., Sinnegger M. J., Glossmann H., Hering S., and Striessnig J. (1998) Familial hemiplegic migraine mutations change α_{1A} Ca^{2+} channel kinetics. *J. Biol. Chem.* **273,** 5586–5590.
40. Hans M., Luvisetto S., Williams M. E., Spagnolo M., Urrutia A., Tottene A., et al. (1999) Functional consequences of mutations in the human α_{1A} calcium channel subunit linked to familial hemiplegic migraine. *J. Neurosci.* **19,** 1610–1619.
41. Kraus R. L., Sinnegger M., Koschak A., Glossmann H., Stenirri S., Carrera P., and Striessnig J. (2000) Three new familial hemiplegic migraine mutants affect P/Q-type Ca^{2+} channel kinetics. *J. Biol. Chem.* **275,** 9239–9243.
42. Jen J. (1999) Calcium channelopathies in the central nervous system. *Curr. Opin. Neurobiol.* **9,** 274–280.
43. Denier C., Ducros A., Vahedi K., Joutel A., Thierry P., Ritz A., et al. (1999) High prevalence of CACNA1A truncations and broader clinical spectrum in episodic ataxia type 2. *Neurology* **52,** 1816–1821.
44. Yue Q., Jen J. C., Nelson S. F., and Baloh R. W. (1997) Progressive ataxia due to a missense mutation in a calcium-channel gene. *Am. J. Hum. Genet.* **61,** 1078–1087.

45. Jodice C., Mantuano E., Veneziano L., Trettel F., Sabbadini G., Calandriello L., et al. (1997) Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. *Hum. Molec. Genet.* **11,** 1973–1978.
46. Friend K. L., Crimmins D., Phan T. G., Sue C. M., Colley A., Fung V. S. C., et al. (1999) Detection of a novel missense mutation and second recurrent mutation in the CACNA1A gene in individuals with EA-2 and FHM. *Hum. Genet.* **105,** 261–265.
47. Denier C., Ducros A., Durr A., Eymard B., Chassande B., and Tournier-Lasserve E. (2001) Missense CACNA1A mutation causing episodic ataxia type 2. *Arch. Neurol.* **58,** 292–295.
48. Guida S., Trettel F., Pagnutti S., Mantuano E., Tottene A., Veneziano L., et al. (2001) Complete loss of P/Q calcium channel activity caused by a CACNA1A missense mutation carried by patients with episodic ataxia type 2. *Am. J. Hum. Genet.* **68,** 759–764.

48a. Zhuchenko O., Bailey J., Bonnen P., Ashizawa T., Stockton D. W., Amos C., Dobyns W. B., Subramony S. H., Zoghbi H. Y., and Lee, C. (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α_{1A}-voltage-dependent calcium channel. *Nature Genet.* **15,** 62–69.

49. Ishikawa K., Fujigasaki H., Saegusa H., Ohwada K., Fujita T., Iwamoto H., Komatsuzaki Y., et al. (1999) Abundant expression and cytoplasmic aggregations of a1A voltage-dependent calcium channel protein associated with neurodegeneration in spinocerebellar ataxia type 6. *Hum. Mol. Genet.* **8,** 1185–1193.
50. Matsuyama Z., Wakamori M., Mori Y., Kawakami H., Nakamura S., and Imoto K. (1999) Direct alteration of the P/Q-type Ca^{2+} channel property by polyglutamine expansion in spinocerebellar ataxia 6. *J. Neurosci.* **19,** RC14 (1–5).
51. Toru S., Murakoshi T., Ishikawa K., Saegusa H., Fujigasaki H., Uchihara T., et al. (2000) Spinocerebellar ataxia type 6 mutation alters P-type calcium channel function. *J. Biol. Chem.* **275,** 10893–10898.
