Acta Pharmacologica Sinica 2006 Jul; 27 (7): 799–812

Blackwell Publishing

Invited review

Molecular basis of inherited calcium channelopathies: role of mutations in pore-forming subunits[1]

Lynn MCKEOWN, Philip ROBINSON, Owen T JONES[2]

Faculty of Life Sciences, the University of Manchester, Manchester, UK

Key words

calcium channels; channelopathies; mutation

[1] Project supported by funds from the Biotechnology and Biological Sciences Research Council UK (No 34/C15752).

[2] Correspondence to Dr Owen T JONES.
Phn 44-0-161-275-5604.
Fax 44-0-161-275-5600.
E-mail owen.t.jones@manchester.ac.uk

Received 2006-05-19
Accepted 2006-05-30

doi: 10.1111/j.1745-7254.2006.00394.x

Abstract

The pore-forming alpha subunits of voltage-gated calcium channels contain the essential biophysical machinery that underlies calcium influx in response to cell depolarization. In combination with requisite auxiliary subunits, these pore subunits form calcium channel complexes that are pivotal to the physiology and pharmacology of diverse cells ranging from sperm to neurons. Not surprisingly, mutations in the pore subunits generate diverse pathologies, termed channelopathies, that range from failures in excitation-contraction coupling to night blindness. Over the last decade, major insights into the mechanisms of pathogenesis have been derived from animals showing spontaneous or induced mutations. In parallel, there has been considerable growth in our understanding of the workings of voltage-gated ion channels from a structure-function, regulation and cell biology perspective. Here we document our current understanding of the mutations underlying channelopathies involving the voltage-gated calcium channel alpha subunits in humans and other species.

Introduction

Voltage-gated calcium channels (VGCCs) are critical determinants of physiological function in both excitable and non-excitable cells[1,2]. The ability of VGCCs to couple changes in membrane potential to the influx of the pivotal "second messenger" calcium (Ca^{2+}) bestow VGCCs with a unique and privileged position among ion channels in the coupling of electrical signaling to intracellular biochemical events. In tissues such as muscle and heart, VGCCs are used for specific functions such as neurotransmitter release and excitation-contraction coupling, respectively. More generally, VGCCs orchestrate cell excitability[3], second messenger signaling[4] and gene expression[5].

Owing to such diverse roles, it is perhaps not too surprising that disruption in VGCC function has been implicated and, in many cases, demonstrated to underlie diverse inherited pathologies ranging from cardiac failure to epilepsy. The goal of this article is to review disparate data on such calcium "channelopathies" from a molecular perspective, focusing on the key pore-forming alpha subunits.

Structure and function of VGCCs

Although the existence of VGCCs had been known for many decades, their emergence as functionally discrete subtypes was not revealed until the pioneering electrophysiological studies of Tsien[6], Lux[7] and many others during the 1980s. Originally, VGCCs were classified into two groups, according to their functional and pharmacological characteristics: T VGCCs [low voltage-activated (LVA) subtype]; and N, L, P/Q, and R VGCCs [high voltage-activated (HVA) subtypes][8] (Table 1). However, molecular cloning, expression and biochemical studies revealed inadequacies in the above classification notation and a more rigorous structure-based nomenclature (Table 1) was subsequently introduced[9].

All VGCCs are large (>400 kDa) heteromers comprised minimally of 3 core subunits α_1, α_2/δ, and β found in a 1:1:1 stoichiometry[8]. Expression studies in *Xenopus* oocytes[10,11] and transfected mammalian cells[12–14] have revealed that the α_1 subunits contain the gating, channel pore and inactivation machinery required for function. However, interactions between α_1 and the auxiliary α_2/δ and β subunits are required for optimal cell surface expression and channel kinetics.

Table 1. Classification and nomenclature of VGCCs. Each channel type is defined by a specific core α1 protein subunit and current yielded, either high voltage-activated (HVA) or low voltage-activated (LVA). Adapted from Catterall *et al*[8] and Ertel EA *et al*[9].

Name	Class	Current type	Alpha subunit	Protein size (kDa)	Ligands
CaV1.1	L	HVA	$\alpha 1_S$	212	Dihydropyridines
CaV1.2	L	HVA	$\alpha 1_C$	240	Dihydropyridines
CaV1.3	L	HVA	$\alpha 1_D$	187	Dihydropyridines
CaV1.4	L	HVA	$\alpha 1_F$	222	Dihydropyridines
CaV2.1	P/Q	HVA	$\alpha 1_A$	257	ω-Agatoxin IVA
CaV2.2	N	HVA	$\alpha 1_B$	262	ω-Conotoxin-GVIA
CaV2.3	R	HVA	$\alpha 1_E$	252	SNX-482
CaV3.1	T	LVA	$\alpha 1_G$	250	Kurtoxin[a]
CaV3.2	T	LVA	$\alpha 1_H$	261	Kurtoxin[a]
CaV3.3	T	LVA	$\alpha 1_I$	224	None

[a] Ligand is low affinity and most probably unreliable.

Additional protein interactions have been identified that appear necessary for trafficking and regulation (Table 2).

A cardinal feature of VGCCs is their extraordinary propensity for diversity. In mammals, 10 α_1, 3 α_2/δ and 4 β subunit genes have been identified. Moreover, most of the RNA transcripts have been shown to undergo alternative splicing, and the number of reported variants is growing rapidly[8]. The precise nature of the α_1, α_2/δ and β gene products in the VGCC complexes define their biophysical characteristics, therefore such diversity has significant functional implications[8]. However, the contribution of any ion channels to integrative physiology also depends on their distribution. Thus, it is notable that specific VGCCs have unique, but often overlapping, patterns of expression in discrete regions of brain and other tissues. Even the distribution over the cell surface can differ[15–22], suggesting that diversity is used to hone voltage-dependent Ca^{2+} influx to the demands of discrete functional compartments[23].

An emerging paradox concerns a subunit, termed γ, first identified as a constituent of the skeletal muscle VGCC but now known to have relatives in heart and brain[24]. Precisely what function these γ subunits serve is unresolved. Expression studies suggest an ability to normalize calcium currents to those resembling endogenous VGCCs[25]. However, not all γ subunits show this effect and one subunit, γ_2, has been shown to interact with the AMPA-subtype of glutamate receptors[26].

Ca^{2+} channelopathies

Inherited defects in VGCCs give rise to some of the most interesting and widely studied channelopathies[27]. Here we summarize, specifically, current information on those defects arising from mutations in the pore-forming alpha subunits in human and other model systems (Table 1).

CaV1.1 ($\alpha 1_S$) The pore-forming subunit of CaV1.1, encoded in humans by the *CACNA1S* (formerly *CACNL1A3*) gene on chromosome 1q 31–32[28], is expressed mainly in skeletal muscle traverse tubules where it mediates excitation-contraction coupling and calcium homeostatsis[29,30].

Muscular dysgenesis in mice is a lethal mutation derived from a frameshift at nucleotide 4010. The resulting deletion of the C-terminus leads to a loss in muscle contraction and was the first mutation reported to affect calcium currents *in vivo*[31,32]. Missense mutations in CACNA1S have been identified in human cases of hypokalemic periodic paralysis (hypoPP) and malignant hyperthermia susceptibility (MHS)[33–38]. The Arg-His or glycine substitutions found in hypoPP (Table 1) are located in the voltage-sensing segments (S4) of domains II and IV, leading to a loss of myotube function[33,39,40]. Arg-His mutations have also been found in patients suffering from MHS. However, although both allelic, MHS and hypoPP appear to be distinct non-overlapping diseases[41]. Genetic studies of MHS have mainly linked associated mutations to the ryanodine receptor (RyR). However, as the RyR comes under the control of CaV1.1 and the R1086H mutation is located in the cytoplasmic loop between transmembrane spanning segments 3 and 4, such mutations might disrupt the functional link between the CaV1.1 and RyR[36].

CaV1.2 ($\alpha 1_C$) CaV1.2 is primarily localized to cardiac or smooth muscle but is also found in endocrine cells and neurones. Functionally, CaV1.2 mediates excitation-contraction coupling in smooth and cardiac muscle, hormone secretion and action potential propagation in sino-atrial and atrio-

Table 2. Summary of major cytoplasmic proteins interacting with VGCC α1 subunits, their binding sites and roles.

Protein	VGCC 1 binding site	Role/effect	Reference
Syntaxin 1	Synprint motif (II–III linker)	SNARE member, vesicle exocytosis	149
SNAP-25	Synprint motif (II–III linker)	SNARE member, vesicle exocytosis	150
Cysteine string protein	Synprint motif (II–III linker)	Vesicle exocytosis	151
Synaptotagmin	Synprint motif (II–III linker)	Vesicle exocytosis	152
CASK	C-terminus	VGCC–vesicle coupling	153
MINT-1	C-terminus	VGCC–vesicle coupling	153
Calmodulin	C-terminus (IQ domain)	Post Ca^{2+}-influx inactivation	154
G-protein βγ subunit	Domain I–II linker, C-terminus	GPCR-coupled inactivation	155
Protein kinase C, protein kinase A	Domain I–II linker, Synprint motif, C- and N-termini	Dependent on channel type and binding site	1
AKAP-79	Domain II–III linker	Postsynaptic L-type channel trafficking	156
Calbindin	C-terminus	Decrease L-type channel activity in pancreatic beta cells	157
CaMKII (Ca^{2+}/calmodulin-dependent kinase II)	C-terminus	Facilitation of Ca^{2+} current	158

ventricular nodes[42].

