Review

Disease causing mutations of calcium channels

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Calcium ions play an important role in the electrical excitability of nerve and muscle, as well as serving as a critical second messenger for diverse cellular functions. As a result, mutations of genes encoding calcium channels may have subtle affects on channel function yet strongly perturb cellular behavior. This review discusses the effects of calcium channel mutations on channel function, the pathological consequences for cellular physiology, and possible links between altered channel function and disease.

Many cellular functions are directly or indirectly regulated by the free cytosolic calcium concentration. Thus, calcium levels must be very tightly regulated in time and space. Intracellular calcium ions are essential secondary messengers and play a role in many functions including, action potential generation, neurotransmitter and hormone release, muscle contraction, neurite outgrowth, synaptic plasticity, calcium-dependent gene expression, and cell death. Calcium ions that control cell activity can be supplied to the cell cytosol from two major sources: the extracellular space or intracellular stores. Voltage-gated and ligand-gated channels are the primary way in which Ca2+ ions enter from the extracellular space. The sarcoplasmic reticulum (SR) in muscle and the endoplasmic reticulum in non-muscle cells are the main intracellular Ca2+ stores: the ryanodine receptor (RyR) and inositol-triphosphate receptor channels are the major contributors of calcium release from internal stores.

Mutations of genes encoding calcium have been implicated in the etiology of a diverse group of nerve and muscle diseases. These mutations have been identified in humans, mice and other organisms. In this review, we will summarize calcium channelopathies of humans and mice. Of the ten calcium channel α1 subunits cloned and sequenced (reviewed in ref. 1), disease-causing mutations have been found in CaV1.4 and CaV2.1 in the nervous system, and CaV1.1 and CaV1.2 in muscle. Mutations in calcium channel auxiliary subunits (α2, δ, β and γ) have also been associated with both human and/or mouse neurological diseases. The disease-causing mutations may provide new insight into the cell biological roles of calcium channels as well as into relationships between structure and function. In addition, understanding how the mutations affect the physiology of the cell could lead to advances in disease treatment by relieving symptoms or slowing the progression of the disease. However, due to the multifaceted functions of calcium in the cell, the correlation between molecular mutation, physiological alterations and disease etiology is neither straightforward nor easily understood. Since calcium is an important intracellular signaling molecule, altered channel function can give rise to widespread changes in cellular function. Indeed, serious diseases result from mutations that cause trivial alterations of calcium currents analyzed in vitro.

Calcium Channelopathies of the Nervous System

Neuronal calcium channels have many cellular functions including control of neurotransmitter release, regulation of gene expression, integration and propagation of postsynaptic signals and neurite outgrowth. In addition to altering these signaling pathways, calcium channel mutations could in principle cause cytotoxicity. In particular, both increased and decreased intracellular Ca2+ have been reported to be cytotoxic to neurons. Neuronal calcium channels have been associated with several dominantly inherited human diseases ranging from visual disorders to migraines, ataxia and seizures. Mice have been described which have recessively-inherited phenotypes attributable to calcium channel defects. Some symptoms associated with human calcium channelopathies are similar to those observed in the mutant mice; thus, the study of these murine disorders may lead to a better understanding of calcium channel function and etiology of calcium channelopathies in humans.

Mutations of CaV1.4 calcium channels. Based on its amino acid sequence, CaV1.4 is an L-type channel, which is highly expressed in retina.6,7 Human incomplete X-linked congenital stationary night blindness (CSNB-2) is a non-progressive, dominantly inherited disorder. Afflicted persons can show a range of symptoms including night blindness, decreased visual acuity, myopia, nystagmus, strabismus and abnormal electroretinograms. These impairments are thought to result from altered synaptic transmission from photoreceptor cells to second-order neurons. CSNB-2 has been linked to more than 50 mutations in the CaV1.4 subunit of a retinal L-type channel. CaV1.4 is expressed highly in retina: strongly in outer and inner nuclear layers and weakly in ganglion cells.8 Mutations

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causing CSNB-2 (Fig. 1) include missense changes, deletions and
frameshifts with premature stops.6-10 The effects of at least nine
CSNB-2 mutations on channel function have been characterized in
heterologous expression systems.11-15 Some of the CaV1.4 channel
mutants (e.g., G369D,11 F742C15) exhibit a hyperpolarizing shift
in the voltage-dependence of activation. Some mutant channels fail
to generate current (W1440X,12 G1007R and R1049W15), even
though these mutant channels produce full-length proteins that are
targeted to the plasma membrane appropriately.15 By contrast, the
CaV1.4 R508Q and L1364H mutated channels exhibit reduced
CaV1.4 channel protein expression.13 In addition, other mutations
(e.g., A928D, G674D) appear not to cause significant alterations in
channel biophysical properties.11