52. Restituito S., Thompson R. M., Eliet J., Raike R. S., Riedl M., Charnet P., and Gomez C. M. (2000) The polyglutamine expansion in spinocerebellar ataxia type 6 causes a β subunit-specific enhanced activation of P/Q-type calcium channels in *Xenopus* oocytes. *J. Neurosci.* **20,** 6394–6403.
53. Burgess D. L. and Noebels J. L. (1999) Single gene defects in mice: the role of voltage-dependent calcium channels in absence models. *Epilepsy Res.* **36,** 111–122.
54. Mori Y., Wakamori M., Oda S., Fletcher C. F., Sekiguchi N., Mori E., et al. (2000) Reduced voltage sensitivity of activation of P/Q-type Ca^{2+} channels is associated with the ataxic mouse mutation *Rolling Nagoya* (tg^{rol}). *J. Neurosci.* **20,** 5654–5662.
55. Zwingman T. A., Neumann P. E., Noebels J. L., and Herrup K. (2001) Rocker is a new variant of the voltage-dependent calcium channel gene CACNA1A. *J. Neurosci.* **21,** 1169–1178.
56. Rhyu I. J., Abbott L. C., Walker D. B., and Sotelo C. (1999) An ultrastructural study of granule cell/Purkinje cell synapses in tottering (tg/tg), leaner (tg^{1a}/tg^{1a}) and compound heterozygous tottering/leaner (tg/tg^{1a}) mice. *Neuroscience* **90,** 717–728.
57. Rhyu I. J., Oda S., Uhm C.-S., Kim H., Suh Y.-S., and Abbott L. C. (1999) Morphological investigation of rolling mouse Nagoya (tg^{rol}/tg^{rol}) cerebellar Purkinje cells: an ataxic mutant revisited. *Neurosci. Lett.* **266,** 49–52.
58. Lau F. C., Abbott L. C., Rhyu I. J., Kim D. S., and Chin H. (1998) Expression of calcium channel α_{1A} mRNA and protein in the leaner mouse (tg^{1a}/tg^{1a}) cerebellum. *Mol. Brain Res.* **59,** 93–99.
59. Doyle J., Ren X., Lennon G., and Stubbs L. (1997) Mutations in the CACNL1A4 calcium channel gene are associated with seizures, cerebellar degeneration, and ataxia in tottering and leaner mutant mice. *Mammalian Genome* **8,** 113–120.
60. Campbell D. B. and Hess E. J. (1999) L-type calcium channels contribute to the tottering mouse dystonic episodes. *Mol. Pharmacol.* **55,** 23–31.
61. Wakamori M., Yamazaki K., Matsunodaira H., Teramoto T., Tanaka I., Niidome T., et al. (1998) Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. *J. Biol. Chem.* **273,** 34,857–34,867.
62. Lorenzon N. M., Lutz C. M., Frankel W. N., and Beam K. G. (1998) Altered calcium channel currents in Purkinje cells of the neurological mutant mouse leaner. *J. Neurosci.* **18,** 4482–4489.
63. Dove L. S., Abbott L. C., and Griffith W. H. (1998) Whole-cell and single-channel analysis of P-type calcium currents in cerebellar Purkinje cells of leaner mutant mice. *J. Neurosci.* **18,** 7687–7699.
64. Qian J. and Noebels J. L. (2000) Presynaptic Ca^{2+} influx at a mouse central synapse with

Ca^{2+} channel subunit mutations. *J. Neurosci.* **20,** 163–170.
65. Plomp J. J., Vergouwe M. N., Van den Maagdenberg A. M., Ferrari M. D., Frants R. R., and Molenaar P. C. (2000) Abnormal transmitter release at neuromuscolar junctions of mice carrying the *tottering* α_{1A} Ca^{2+} channel mutation. *Brain* **123,** 463–471.
66. Caddick S. J., Wang C., Fletcher C. F., Jenkins N. A., Copeland N. G., and Hosford D. A. (1999) Excitatory but not inhibitory synaptic transmission is reduced in lethargic ($Cacnb4^{lh}$) and tottering ($Cacnala^{tg}$) mouse thalami. *J. Neurophysiol.* **81,** 2066–2074.