The CaV1.2 subunit is encoded by the *CACNA1C* (formerly *CACNL1A1*) gene on chromosome 12p13.3[43]. In mice, knockout of the CaV1.2 gene is lethal due to cardiac dysfunction (Seisenberger *et al* 2000). However, two *de novo* missense mutations in CaV1.2 in humans result in Timothy syndrome, a multi-system disorder including syndactyly, immune deficiency, long QT syndrome and ventricular arrhythmias during infancy[44]. These gain of function phenotypes arise from highly conserved glycine substitutions for either Arg at 406 or Ser at 402[44]. Mutation G406 is located in alternatively spliced exon 8A, at the cytoplasmic face of the transmembrane segment S6 of domain I, whereas mutation G402 is located within the transmembrane region. As glycine can act as a hinge-point in α helices, such mutations have been suggested to disrupt the activation gate[44]. Further support for the role of CaV1.2 in cardiac development and dysfunction comes from studies on zebrafish whose embryos can survive without blood flow for several days. In their study, Rottbauer *et al*[45] mapped the genetic mutations responsible for the zebrafish isl lethal mutant. Mutants expressing two isl nonsense mutations at M379 or M458 (which both caused premature truncation of CaV1.2) present with abnormal heart growth during development.

CaV1.3 ($\alpha 1_D$) The pore-forming subunit of the CaV1.1 channel is mainly expressed in endocrine cells of the pituitary and adrenal chromaffin cells, but is also found in sensory cells and in low densities in atrial muscle, heart and neurons. Originally classed as the neuroendocrine L-type channel, CaV1.3 plays a role in hormone secretion, mood behaviour, and control of cardiac rhythm at rest[46].

The CaV1.3 subunit is encoded by the *CACNA1D* (formerly *CACNL1A2*) gene on chromosome 3p14.3[47]. Insights into the role of Cav1.3 in cardiac tissue have largely evolved due to electrophysiology studies on cells from knockout mice[48] and cardiac cells from human patients[49]. Although no human gene defect has been reported, animal models have provided useful insights into potential deficits. In one study, Wappl *et al* created a mouse model in which the high dihydropyridine sensitivity of CaV1.2 subunits was eliminated by replacement of Thr1066 in helix IIIS5 with a tyrosine residue[50]. As the distribution of CaV1.2 and 1.3 often overlap, and they both mediate L-type currents, the creation of this mouse model allowed Sinnegger-Brauns *et al*, to isolate the function of CaV1.3 in brain, pancreatic beta cells and the cardiovascular system[51]. These studies ruled out a direct role for CaV1.3 in insulin secretion, cardiac inotropy, and arterial smooth muscle contractility but suggested it might play a role in depression. Although no linkage of CaV1.3 channel mutations has been reported for human inherited diseases, mice carrying a targeted null allele display profound congenital deafness, thus providing insight into the molecular basis of CaV1.3 function in auditory processing[52,53]. Interestingly, the *ise*, mutant form of zebrafish larvae displays a deafness-imbalance phenotype, arising through two mutations (R1250X and R284C) in a gene encoding for the CaV1.3 channel[54]. The first mutation involves exchange of an Arg codon for a stop codon at position 1250 and results in a nonsense mutation in domain IVS4 that disrupts the transmembrane region and removes the

Table 3. The position of inherited and *de novo* mutations in α1 genes and their resulting phenotypes. Mutation refers to amino acid unless followed by (n) for nucleotide. Ic, intracellular loops; Ec, extracellular loops.

Channel	Gene	Mutation	Type	Location	Inherited disease	Reference
CaV1.1	*CACNA1S*	R528H	Missense	IIS4	HypoPP	33–35
		R528G	Missense	IIS4	HypoPP	38
		R1239G	Missense	IVS4	HypoPP	42
		R1239H	Missense	IVS4	HypoPP	133
		R1086H	Missense	Linker III–IV	MHS	36,37
CaV1.2	*CACNA1C*	G406R	Missense	IS6	Timothy syndrome	44,50
		G402S	Missense		Timothy syndrome	44,50
CaV1.4	*CACNA1D*	S229P	Missense	Ic loop IS4–S5	XLCSNB	133
		341delC	Frameshift	Linker I II	XLCSNB	55
		G369D	Missense	IS6	XLCSNB	56,133
		R508Q	Missense	Linker I II	XLCSNB	56,134
		I745T	Missense	IIS6	XLCSNB	136
		R680X	Nonsense	Pore IIS5–S6	XLCSNB	57
		R830X	Nonsense	Ec loop IIIS1–S2	XLCSNB	55
		R958X	Nonsense	IIIS4	XLCSNB	56
		991insC	Frameshift	Pore IIIS5–S6	XLCSNB	55
		R1049W	Missense	Pore IIIS5–S6	XLCSNB	56
		L1068P	Missense	Pore IIIS5–S6	XLCSNB	133
		1159delC	Frameshift	Ec loop IVS1–S2	XLCSNB	55
		R1234X	Nonsense	IVS4	XLCSNB	55
		S1254I	Missense	IVS4	XLCSNB	57
		R1285S	Missense	IVS4	XLCSNB	57
		Q1348X	Nonsense	Pore IVS5–S6	XLCSNB	56
		L1364H	Missense	Pore IVS5–S6	XLCSNB	56,134
		W1386X	Nonsense	C terminal	XLCSNB	55
		W1440X	Nonsense	C terminal	XLCSNB	133
		1516delC	Frameshift	C-terminal	XLCSNB	135
		K1591X	Nonsense	C-terminal	XLCSNB	56
		R1919H	Missense	C-terminal	XLCSNB	57
		3133insC (n)	Frameshift	Pore IIIS5–S6	XLCSNB	56
		3658–3669(n)	Deletion	IVS2	XLCSNB	56
CaV2.1	CACNA1A	R192Q	Missense	IS4	FHM	85
		R195K	Missense	IS4	FHM	95
		S218L	Missense	Ic loop IS4–S5	FHM	137
		R583Q	Missense	IIS4	FHM	138
		T666M	Missense	Pore IIS5–S6	FHM	85
		V714A	Missense	IIS6	FHM	85
		D715E	Missense	IIS6	FHM	94
		K1336E	Missense	Ec loop III S3–S4	FHM	94
		Y1385C	Missense	IIIS5	FHM	139
		V1457L	Missense	Pore IIIS5–S6	FHM	140
		R1668W	Missense	IVS4	FHM	95
		L1682P	Missense	IVS4	FHM	141
		W1684R	Missense	IVS4	FHM	95
		V1696I	Missense	IVS5	FHM	95
		H253Y	Missense	Pore IS5–S6	EA-2	92

(continue)

Channel	Gene	Mutation	Type	Location	Inherited disease	Reference
		SS	Missplice	IIS2	EA-2	142
		2145del 8(n)	Frameshift	IIS5	EA-2	92
		2317del8(n)	Frameshift	Pore IIS5–S6	EA-2	142,92
		FS1067X	Frameshift	Linker II–III	EA-2	143
		FS1144X	Frameshift	Linker II–III	EA-2	144
		FS1294X	Frameshift	IIIS1	EA-2	85,142
		R1279X	Nonsense	IIIS2	EA-2	145
		SS	Missplice	IIIS3	EA-2	85
		4451delC(n)	Frameshift	IIIS5	EA-2	92
		SS	Missplice	Pore IIIS5–S6	EA-2	142
		Y1444X	Nonsense	Pore IIIS5–S6	EA-2	142
		R1547X	Nonsense	Linker III–IV	EA-2	142
		5056del8 (n)	Frameshift	IVS1–S2	EA-2	142
		5123del	Frameshift	IVS2	EA-2	143
		R1662H	Missense	IVS4	EA-2	146
		H1736L	Missense	IVS5–S6	EA-2	147
		E1757K	Missense	Pore IVS5–S6	EA-2	148
		SS	Missplice	IVS3	EA-2	87
		7213 GAG (n)	Expansion	C-terminal	Spinocerebellar ataxia type 6	96
		R1664Q	Missense	IVS4		149
CaV3.2	CACNA1B	F161L	Missense	IS2–IS3	Childhood absence epilepsy	126
		E282K	Missense	Pore IS5–S6	Childhood absence epilepsy	126
		C456S	Missense	Linker I–II	Childhood absence epilepsy	126
		G499S	Missense	Linker I–II	Childhood absence epilepsy	126
		P648L	Missense	Linker I–II	Childhood absence epilepsy	126
		R744Q	Missense	Linker I–II	Childhood absence epilepsy	126
		A748V	Missense	Linker I–II	Childhood absence epilepsy	126
		G773D	Missense	Linker I–II	Childhood absence epilepsy	126
		G784S	Missense	Linker I–II	Childhood absence epilepsy	126
		V831M	Missense	IIS2	Childhood absence epilepsy	126
		G848S	Missense	IIS3	Childhood absence epilepsy	126
		D1463N	Missense	Pore IIIS5–S6	Childhood absence epilepsy	126
		A480T	Missense	Linker I–II	IGE	128
		P618L	Missense	Linker I–II	IGE	128
		G775D	Missense	Linker I–II	IGE	128

carboxy-terminal tail. The second mutation substitutes a highly conserved Arg for a Cys residue at position 284 within the extracellular region of the pore loop between S5 and S6 of domain I.