The functional effects of altered CaV1.4 channel expression have
been studied in a mouse model of CSNB-2. The nob2 (no b-wave 2)
mouse is a naturally occurring mutant that carries a null mutation
in the Cacna1f gene.16 Nob2 mice demonstrate a significant decrease
in depolarization-induced calcium influx into photoreceptor termi-
nals.16 Nob2 photoreceptors respond with a normal hyperpolarization
to light; however, transmission from photoreceptors to second-order
neurons is significantly lost.17 These results support the concept that
CaV1.4 is the primary voltage-gated calcium channel mediating tonic
glutamate release from rods and cones.17 Physically, the retinas of
nob2 mice show abnormal outgrowth of rod bipolar cell dendrites
and horizontal cell processes into the outer nuclear layer (ONL) and
ectopic synaptic contacts with rod photoreceptors in the ONL.18

A second visual disorder has been associated with mutations in
CaV1.4.19 This disease was described in a single pedigree and is
similar to, but distinct from, CSNB-2. Clinically, the phenotype
in heterozygous male individuals is more severe than that typically
seen in CSNB-2, and female heterozygotes exhibit clinical signs
and ERG abnormalities (heterologous CSNB-2 females do not).

Figure 1. Mutations in CaV1.4 causing incomplete x-linked congenital night blindness. Schematic representation of the CaV1.4 subunit membrane topology
and locations of mutations. Both the amino and carboxyl terminals are intracellular; polypeptide has four homologous repeats (I–IV); each of which contains
six putative transmembrane segments (S1–S6). Circles indicated mutations associated with CSNB-2, and star indicates mutation (I745T)20 causing related
visual defect. In this and all subsequent figures mutations are indicated by the number of the affected amino acid. Superscript numbers indicate the references
in which the mutations were identified and described. Amino acid numbering as in each reference cited.

Mutations in CaV2.1 calcium channels. CaV2.1 channels are
expressed in cell bodies, dendrites and presynaptic terminals of most
central neurons21 with highest expression in cerebellar neurons.21-25
As a result of alternative splicing, the CaV2.1 gene produces both
P- and Q-type currents (as defined by current kinetics and sensitivity
to ω-Aga-IVA),26 which mediate synaptic neurotransmitter release
in many cell types (reviewed in refs. 27 and 28). Important in regard
to the channelopathies described in this section is that alternative
splicing of several regions of the CaV2.1 gene (Fig. 2) is known
to produce striking alterations in the properties of the channel. One
alternative splicing inserts a valine residue in the domain I–II linker
and causes alterations in inactivation and modulation by second
messengers. A second alternative splicing inserts an asparagine and
proline (+NP) into the S3–S4 extracellular linker in domain IV of
CaV2.1. This insertion alters the voltage-dependence of activation
and the sensitivity to ω-Aga-IVA. The ±NP splice isoforms display
differential expression in the CNS.26 For example, most cerebellar
transcripts lack the NP insertion, while in the hippocampus the
+NP and -NP isoforms are about equally expressed. The differential
expression patterns of CaV2.1 isoforms may mean that a particular
CaV2.1 mutation could have distinct functional consequences in
different cell types. In addition, there are a number of alternatively
spliced isoforms of the C-terminal domain of the CaV2.1 subunit,
including two major isoforms that have been designated α1A-1

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and α1A-2 in humans\textsuperscript{29} and B1-I and B1-II in rabbits\textsuperscript{32} and rats.\textsuperscript{23} Because of this alternative splicing, some mutations of CaV2.1 are present in certain C-terminal isoforms, as described below for spinocerebellar ataxia-6.

Mutations in the CaV2.1 gene have been shown to produce a spectrum of human autosomal-dominant neurological disorders: familial hemiplegic migraine, episodic ataxia type-2, spinocerebellar ataxia-6, episodic-and-progressive ataxia and generalized epilepsy. Some of these diseases have overlapping phenotypes; where some mutations can cause migraine or ataxia or cause migraine, ataxia and epilepsy. Defects in the gene encoding the CaV2.1 calcium channel subunit are also responsible for the recessively-inherited phenotypes of tottering, leaner, rolling Nagoya and rocker in mice. In each of these diseases, alteration of the CaV2.1 gene results in a host of neurological aberrations. In general, CaV2.1 mutations have pronounced effects on the cerebellum, which is not surprising given that CaV2.1 is highly expressed in cerebellar neurons. In particular, about 90% of rat Purkinje cell calcium current is blocked with high affinity by the toxin ω-Aga-IVA,\textsuperscript{30} indicating that this large fraction of current arises from CaV2.1.\textsuperscript{26} The diseases associated with mutations in the CaV2.1 subunit are discussed below.

Familial hemiplegic migraine (FHM) is a dominantly inherited human disorder with symptoms that include headaches accompanied by aura and hemiparesis (which is typically transient hours to days), ataxia and nystagmus. In some families, cerebellar atrophy may also occur. The onset of this disorder usually occurs in childhood or adolescence. FHM can be accompanied by ataxia and in some cases by childhood epilepsy.\textsuperscript{31} Recent reviews of migraine etiology suggest possible mechanisms by which calcium channels contribute to migraine.\textsuperscript{32} Genetic studies of FHM patients have identified 15 missense mutations causing defects in the CaV2.1 subunit\textsuperscript{33-40} (Fig. 2), with T666M being the most common mutation.\textsuperscript{36} These missense mutations affect highly conserved residues\textsuperscript{33} and are widely distributed throughout the protein.