67. Ayata C., Shimizu-Sasamata M., Lo E. H., Noebels J. L., and Moskowitz (2000) Impaired neurotransmitter release and elevated threshold for cortical spreading depression in mice with mutations in the α_{1A} subunit of P/Q type calcium channels *Neuroscience* **95,** 639–645.
68. Jun K., Piedras-Renteria E. S., Smith S. M., Wheeler D. B., Lee S. B., Lee T. G., et al. (1999) Ablation of P/Q-type Ca^{2+} channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the α_{1A} -subunit. *Proc. Natl. Acad. Sci. USA* **96,** 15245–15250.
69. Fletcher C. F., Tottene A., Lennon V. A., Wilson S. M., Dubel S. J., Paylor R., et al. (2001) Dystonia and cerebellar atrophy in cacna1a null mice lacking P/Q calcium channel activity. *FASEB J.* **15,** 1288–1290.
70. Burgess D. L., Jones J. M., Meisler M. H., and Noebels J. L. (1997) Mutation of the Ca^{2+} channel b subunit gene Cehb4 is associated with ataxia and seizures in the lethargic (lh) mouse. *Cell* **88,** 385–392.
71. Volsen S. G., Day N. C., McCormack A. L., Smith W., Craig P. J., Beattie R. E., et al. (1997) The expression of voltage-dependent calcium channel beta subunits in human cerebellum. *Neuroscience* **80,** 161–174.
72. McEnery M. W., Copeland T. D., and Vance C. L. (1998) Altered expression and assembly of N-type calcium channel α_{1B} and β subunits in epileptic lethargic (lh/lh) mouse. *J. Biol. Chem.* **273,** 21,435–21,438.
73. Letts V. A., Felix R., Biddlecome G. H., Arikkath J., Mahaffey C. L., Valenzuela A., et al. (1998) The mouse stargazer gene encodes a neuronal Ca^{2+}-channel γ subunit. *Nature Genet.* **19,** 340–347.
74. Hashimoto K., Fukaya M., Qiao X., Sakimura K., Watanabe M., and Kano M. (1999) Impairment of AMPA receptor function in cerebellar granule cells of ataxic mutant mouse Stargazer. *J. Neurosci.* **19,** 6027–6036.
75. Chen L., Bao S., Qiao X., and Thompson R. F. (1999) Impaired cerebellar synapse maturation in waggler, a mutant mouse with a disrupted neuronal calcium channel γ subunit. *Proc. Natl. Acad. Sci. USA* **96,** 12132–12137.
76. Chen L., Chetkovich D. M., Petralia R. S., Sweeney N. T., Kawasaki Y., Wenthold R. J., et al. (2000) Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408,** 936–943.
77. Elliot M. A., Peroutka S. J., Welch S., and May E. F. (1996) Familial hemiplegic migraine, nystagmus, and cerebellar atrophy. *Ann. Neurol.* 1996, 100–106.
78. Ishikawa K., Watanabe M., Yoshizawa K., Fujita T., Iwamoto H., Yoshizawa T., et al. (1999) Clinical, neuropathological, and molecular study in two families with spinocerebellar ataxia type 6 (SCA6). *J. Neurol. Neurosurg. Psychiatry* **67,** 86–89.
79. Ptacek L. J. (1998) The place of migraine as a channelopathy. *Curr. Opin. Neurobiol.* **11,** 217–226.
80. Welch K. M. A. (1998) Current opinions in headache pathogenesis: introduction and synthesis. *Curr. Opin. Neurol.* **11,** 193–197.
81. Hadjikhani N., Sanchez del Rio M., Wu O., Schwartz D., Bakker D., Fischl B., et al. (2001) Mechanisms of migraine aura revealed by functional MRI in human visual cortex. *Proc. Natl. Acad. Sci. USA* **98,** 4687–4692.