CaV1.4 ($\alpha 1_F$) The CaV1.4 subunit, encoded in humans by the gene *CACNA1F*, maps to chromosome Xp11.4[55] and is mainly expressed in retinal rods and bipolar cells, spinal cord, adrenal gland and mast cells. As its main cellular function is thought to be neurotransmitter release from photoreceptors, it is no surprize that mutations in this channel are involved with inherited diseases of the eye.

The locus for X-linked congenital stationary night blindness type 2 (XLCSNB-2) was mapped to the *CACNA1F* gene[55,56] and several mutations have since been identified. In case studies of patients with XLCSNB, over 73 *CACNA1F* mutations have been detected, of which 51% are nonsense mutations, 32% missense mutations and 8% frameshifts[57]. A list of several of the mutations identified and their positions within the channel is given in Table 2. Very recently, Wei and Hemmings detected a genetic association between schizophrenic patients and the *CACNA1F* locus, although the exact mutations are unknown[58]. The prevalence of visual abnormalities in schizophrenia makes an association with *CACNA1F* especially interesting.

CaV2.1 ($\alpha 1_A$) The P/Q-type calcium channel CaV2.1 (alpha1A) represents one of the most important channels both from a physiological perspective and its role in channelopathy. Found throughout the nervous system,

CaV2.1 is considered to be the primary VGCC controlling fast neurotransmitter release, especially at excitatory synapses. Not surprisingly, CaV2.1 is found in high concentrations in the presynaptic nerve terminus, where it exists in discrete release sites that are more efficiently coupled to the vesicle release machinery than other VGCCs[59–62]. However, CaV2.1 is also found throughout the dendritic arbour, especially in cerebellar Purkinje cells, where it contributes to integrative dendritic physiology[63].

Historically, one of the first lines of evidence linking CaV2.1 to neurological disorders came from studies of the tottering (*tg*) mouse[64]. The *tg* mouse is a neurological mutant displaying ataxia, and involuntary spasms indicative of tonic-clonic seizures as well as neurophysiological signs of absence epilepsy[65]. The underlying defect in *tg* mice has been identified, through positional cloning, as a point mutation (P601L) in *cacna1a*, the mouse CaV2.1 gene[64]. Using whole cell patch clamp methods, Wakamori *et al* found significant (40%) decreases in P-type (Ba^{2+}) currents in dissociated Purkinje cells obtained from *tg*/*tg* versus wild-type (*wt*) mice that could be replicated in a simple heterologous expression system[66]. Surprisingly, they found no change in the voltage-dependence of activation or inactivation, single channel conductance or reversal potential, suggesting that the decreased current density is not due to impaired ion conductance or activation/inactivation mechanisms.

Three other mouse mutations showing varying degrees of seizure activity have been identified that map to the *cacna1a* locus rocker (*rkr*), tottering leaner (tg^{la}) and rolling Nagoya (tg^{rol}) [64,67,68]. Remarkably, each mutant mouse shows considerable differences in the extent and times of onset of seizure, cerebellar atrophy and ataxia. Thus *tg*, tg^{la} and *rkr* but not tg^{rol} mice show seizure activity, whereas tg^{la}, *rkr* and tg^{rol} but not *tg* mice show marked ataxia[65,67-69].

Rocker arises through a point mutation within the extracellular S_5–S_6 region (T1310K) of domain III[67]. Surprisingly, the precise effect of the *rkr* mutation on P/Q currents has not been forthcoming, but is likely to resemble those in *tg* mice. A distinct mutation in domain III, (R1262G), within the S_4 voltage sensor, occurs in tg^{rol} mice[68]. This mutation displays a marked reduction in the voltage sensitivity of channel activation. The tg^{la} mutant has absence seizures, severe ataxia and cerebellar damage[70]. In tg^{la}, a point mutation at a splice/donor consensus sequence leads to aberrant RNA splicing in the region encoding the carboxy terminus of the α_{1A} subunit. As a result, translation yields two primary protein products corresponding to truncated CaV2.1 subunits bearing a novel and distinct C-termini. Electrophysiological studies on Purkinje cells show that the major deficit caused by the tg^{la} mutation is a 60% reduction in P/Q-type currents or current densities compared to the wild-type mouse[66,71,72]. Single channel recordings suggest the decrease in current densities is not due to effects on either the channel conductance or lifetimes[72], but due to effects on channel opening probability (P_o) or, more likely, a decrease in channel densities at the cell surface, perhaps due to a trafficking defect. Interestingly, in transfected cells, only the $tg^{la}{}_{short}$ form shows a significant reduction in current density[66].

Just how the phenotypes of these spontaneous mouse mutants arise is unclear but might give insight into the analogous human conditions[73]. In general, aberrant activity of CaV2.1 channels cannot be functionally compensated for at many central synapses. Apart from the 'gain of function' FHM mutations (below), impairment of neurotransmission appears to be the rule for CaV2.1 knockout[74–76], *tg*/*tg*[77] and *tg*/tg^{rol}[78,79]. However, at the climbing fiber-Purkinje cell synapse, evoked glutamate release is similar between wild-type and CaV2.1$^{-/-}$, tg^{rol}/tg^{rol} or *tg*/*tg* mice[76,78]. Inhibitory transmission does not appear to be affected[80]. An obvious complication is the degree to which alternate VGCCs can stand in for the aberrant CaV2.1 channels and to what extent this might contribute to the neurological phenotype. Based on their similar trafficking and biophysical properties, the most likely replacements are expected to be the CaV2.2 VGCCs. Indeed, compensation by CaV2.2 has been documented at the calyx of Held synapse in CaV, CaV2.1$^{-/-}$ knockout mice and other mutants[74,77,78,81]. However, there is also evidence for upregulation of both CaV2.2 and CaV2.3 at the neuromuscular junction of CaV2.1$^{-/-}$ mice[82].

In humans, the gene encoding CaV2.1 is designated as *CACNA1A* (formerly known as *CACNLA4*) and is localized to a large 300 kb region containing 47 exons at chromosome position 19p13[83]. Whereas gene expression yields a primary transcript of 9.8 kb, several splice variants have been identified[84], most notably an isoform that differs in exon 37 by 97 nucleotides.

In humans, the cardinal mutation associated with CaV2.1 is a rare disorder termed episodic ataxia type 2 (EA-2) that causes paroxysmal attacks of cerebellar ataxia that can last for several days. In 1996, Ophoff *et al* identified 2 mutations in unrelated patients displaying EA-2 that mapped to the *CACNA1A* gene[85]. One mutation involves a base deletion and the other occurs at a splice junction site, but both are predicted to lead to a frameshift such that the CaV2.1 protein is truncated prematurely after the S1 region of domain III (Figure 1). The partially complete channel is, thus, predicted to be non-functional or to be incorrectly folded and trafficked. More recently, additional familial EA-2 mutations have been

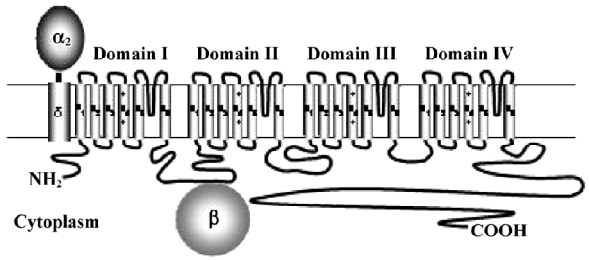

Figure 1. The predicted structure of a prototypical VGCC consists of an α1 pore-forming subunit and at least two auxiliary/regulatory subunits, β and α2d. The α1 subunit is a transmembrane protein organized into 4 repeating domains (I–IV), each containing 6 hydrophobic transmembrane regions (S1–S6). Segments S5 and S6 of each domain line the channel pore (P loop) and S4 is designated as the voltage sensor.

identified that induce mis-splicing either through a G-A substitution at an intron-exon boundary or, especially interesting, through a 4 bp ACGT deletion within an intron (the first described for *CACNA1A*), which might unmask a cryptic splice downstream[86]. Typically, clinical signs of EA-2 are evident prior to adulthood. However, just recently, a case of late-onset (aged 61 years) EA-2 has been identified involving a 9 bp insertion in the cytoplasmic domain II–III linker. To date this is the largest insertion reported for the human gene[87]. Quite what the mutation does is unclear. Although it lies within the II–III linker, it is just downstream of the synprint region for SNAP-25/syntaxin binding. Expression studies indicate a significant (approximately 82%) reduction in current and a 20 mV depolarization shift in activation threshold with some change in activation and inactivation kinetics. Together, these data might be indicative of an effect on gating. Why the clinical symptoms appeared so late in this patient is unclear. An examination of compensatory expression of other VGCCs in this patient would certainly be interesting. A novel *CACNA1A* mutation, IVS36-2A>G, at the 3' acceptor splice site of intron 36 was identified by sequencing[88]. It is the first described *CACNA1A* acceptor splice site mutation and the most C-terminal EA-2-causing mutation reported to date.

Another highly debilitating *CACNA1A* channelopathy is the rare autosomal dominant disorder familial hemiplegic migraine (FHM). Characterized by intense attacks of migraine with aura, often lasting for several days, FHM has often been misdiagnosed as epilepsy or stroke[89]. Cerebellar dysfunction has also been noted in some families. Following the initial work by Ophoff *et al*[85], several missense mutations have been found within *CACNA1A* that lead to increased calcium influx through the expressed channels[90]. However, the precise biophysical characteristics conferred by the mutations on the CaV2.1 channel are not identical. Thus, single channel recordings on expressed channels show changes in gating with mutants T666M, V714A, and I1819L, but not R192Q. Recovery from inactivation can be slower (T666M) or faster (V714A and I1819L) compared to wild-type CaV2.1[91]. Subsequent studies have found an increased propensity for activation at weakly depolarizing potentials for three additional FHM-associated mutants[92]. Powerful insights into the neuropathology of FHM have come from a recent study by Van den Maagdenberg *et al*[93] who generated a transgenic mouse model bearing the human *CACNA1A* mutation R192Q. Recordings from cerebellar granule cells showed increased CaV2.1 channel current densities, which activated at more negative voltages in R192Q mice than wild-type channels. Significantly, the R192Q mice showed enhanced neurotransmission and susceptibility to cortical spreading depression. Taken together the above suggests that FHM is a *CACNA1A* channelopathy that arises through a gain of function that enhances neurotransmitter release.

As FHM and EA-2 both involve *CACNA1A*, an interesting question concerns the extent to which they overlap. In this respect it is notable that approximately 20% of FHM cases show signs of mild cerebellar ataxia[94]. Although cross-correlational prediction is not straightforward, 83% of patients with six missense mutations associated with migraine also showed ataxia and or nystagmus[95].

A third clinical disorder associated with mutations in CaV2.1 has been identified. Termed Spinocerebellar ataxia type 6 (SCA6), this disorder appears in early middle age (30–40 years) and is characterized by a mild progressive (over a subsequent 25 years) cerebellar atrophy causing dysarthria, nystagmus, ataxia, loss of gait and sometimes death[96]. Unlike EA-2 and FHM, SCA6 appears to arise through a shift in the reading frame and triplet (CAG) repeat expansion at the distal carboxy terminus[97]. Based on a study by Ishikawa *et al*[98] the critical size of the CAG encoded polyglutamine stretch appears to be 19 repeats, with cases showing longer stretches having poorer neurological outcomes and earlier disease onset. Precisely what functional effects the SCA6 remains unclear. In a model of SCA6, elongation of the polyglutamine tract in SCA6 CaV2.1 caused a concomitant hyperpolarizing shift of voltage-dependent inactivation to more negative potentials, suggesting an overall reduction of calcium influx might contribute to SCA6 symptomology. However, just recently, compelling evidence has been presented that a portion of the CaV2.1 carboxy-terminus is cleaved *in vivo* and can enter the nucleus by virtue of nuclear localization signals[99]. Although the wild-type carboxy ter-

minal fragment is weakly toxic, a fragment containing an expanded polyglutamine tract (Q33) corresponding to SCA6 is highly toxic to Purkinje neurons and other cells. Thus, SCA6 might share a similar mechanism of action with some other expansion disorders where pathogenesis requires entry of a polyglutamine-containing fragment into the nucleus.

Given the above, one would anticipate that mutations in the human CaV2.1 gene are associated with epilepsy[100]. Nevertheless, the evidence has been slow to emerge. One early study found no statistically significant evidence that genetic variants of the *CACNA1A* gene might play a causative role in common forms of idiopathic generalized epilepsy (IGE)[101]. Moreover, reports of an allelic association of a silent single nucleotide polymorphism (SNP8) with IGE[102,103] have been refuted[104]. Nevertheless, there is growing recognition that in some cases patients with EA-2 also show an epilepsy phenotype most usually of a primary generalized nature[105,106].

The first human EA-2-epilepsy case was described in 2001 by Jouvenceau *et al* in an 11-year-old who showed frequent episodes of ataxia and poorly controlled absence seizures, and generalized tonic–clonic seizures[105]. The underlying mutation was found to lie in exon 36 (C5733T) giving rise to a premature truncation behind the domain IV S6, and, thus, complete loss of the C-terminus. In an expression system, the primary effect of this mutation is a massive loss of functional channels at the cell surface. However, it is interesting to note that the mutation appears to have a dominant-negative effect when co-expressed with wild-type CaV2.1 (as anticipated for the heterozygous state). It is our contention that it is only a matter of time before further epilepsy-associated *CANA1A* mutations are documented.

CaV2.2 ($\alpha 1_B$) Given its established role alongside CaV2.1 in neurotransmitter release, it is remarkable that mutations in the *CACNA1B* gene (locus 9q34) have not been identified in the human population. Based on studies in CaV2.2$^{-/-}$ knockout mice one would anticipate problems in nociception[107], decreases in sympathetic nervous system function[108] and alterations in response to ethanol[109] and anaesthetics[110].

CaV2.3 ($\alpha 1_E$) The CaV2.3 channel (*CACNA1E*) (locus 1q25–q31)[83] is primarily localized to the somata and dendrites of central neurones. However, such channels are also found in the nerve terminals of central synapses[111,112] where they might participate in transmitter release[113]. Although no human mutations have been identified in CaV2.3, observations in knockout mice by Jing *et al* predict that mutations impairing this VGCC are likely to affect glucose-stimulated insulin release from pancreatic beta cells by facilitating the global entry of calcium needed for granule replenishment[114].

CaV3.1 ($\alpha 1_G$) The CaV3.1 subunit gene *CACNA1G*, located on chromosome 17q22[115] is thought to encode a T-type (ie low threshold) VGCC. This channel is highly expressed in brain, especially on dendrites, and it is considered to be the primary T-channel in the thalamus[116]. However, CaV3.1 is also found in the ovary, placenta and heart[117] To date no mutations have been identified in inherited human diseases. However, studies on knockout mice indicate reduced sleep patterns[118,119], bradycardia and delayed atriventricular conduction[5,120].

CaV3.2 ($\alpha 1_H$) In humans, the CaV3.2 gene (*CACNA1H*) has been mapped to chromosomal locus 16p13.3[6,121]. This subunit appears to be widely expressed in brain (especially the neocortex), kidney, smooth muscle, liver and heart. Targeted knockout studies in the rat nociceptive root ganglion suggest CaV3.2 plays a role in nociception[7,122]. Knockout mice show constitutively constricted coronary arterioles and focal myocardial fibrosis[123], and CaV3.1 knockouts in human spermatazoa demonstrate that CaV3.2 is a key player in the T-type current accompanying the acrosome reaction[124]. Mutations in *CACNA1H* are now thought to underlie diverse epilepsies. Thus, in 2003, Chen *et al* found 12 missense mutations in 14 patients with childhood absence epilepsy[125], and several of the 12 appeared to promote calcium influx during activation[126]. Even more recently, a study by Heron *et al*[127] identified 3 missense mutations and a single nonsense mutation in *CACNA1H* in a subset of patients with IGE. On expression, these latter mutations yield statistically significant changes in the kinetics of activation and inactivation of the CaV3.2 channel. Interestingly, many of these mutations lie in the same domain I–II linker region. Even more significant is the possibility that mutations in this region might block the selective inhibition of this VGCC by G-protein $\beta 2\alpha 2$ subunits[128]. Changes in T-type channels have long been implicated in epilepsy[100].

CaV3.3 ($\alpha 1_I$) In humans, the *CACNA1I* gene maps to chromosome position 22q12.3 [129]. In common with the other T-type VGCCs it is expressed highly in brain[130] where it is thought to play a role in thalamic oscillation[131]. Little is known about possible CaV3.3 gene defects, either in the human population or inferred from knockout animals.

Summary

With just a few exceptions, pathological mutations have now been identified in every VGCC pore-forming alpha subunit in humans. In many cases the pathology can be predicted on the basis of the tissue patterns of gene expression. Studies on spontaneous mouse mutants provide important

clues to the human condition and, in the case of knockout or knockin mutants, might predict disease phenotype and outcome. What is especially striking is the extent to which small differences in function can have major and very distinct effects on behavior. Such phenotypic pleiotropy does not simply reflect gain or loss of VGCC function, but more subtle effects on biophysical parameters such as pore conduction, gating and inactivation kinetics. In some cases, effects could be attributed to sites of interaction with proteins involved in channel regulation. However, a largely unexplored area is the extent to which VGCC mutations affect channel trafficking to and from the cell surface. Just recently, Papazian's group[132] identified mutations in *tg*/*tg* mice that appear to disrupt trafficking of CaV2.1 to the cell surface. Preliminary work in our laboratory suggests that similar effects might occur in other VGCC mutants. Whatever their origin, it is clear that the list of VGCC mutations identified in the human population will continue to expand. It is our contention that a detailed understanding of the structural and functional basis of such mutations is essential for treating the disorders they manifest.

References

1 Felix R. Molecular regulation of voltage-gated Ca^{2+} channels. J Recept Signal Transduct Res 2005; 25 :57–71.

2 Lacinova L. Voltage-dependent calcium channels. Gen Physiol Biophys 2005; 24(Suppl 1): 1–78.

3 Autret L, Mechaly I, Scamps F, Valmier J, Lory P, Desmadryl G. The involvement of CaV3.2/alpha1H T-type calcium channels in excitability of mouse embryonic primary vestibular neurones. J Physiol 2005; 567(Pt 1): 67–78.

4 Rizzuto R, Pozzan T. Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. Physiol Rev 2006; 86: 369–408.

5 Weick JP, Groth RD, Isaksen AL, Mermelstein PG. Interactions with PDZ proteins are required for L-type calcium channels to activate cAMP response element-binding protein-dependent gene expression. J Neurosci 2003; 23: 3446–56.

6 Fox AP, Nowycky MC, Tsien RW. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. J Physiol 1987; 394: 149–72.

7 Yaari Y, Hamon B, Lux HD. Development of two types of calcium channels in cultured mammalian hippocampal neurons. Science 1987; 235: 680–2.

8 Catterall WA, Perez-Reyes E, Snutch TP, Striessnig J. International Union of Pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev 2005; 57: 411–25.

9 Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, *et al.* Nomenclature of voltage-gated calcium channels. Neuron 2000; 25: 533–5.

10 Mori Y, Friedrich T, Kim MS, Mikami A, Nakai J, Ruth P, *et al.* Primary structure and functional expression from complementary DNA of a brain calcium channel. Nature 1991; 350: 398–402.

11 Williams ME, Brust PF, Feldman DH, Patthi S, Simerson S, Maroufi A, *et al*. Structure and functional expression of an ω-conotoxin-sensitive human N-type calcium channel. Science 1992; 257: 389–95.

12 Williams ME, Feldman DH, McCue AF, Brenner R, Velicelebi G, Ellis SB, *et al*. Structure and functional expression of α1, α2, and β subunits of a novel human neuronal calcium channel subtype. Neuron 1992; 8: 71–84.

13 Fujita Y, Mynlieff M, Dirksen RT, Kim M, Niidome T, Nakai J, *et al*. Primary structure and functional expression of the ω-conotoxin-sensitive N-type calcium channel from rabbit brain. Neuron 1993; 10: 585–98.

14 Stea A, Dubel SJ, Pragnell M, Leonard JP, Campbell KP, Snutch TP. A β-subunit normalizes the electrophysiological properties of a cloned N-type calcium channel. Neuropharmacology 1993; 32: 1103–16.

15 Jones OT, Kunze DL, Angelides KJ. Localization and mobility of omega-conotoxin-sensitive Ca^{2+} channels in hippocampal CA1 neurons. Science 1989; 244: 1189–93.

16 Westenbroek RE, Ahlijanian MK, Catterall WA. Clustering of L-type Ca^{2+} channels at the base of major dendrites in hippocampal pyramidal neurons. Nature 1990; 347: 281–4.

17 Westenbroek RE, Hell JW, Warner C, Dubel SJ, Snutch TP, Catterall WA. Biochemical properties and subcellular distribution of an N-type calcium channel alpha 1 subunit. Neuron 1992; 9: 1099–15.

18 Hell JW, Westenbroek RE, Warner C, Ahlijanian MK, Prystay W, Gilbert MM, *et al*. Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel alpha 1 subunits. J Cell Biol 1993; 123: 949–62.

19 Haydon PG, Henderson E, Stanley EF. Localization of individual calcium channels at the release face of a presynaptic nerve terminal. Neuron 1994; 13: 1275–80.

20 Mills LR, Niesen CE, So AP, Carlen PL, Spigelman I, Jones OT. N-type Ca^{2+} channels are located on somata, dendrites, and a subpopulation of dendritic spines on live hippocampal pyramidal neurons. J Neurosci 1994; 14: 6815–24.

21 Elliott EM, Malouf AT, Catterall WA. Role of calcium channel subtypes in calcium transients in hippocampal CA3 neurons. J Neurosci 1995; 15: 6433–44.

22 Westenbroek RE, Sakurai T, Elliott EM, Hell JW, Starr TVB, Snutch TP, *et al*. Immunochemical identification and subcellular distribution of the α_{1A} subunits of brain calcium channels. J Neurosci 1995; 15: 6403–18.

23 Johnston D, Magee JC, Colbert CM, Christie BR. Active properties of neuronal dendrites. Annu Rev Neurosci 1996; 19: 165–86.

24 Klugbauer N, Dai S, Specht V, Lacinova L, Marais E, Bohn G, *et al*. A family of gamma-like calcium channel subunits. FEBS Lett 2000; 470: 189–97.

25 Dascal N, Chilcott G, Lester HA. Recording of voltage and Ca^{2+}-dependent currents in *Xenopus* oocytes using an intracellular perfusion method. J Neurosci Methods 1991; 39: 29–38.

26 Chen L, Chetkovich DM, Petralla RS, Sweeney NT, Kawasaki Y, Wenthold RJ, *et al*. Stargazin regulates synaptic targeting of AMPA receptors by two distinct mechanisms. Nature 2000;

408: 936–43.
27 Pietrobon D. Calcium channels and channelopathies of the central nervous system. Mol Neurobiol 2002; 25: 31–50.
28 Gregg RG, Couch F, Hogan K, Powers PA. Assignment of the human gene for the alpha 1 subunit of the skeletal muscle DHP-sensitive Ca^{2+} channel (CACNL1A3) to chromosome 1q31-q32. Genomics 1993;15: 107–12.
29 Garcia J, McKinley K, Appel SH, Stefani E. Ca^{2+} current and charge movement in adult single human skeletal muscle fibres. J Physiol 1992; 454: 183–96.
30 Flucher BE, Franzini-Armstrong C. Formation of junctions involved in excitation-contraction coupling in skeletal and cardiac muscle. Proc Natl Acad Sci USA 1996; 93: 8101–6.
31 Beam KG, Knudson CM, Powell JA. A lethal mutation in mice eliminates the slow calcium current in skeletal muscle cells. Nature 1986; 320:168–170.
32 Chaudhari N. A single nucleotide deletion in the skeletal muscle-specific calcium channel transcript of muscular dysgenesis (mdg) mice. J Biol Chem 1992; 267: 25636–9.
33 Ptacek LJ, Tawil R, Griggs RC, Engel AG, Layzer RB, Kwiecinski H, *et al*. Dihydropyridine receptor mutations cause hypokalemic periodic paralysis. Cell 1994; 77: 863–8.
34 Jurkat-Rott K, Lehmann-Horn F, Elbaz A, Heine R, Gregg RG, Hogan K, *et al*. A calcium channel mutation causing hypokalemic periodic paralysis. Hum Mol Genet 1994; 3:1415–9.
35 Elbaz A, Vale-Santos J, Jurkat-Rott K, Lapie P, Ophoff RA, Bady B, *et al*. Hypokalemic periodic paralysis and the dihydropyridine receptor (CACNL1A3): genotype/phenotype correlations for two predominant mutations and evidence for the absence of a founder effect in 16 caucasian families. Am J Hum Genet 1995; 56: 374–80.
36 Monnier N, Procaccio V, Stieglitz P, Lunardi J. Malignant-hyperthermia susceptibility is associated with a mutation of the alpha 1-subunit of the human dihydropyridine-sensitive L-type voltage-dependent calcium-channel receptor in skeletal muscle. Am J Hum Genet 1997; 60: 1316–25.
37 Stewart SL, Hogan K, Rosenberg H, Fletcher JE. Identification of the Arg1086His mutation in the alpha subunit of the voltage-dependent calcium channel (CACNA1S) in a North American family with malignant hyperthermia. Clin Genet 2001; 59: 178–84.
38 Wang Q, Liu M, Xu C, Tang Z, Liao Y, Du R, *et al*. Novel CACNA1S mutation causes autosomal dominant hypokalemic periodic paralysis in a Chinese family. J Mol Med 2005; 83: 203–8.
39 Sipos I, Jurkat-Rott K, Harasztosi C, Fontaine B, Kovacs L, Melzer W, *et al*. Skeletal muscle DHP receptor mutations alter calcium currents in human hypokalaemic periodic paralysis myotubes. J Physiol 1995; 483: 299–306.
40 Lehmann-Horn F, Sipos I, Jurkat-Rott K, Heine R, Brinkmeier H, Fontaine B, *et al*. Altered calcium currents in human hypokalemic periodic paralysis myotubes expressing mutant L-type calcium channels. Soc Gen Physiol Ser 1995; 50: 101–13.
41 Marchant CL, Ellis FR, Halsall PJ, Hopkins PM, Robinson RL. Mutation analysis of two patients with hypokalemic periodic paralysis and suspected malignant hyperthermia. Muscle Nerve 2004; 30: 114–7.
42 Beam KG, Knudson CM, Powell JA. A lethal mutation in mice eliminates the slow calcium current in skeletal muscle cells. Nature 1986; 320: -168–70.
43 Sun W, McPherson JD, Hoang DQ, Wasmuth JJ, Evans GA, Montal M. Mapping of a human brain voltage-gated calcium channel to human chromosome 12p13-pter. Genomics 1992; 14: 1092-4.
44 Splawski I, Timothy KW, Decher N, Kumar P, Sachse FB, Beggs AH, *et al*. Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. Proc Natl Acad Sci USA 2005; 102: 8089–96.
45 Rottbauer W, Baker K, Wo ZG, Mohideen MA, Cantiello HF, Fishman MC. Growth and function of the embryonic heart depend upon the cardiac-specific L-type calcium channel alpha1 subunit. Dev Cell 2001; 1: 265–75.
46 Zhang Z, He Y, Tuteja D, Xu D, Timofeyev V, Zhang Q, *et al*. Functional roles of CaV1.3(alpha1D) calcium channels in atria: insights gained from gene-targeted null mutant mice. Circulation 2005; 112: 1936–44.
47 Seino S, Yamada Y, Espinosa R 3rd, Le Beau MM, Bell GI. Assignment of the gene encoding the alpha 1 subunit of the neuroendocrine/brain-type calcium channel (CACNL1A2) to human chromosome 3, band p14.3. Genomics 1992; 13: 1375–7.
48 Matthes J, Yildirim L, Wietzorrek G, Reimer D, Striessnig J, Herzig S. Disturbed atrio-ventricular conduction and normal contractile function in isolated hearts from Cav1.3-knockout mice. Naunyn Schmiedebergs Arch Pharmacol 2004; 369: 554–62.
49 Mangoni ME, Couette B, Bourinet E, Platzer J, Reimer D, Striessnig J, *et al*. Functional role of L-type Cav1.3 Ca^{2+} channels in cardiac pacemaker activity. Proc Natl Acad Sci USA 2003; 100: 5543–8.
50 Wappl E, Mitterdorfer J, Glossmann H, Striessnig J. Mechanism of dihydropyridine interaction with critical binding residues of L-type $Ca2^{+}$ channel alpha 1 subunits. J Biol Chem 2001; 20; 276: 12730–5.
51 Sinnegger-Brauns MJ, Hetzenauer A, Huber IG, Renstrom E, Wietzorrek G, Berjukov S, *et al*. Isoform-specific regulation of mood behavior and pancreatic beta cell and cardiovascular function by L-type Ca^{2+} channels. J Clin Invest 2004; 113: 1430–9.
52 Platzer J, Engel J, Schrott-Fischer A, Stephan K, Bova S, Chen H, *et al*. Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca^{2+} channels. Cell 2000; 102: 89–97.
53 Dou H, Vazquez AE, Namkung Y, Chu H, Cardell EL, Nie L, *et al*. Null mutation of alpha1D Ca^{2+} channel gene results in deafness but no vestibular defect in mice. J Assoc Res Otolaryngol 2004; 5: 215–26.
54 Sidi S, Busch-Nentwich E, Friedrich R, Schoenberger U, Nicolson T. gemini encodes a zebrafish L-type calcium channel that localizes at sensory hair cell ribbon synapses. J Neurosci 2004; 24: 4213–23.
55 Bech-Hansen NT, Naylor MJ, Maybaum TA, Pearce WG, Koop B, Fishman GA, *et al*. Loss-of-function mutations in a calcium-channel alpha1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. Nat Genet 1998; 19: 264–7.
56 Strom TM, Nyakatura G, pfelstedt-Sylla E, Hellebrand H, Lorenz B, Weber BH, *et al*. An L-type calcium-channel gene mutated in

incomplete X-linked congenital stationary night blindness. Nat Genet 1998; 19: 260–3.
57 Zeitz C, Minotti R, Feil S, Matyas G, Cremers FP, Hoyng CB, *et al*. Novel mutations in CACNA1F and NYX in Dutch families with X-linked congenital stationary night blindness. Mol Vis 2005; 11: 179–83.
58 Wei J, Hemmings GP. A further study of a possible locus for schizophrenia on the X chromosome. Biochem Biophys Res Commun 2006; 344: 1241–5.
59 Matsushita K, Wakamori M, Rhyu IJ, Arii T, Oda S, Mori Y, *et al*. Bidirectional alterations in cerebellar synaptic transmission of tottering and rolling Ca^{2+} channel mutant mice. J Neurosci 2002; 22: 4388–98.
60 Qian J, Noebels JL. Presynaptic Ca(2+) influx at a mouse central synapse with Ca(2+) channel subunit mutations. J Neurosci 2000; 20:163–70.
61 Mintz IM, Sabatini BL, Regehr WG. Calcium control of transmitter release at a cerebellar synapse. Neuron 1995; 15: 675–88.
62 Wu LG, Westenbroek RE, Borst JGG, Catterall WA, Sakmann B. Calcium channel types with distinct presynaptic localization couple differentially to transmitter release in single calyx-type synapses. J Neurosci 1999; 19: 726–36.
63 Llinas RR, Sugimori M, Cherksey B. Voltage-dependent calcium conductances in mammalian neurons. The P channel. Ann NY Acad Sci 1989; 560: 103–11.
64 Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes R, Frankel WN, *et al*. Absence epilepsy in tottering mutant mice is associated with calcium channel defects. Cell 1996; 87: 607–17.
65 Noebels JL, Sidman RL. Inherited epilepsy: spike-wave and focal motor seizures in the mutant mouse tottering. Science 1979; 204:1334–6.
66 Wakamori M, Yamazaki K, Matsunodaira H, Teramoto T, Tanaka I, Niidome T, *et al*. Single tottering mutations responsible for the neuropathic phenotype of the P-type calcium channel. J Biol Chem 1998; 273: 34857-67.
67 Zwingman TA, Neumann PE, Noebels JL, Herrup K. Rocker is a new variant of the voltage-dependent calcium channel gene Cacna1a. J Neurosci 2001; 21:1169–78.
68 Mori Y, Wakamori M, Oda S, Fletcher CF, Sekiguchi N, Mori E, *et al*. 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 2000; 20: 5654–62.
69 Noebels JL. The biology of epilepsy genes. Annu Rev Neurosci 2003; 26: 599–625.
70 Tsuji S, Meier H. Evidence for allelism of leaner and tottering in the mouse. Genet Res 1971; 17: 83–88.
71 Lorenzon NM, Lutz CM, Frankel WN, Beam KG. Altered calcium channel currents in Purkinje cells of the neurological mutant mouse leaner. J Neurosci 1998;18: 4482–9.
72 Dove LS, Abbott LC, Griffith WH. Whole-cell and single-channel analysis of P-type calcium currents in cerebellar Purkinje cells of leaner mutant mice. J Neurosci 1998; 18: 7687–99.
73 Hess EJ. Migraines in mice? Cell 1996; 87: 1149–51.
74 Jun K, Piedras-Renteria ES, Smith SM, Wheeler DB, Lee SB, Lee TG, *et al*. Ablation of P/Q-type Ca^{2+} channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the a1A -subunit. Proc Natl Acad Sci USA 1999; 96: 15245–50.
75 Inchauspe CG, Martini FJ, Forsythe ID, Uchitel OD. Functional compensation of P/Q by N-type channels blocks short-term plasticity at the calyx of held presynaptic terminal. J Neurosci 2004; 24: 10379–83
76 Miyazaki T, Hashimoto K, Shin HS, Kano M, Watanabe M. P/Q-type Ca^{2+} channel alpha1A regulates synaptic competition on developing cerebellar Purkinje cells. J Neurosci 2004; 24: 1734–43.
77 Qian J, Noebels JL. Presynaptic Ca^{2+} influx at a mouse central synapse with Ca^{2+} channel subunit mutations. J Neurosci 2000; 20:163-70.
78 Matsushita K, Wakamori M, Rhyu IJ, Arii T, Oda S, Mori Y, *et al*. Bidirectional alterations in cerebellar synaptic transmission of tottering and rolling Ca^{2+} channel mutant mice. J Neurosci 2002; 22: 4388–98.
79 Caddick SJ, Wang C, Fletcher CF, Jenkins NA, Copeland NG, Hosford DA. Excitatory but not inhibitory synaptic transmission is reduced in lethargic (Cacnb4lh) and tottering (Cacna1atg) mouse thalami. J Neurophysiol 1999; 81: 2066–74.
80 Leenders AG, van den Maagdenberg AM, Lopes da Silva FH, Sheng ZH, Molenaar PC, Ghijsen WE. Neurotransmitter release from tottering mice nerve terminals with reduced expression of mutated P- and Q-type Ca^{2+}-channels. Eur J Neurosci 2002; 15: 13–8.
81 Zhou YD, Turner TJ, Dunlap K. Enhanced G protein-dependent modulation of excitatory synaptic transmission in the cerebellum of the Ca^{2+} channel-mutant mouse, tottering. J Physiol 2003; 547: 497–507.
82 Pagani R, Song M, McEnery M, Qin N, Tsien RW, Toro L, *et al.* Differential expression of alpha 1 and beta subunits of voltage dependent Ca^{2+} channel at the neuromuscular junction of normal and P/Q Ca^{2+} channel knockout mouse. Neuroscience 2004; 123: 75–85.
83 Diriong S, Lory P, Williams ME, Ellis SB, Harpold MM, Taviaux S. Chromosomal localization of the human genes for alpha-1A, alpha-1B, and alpha-1E voltage-dependent Ca^{2+} channel subunits. Genomics 1995; 30: 605–9.
84 Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, *et al.* Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha (1A)-voltage-dependent calcium channel. Nat Genet 1997; 15: 62–9.
85 Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SMG, *et al*. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca(2+) channel gene CACNL1A4. Cell 1996; 87: 543–52.
86 Eunson LH, Graves TD, Hanna MG. New calcium channel mutations predict aberrant RNA splicing in episodic ataxia. Neurology 2005; 65: 308–10.
87 Imbrici P, Jaffe SL, Eunson LH, Davies NP, Herd C, Robertson R, *et al*. Dysfunction of the brain calcium channel CaV2.1 in absence epilepsy and episodic ataxia. Brain 2004; 127: 2682–92.
88 Kaunisto MA, Harno H, Kallela M, Somer H, Sallinen R, Hamalainen E, *et al*. Novel splice site CACNA1A mutation causing episodic ataxia type 2. Neurogenetics 2004; 5: 69–73.

89 Wessman M, Kaunisto MA, Kallela M, Palotie A. The molecular genetics of migraine. Ann Med 2004; 36: 462–73.
90 Trettel F, Mantuano E, Calabresi V, Veneziano L, Olsen AS, Georgescu A, *et al.* A fine physical map of the CACNA1A gene region on 19p13.1-p13.2 chromosome. Gene 2000; 241: 45–50.
91 Kraus RL, Sinnegger MJ, Glossmann H, Hering S, Striessnig J. Familial hemiplegic migraine mutations change alpha(1A)Ca (2+) channel kinetics. J Biol Chem 1998; 273: 5586–90.
92 Kraus RL, Sinnegger MJ, Koschak A, Glossmann H, Stenirri S, Carrera P, *et al*. Three new familial hemiplegic migraine mutants affect P/Q-type Ca(2+) channel kinetics. J Biol Chem 2000; 275: 9239–43.
93 van den Maagdenberg AMJM, Pietrobon D, Pizzorusso T, Kaja S, Broos LAM, Cesetti T, *et al.* A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. Neuron 2004; 41: 701–10.
94 Ducros A, Denier C, Joutel A, Vahedi K, Michel A, Darcel F, *et al*. Recurrence of the T666M calcium channel CACNA1A gene mutation in familial hemiplegic migraine with progressive cerebellar ataxia. Am J Hum Genet 1999; 64: 89–98.
95 Ducros A, Denier C, Joutel A, Cecillon M, Lescoat C, Vahedi K, *et al.* The clinical spectrum of familial hemiplegic migraine associated with mutations in a neuronal calcium channel. New Engl J Med 2001; 345: 17–24.
96 Manto MU. The wide spectrum of spinocerebellar ataxias (SCAs). Cerebellum 2005; 4: 2–6.
97 Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, *et al.* Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha (1A)-voltage-dependent calcium channel. Nat Genet 1997; 15: 62–9.
98 Ishikawa K, Tanaka H, Saito M, Ohkoshi N, Fujita T, Yoshizawa K, *et al*. Japanese families with autosomal dominant pure cerebellar ataxia map to chromosome 19p13.1-p13.2 and are strongly associated with mild CAG expansions in the spinocerebellar ataxia type 6 gene in chromosome 19p13.1. Am J Hum Genet 1997; 61: 336–46.
99 Kordasiewicz HB, Thompson RM, Clark HB, Gomez CM. C-termini of P/Q-type Ca^{2+} channel {alpha}1A subunits translocate to nuclei and promote polyglutamine-mediated toxicity. Hum Mol Genet 2006;15: 1587–99.
100 Jones OT. Ca^{2+} channels and epilepsy. Eur J Pharmacol 2002; 447: 211–25.
101 Sander T, Peters C, Janz D, Bianchi A, Bauer G, Wienker TF, *et al*. The gene encoding the alpha1A-voltage-dependent calcium channel (CACN1A4) is not a candidate for causing common subtypes of idiopathic generalized epilepsy. Epilepsy Res 1998; 29: 115–22
102 Chioza B, Wilkie H, Nashef L, Blower J, McCormick D, Sham P, *et al.* Association between the alpha-1A calcium channel gene CACNA1A and idiopathic generalized epilepsy. Neurology 2001; 56: 1245–6.
103 Chioza B, Nashef L, Asherson P, Makoff A. CACNA1A and P/Q-type calcium channels in epilepsy. Lancet 2002; 359: 258.
104 Sander T, Toliat MR, Heils A, Becker C, Nurnberg P. Failure to replicate an allelic association between an exon 8 polymorphism of the human alpha(1A) calcium channel gene and common syndromes of idiopathic generalized epilepsy. Epilepsy Res 2002; 49: 173–7.
105 Jouvenceau A, Eunson LH, Spauschus A, Ramesh V, Zuberi SM, Kullmann DM, *et al*. G.Human epilepsy associated with dysfunction of the brain P/Q-type calcium channel. Lancet 2001; 358: 801–7.
106 Strupp M, Kalla R, Dichgans M, Freilinger T, Glasauer S, Brandt T. Treatment of episodic ataxia type 2 with the potassium channel blocker 4-aminopyridine. Neurology 2004; 62: 1623–5.
107 Saegusa H, Matsuda Y, Tanabe T. Effects of ablation of N- and R-type Ca(2+) channels on pain transmission. Neurosci Res 2002; 43: 1–7.
108 Ino M, Yoshinaga T, Wakamori M, Miyamoto N, Takahashi E, Sonoda J, *et al*. Functional disorders of the sympathetic nervous system in mice lacking the alpha 1B subunit (Cav 2.2) of N-type calcium channels. Proc Natl Acad Sci USA 2001; 98: 5323–8.
109 Newton PM, Orr CJ, Wallace MJ, Kim C, Shin HS, Messing RO. Deletion of N-type calcium channels alters ethanol reward and reduces ethanol consumption in mice. J Neurosci 2004; 24: 9862–9.
110 Takei T, Saegusa H, Zong S, Murakoshi T, Makita K, Tanabe T. Increased sensitivity to halothane but decreased sensitivity to propofol in mice lacking the N-type Ca^{2+} channel. Neurosci Lett 2003; 350: 41–5.
111 Kamp MA, Krieger A, Henry M, Hescheler J, Weiergraber M, Schneider T. Presynaptic 'Ca2.3-containing' E-type Ca channels share dual roles during neurotransmitter release. Eur J Neurosci 2005; 21: 1617–25.
112 Breustedt J, Vogt KE, Miller RJ, Nicoll RA, Schmitz D. Alpha1E-containing Ca^{2+} channels are involved in synaptic plasticity. Proc Natl Acad Sci USA 2003; 100: 12450–5.
113 Saegusa H, Matsuda Y, Tanabe T. Effects of ablation of N- and R-type Ca(2+) channels on pain transmission. Neurosci Res 2002; 43:1–7.
114 Jing X, Li DQ, Olofsson CS. Salehi A, Surve VV, Caballero J, *et al.* CaV2.3 calcium channels control second-phase insulin release. J Clin Invest 2005; 115: 146–54.
115 Mittman S, Guo J, Agnew WS. Structure and alternative splicing of the gene encoding alpha1G, a human brain T calcium channel alpha1 subunit. Neurosci Lett 1999; 274:143–6.
116 Yunker AM, Sharp AH, Sundarraj S, Ranganathan V, Copeland TD, McEnery MW. Immunological characterization of T-type voltage-dependent calcium channel CaV3.1 (alpha 1G) and CaV3.3 (alpha 1I) isoforms reveal differences in their localization, expression, and neural development. Neuroscience 2003; 117: 321–35.
117 Mizuta E, Miake J, Yano S, Furuichi H, Manabe K, Sasaki N, *et al.* Subtype switching of T-type Ca^{2+} channels from Cav3.2 to Cav3.1 during differentiation of embryonic stem cells to cardiac cell lineage. Circ J 2005; 69: 1284–9.
118 Anderson MP, Mochizuki T, Xie J, Fischler W, Manger JP, Talley EM, *et al.* Thalamic Cav3.1 T-type Ca^{2+} channel plays a crucial role in stabilizing sleep. Proc Natl Acad Sci USA 2005; 102: 1743–8.
119 Lee J, Kim D, Shin HS. Lack of delta waves and sleep disturbances during non-rapid eye movement sleep in mice lacking alpha1G-subunit of T-type calcium channels. Proc Natl Acad

Sci USA 2004; 101: 18195–9.
120 Mangoni ME, Traboulsie A, Leoni AL, Couette B, Marger L, Le Quang K, *et al*. Bradycardia and slowing of the atrioventricular conduction in mice lacking CaV3.1/{alpha}1G T-type calcium channels. Circ Res 2006; 98: 1422–30.
121 Cribbs LL, Lee JH, Yang J, Satin J, Zhang Y, Daud A, *et al*. Cloning and characterization of alpha1H from human heart, a member of the T-type Ca^{2+} channel gene family. Circ Res 1998; 83: 103–9.
122 Bourinet E, Alloui A, Monteil A, Barrere C, Couette B, Poirot O, *et al*. Silencing of the CaV3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception. EMBO J 2005; 24: 315–24.
123 Chen CC, Lamping KG, Nuno DW, Barresi R, Prouty SJ, Lavoie JL, *et al*. Abnormal coronary function in mice deficient in alpha1H T-type Ca^{2+} channels. Science 2003; 302: 1416–8.
124 Stamboulian S, Kim D, Shin HS, Ronjat M, De WM, Arnoult C. Biophysical and pharmacological characterization of spermatogenic T-type calcium current in mice lacking the CaV3.1 (alpha1G) calcium channel: CaV3.2 (alpha1H) is the main functional calcium channel in wild-type spermatogenic cells. J Cell Physiol 2004; 200: 116–24.
125 Chen Y, Lu J, Pan H, Zhang Y, Wu H, Xu K, *et al*. Association between genetic variation of CACNA1H and childhood absence epilepsy. Ann Neurol 2003; 54: 239–43
126 Khosravani H, Altier C, Simms B, Hamming KS, Snutch TP, Mezeyova J, *et al*. Gating effects of mutations in the Cav3.2 T-type calcium channel associated with childhood absence epilepsy. J Biol Chem 2004; 279: 9681–84
127 Heron SE, Phillips HA, Mulley JC, Mazarib A, Neufeld MY, Berkovic SF, *et al*. Genetic variation of CACNA1H in idiopathic generalized epilepsy. Ann Neurol 2004; 55: 595-6.
128 Wolfe JT, Wang H, Howard J, Garrison JC, Barrett PQ. T-type calcium channel regulation by specific G-protein beta-gamma subunits. Nature 2003; 424: 209–13,
129 Mittman S, Guo J, Emerick MC, Agnew WS. Structure and alternative splicing of the gene encoding alpha-1I, a human brain T calcium channel alpha-1 subunit. Neurosci Lett 1999; 269: 121–4,
130 Talley EM, Cribbs LL, Lee JH, Daud A, Perez-Reyes E, Bayliss DA. Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. J Neurosci 1999;19: 1895–911.
131 Perez-Reyes E. Molecular physiology of low-voltage-activated t-type calcium channels. Physiol Rev 2003; 83: 117–61.
132 Wan J, Khanna R, Sandusky M, Papazian DM, Jen JC, Baloh RW. CACNA1A mutations causing episodic and progressive ataxia alter channel trafficking and kinetics. Neurology 2005; 64: 2090–7.
133 Hoda JC, Zaghetto F, Koschak A, Striessnig J. Congenital stationary night blindness type 2 mutations S229P, G369D, L1068P, and W1440X alter channel gating or functional expression of Ca(V)1.4 L-type Ca^{2+} channels. J Neurosci 2005; 25: 252–9.
134 Hoda JC, Zaghetto F, Singh A, Koschak A, Striessnig J. Effects of congenital stationary night blindness type 2 mutations R508Q and L1364H on CaV1.4 L-type Ca^{2+} channel function and expression. J Neurochem 2006; 96: 1648–58.
135 Jacobi FK, Hamel CP, Arnaud B, Blin N, Broghammer M, Jacobi PC, Apfelstedt-Sylla E, Pusch CM. A novel CACNA1F mutation in a french family with the incomplete type of X-linked congenital stationary night blindness. Am J Ophthalmol 2003; 135: 733–6.
136 Sun W, McPherson JD, Hoang DQ, Wasmuth JJ, Evans GA, Montal M. Mapping of a human brain voltage-gated calcium channel to human chromosome 12p13-pter. Genomics 1992; 14: 1092–1094.
137 Kors EE, Terwindt GM, Vermeulen FL, Fitzsimons RB, Jardine PE, Heywood P, *et al*. Delayed cerebral edema and fatal coma after minor head trauma: role of the CACNA1A calcium channel subunit gene and relationship with familial hemiplegic migraine. Ann Neurol 2001; 49: 753–60.
138 Battistini S, Stenirri S, Piatti M, Gelfi C, Righetti PG, Rocchi R, *et al*. A new CACNA1A gene mutation in acetazolamide-responsive familial hemiplegic migraine and ataxia. Neurology 1999; 53: 38–43.
139 Vahedi K, Denier C, Ducros A, Bousson V, Levy C, Chabriat H, *et al*. CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy. Neurology 2000; 55: 1040–2.
140 Carrera P, Piatti M, Stenirri S, Grimaldi LM, Marchioni E, Curcio M, *et al*. Genetic heterogeneity in Italian families with familial hemiplegic migraine. Neurology 1999; 53: 26–33.
141 Gardner K. The genetic basis of migraine: how much do we know? Can J Neurol Sci 1999; 26(Suppl 3): S37–43.
142 Denier C, Ducros A, Vahedi K, Joutel A, Thierry P, Ritz A, *et al*. High prevalence of CACNA1A truncations and broader clinical spectrum in episodic ataxia type 2. Neurology 1999; 52: 1816–21.
143 Scoggan KA, Chandra T, Nelson R, Hahn AF, Bulman DE. Identification of two novel mutations in the CACNA1A gene responsible for episodic ataxia type 2. J Med Genet 2001; 38: 249–53.
144 Matsuyama Z, Murase M, Shimizu H, Aoki Y, Hayashi M, Hozumi I, *et al*. A novel insertion mutation of acetazolamide-responsive episodic ataxia in a Japanese family. J Neurol Sci 2003; 210: 91–3.
145 Yue Q, Jen JC, Thwe MM, Nelson SF, Baloh RW. *De novo* mutation in CACNA1A caused acetazolamide-responsive episodic ataxia. Am J Med Genet 1998; 77: 298–301.
146 Friend KL, Crimmins D, Phan TG, Sue CM, Colley A, Fung VS, *et al*. Detection of a novel missense mutation and second recurrent mutation in the CACNA1A gene in individuals with EA-2 and FHM. Hum Genet 1999; 105: 261–5.
147 Spacey SD, Hildebrand ME, Materek LA, Bird TD, Snutch TP. Functional implications of a novel EA-2 mutation in the P/Q-type calcium channel. Ann Neurol 2004; 56: 213–20.
148 Tonelli A, D'Angelo MG, Salati R, Villa L, Germinasi C, Frattini T, *et al*. Early onset, non fluctuating spinocerebellar ataxia and a novel missense mutation in CACNA1A gene. J Neurol Sci 2006; 241: 13–7.
149 Jarvis SE, Barr W, Feng ZP, Hamid J, Zamponi GW. Molecular determinants of syntaxin 1 modulation of N-type calcium channels. J Biol Chem 2002; 277: 44399–407.
150 Wiser O, Bennett MK, Atlas D. Functional interaction of syntaxin and SNAP-25 with voltage-sensitive L- and N-type Ca^{2+} channels. EMBO J 1996; 15: 4100–10.

151 Magga JM, Jarvis SE, Arnot MI, Zamponi GW, Braun JE. Cysteine string protein regulates G protein modulation of N-type calcium channels. Neuron 2000; 28: 195–204.

152 Sheng ZH, Yokoyama CT, Catterall WA. Interaction of the synprint site of N-type Ca^{2+} channels with the C2B domain of synaptotagmin I. Proc Natl Acad Sci USA 1997; 94: 5405–10.

153 Maximov A, Sudhof TC, Bezprozvanny I. Association of neuronal calcium channels with modular adaptor proteins. J Biol Chem 1999; 274: 24453–6.

154 Erickson MG, Alseikhan BA, Peterson BZ, Yue DT. Preassociation of calmodulin with voltage-gated Ca^{2+} channels revealed by FRET in single living cells. Neuron 2001; 31: 973–85.

155 Strock J, Diverse-Pierluissi MA. Ca^{2+} channels as integrators of G protein-mediated signaling in neurons. Mol Pharmacol 2004; 66: 1071–6.

156 Altier C, Dubel SJ, Barrere C, Jarvis SE, Stotz SC, Spaetgens RL, *et al*. Trafficking of L-type calcium channels mediated by the postsynaptic scaffolding protein AKAP79. J Biol Chem 2002; 277: 33598–603.

157 Lee D, Obukhov AG, Shen Q, Liu Y, Dhawan P, Nowycky MC, *et al*. Calbindin-D(28k) decreases L-type calcium channel activity and modulates intracellular calcium homeostasis in response to K(+) depolarization in a rat beta cell line RINr1046-38. Cell Calcium 2006; 39: 475–85.

158 Hudmon A, Schulman H, Kim J, Maltez JM, Tsien RW, Pitt GS. CaMKII tethers to L-type Ca^{2+} channels, establishing a local and dedicated integrator of Ca^{2+} signals for facilitation. J Cell Biol 2005; 171: 537–47.