Heterologous expression of CaV2.1 containing FHM mutations has revealed changes in both macroscopic P/Q-type current density (which could arise from changes in surface protein expression, single channel conductance or open probability) and specific channel properties. Hans et al.\textsuperscript{41} and Tottene et al.\textsuperscript{42} analyzed human CaV2.1 (specifically, α1A-2) coexpressed with α2δ and either β2a or β3s in HEK293 cells, whereas Kraus et al.\textsuperscript{43,44} expressed rabbit CaV2.1 (specifically, B2) together with α8 and β1s subunits in Xenopus oocytes or HEK293 cells (for simplicity, the rabbit mutations are renumbered according to the human sequence). Additionally, Tottene et al.\textsuperscript{42} analyzed expression of human α1A-2 in cerebellar granule cells from CaV2.1 null mice. In the human channel studies in HEK293 cells, the current density was increased for R192Q and decreased for T666M, V714A, I1811L\textsuperscript{41} and V1457L.\textsuperscript{42} Interestingly, in cerebellar granule cells, all of the five mutations above caused a reduction in the density of whole-cell calcium current.\textsuperscript{42} At the single channel level (human α1A-2 in HEK293 cells), open probability was increased for R192Q, T666M, V714A, I1811L\textsuperscript{41} and V1457L.\textsuperscript{42} Of these five mutations, single channel conductance was decreased for T666M and V714A\textsuperscript{41} and V1457L.\textsuperscript{42} Thus, Tottene et al.\textsuperscript{42} suggest that common features of FHM causing mutations are (i) a hyperpolarizing shift in the single channel p\textsubscript{o} versus voltage relationship, and (ii) a decrease in maximal current density at the whole cell level. A number of additional changes have been found in both the human and rabbit channel experiments. The mutations R538Q, T666M, V714A, D715E, V1457L and I1811L all significantly shifted the voltage-dependence of activation to more hyperpolarized potentials (T666M, V714A, I1811L;\textsuperscript{45} R538Q, D715E, V1457L;\textsuperscript{44,46} V714A, I1815L;\textsuperscript{41} V1457L).\textsuperscript{42} This would result in an increase in the calcium current in response to weak depolarizations. Two mutations (R538Q and D715E) caused a reduction in calcium current during pulse trains as a result of faster inactivation for D715E and slower recovery from inactivation for R538Q.\textsuperscript{44} This could alter calcium influx especially during high, but not low, neuronal activity, thus imposing the episodic nature of FHM with attacks triggered by sensory or emotional stimuli.\textsuperscript{44} Confocal microscopy and western blot analyses suggest that at least in the case of the T666M mutation (the most common mutation), the decrease in current density is due to a reduced number of channels available for voltage-dependent gating and not due to a reduced number of channels expressed at the protein level.
the plasma membrane. The FHM mutation S218L appears to have the greatest effects on channel function and has been associated with additional clinical features. FHM patients carrying the S218L mutation exhibit characteristic FHM attacks triggered by minor head trauma which are accompanied by deep or fatal coma and long-lasting severe cerebral edema. The biophysical alterations associated with S218L are (i) a shift in voltage-dependence of activation to voltages at or near resting membrane potential, and (ii) a significant decrease in the rate of inactivation and a faster recovery from inactivation.

The effects on channel function of some FHM mutations depend on the identity of the co-expressed \( \beta \) subunit, which can help to explain inconsistencies in reported effects from different neuronal cell types and experimental expression systems. For example, \( \text{Ca}_v\cdot2.1 \) K1336E currents showed a negative shift in the voltage-dependence of inactivation only when co-expressed with the \( \beta_3 \) subunit.

A knock-in mouse carrying the human FHM mutation R192Q exhibited multiple gain-of-function effects: increased current density in cerebellar granule cells, increased transmission at the neuromuscular junction, and reduced threshold and increased velocity of cortical spreading depression (CSD) in the intact animal. These authors concluded that the increased susceptibility for CSD and aura in migraine might be due to cortical hyperexcitability.

**Episodic ataxia-2 (EA-2)** is a dominantly-inherited human disorder, which results in symptoms of ataxia, nystagmus, dysarthria and vertigo. Cerebellar atrophy is common, and 50% of EA-2 patients report migraine-like symptoms. The ataxia is provoked by stress, exercise or fatigue and lasts hours to days. Between attacks, nystagmus on lateral or vertical gaze is the most prominent sign. A common feature is responsiveness of symptoms to acetazolamide. The onset of this disorder is usually during late childhood or adolescence. Affected individuals show a broad range of phenotypes (including non-ataxic manifestations), and of the severity of both episodic and permanent neurologic abnormalities. Jen et al. described the presence of great variability in penetrance and symptoms within a single large pedigree, which suggests that environmental, hormonal and/or genetic factors other than \( \text{Ca}_v\cdot2.1 \) are important for the phenotype of EA-2.

More than 20 mutations causing EA-2 (Fig. 3) have been identified in the \( \text{Ca}_v\cdot2.1 \) subunit. Originally, the mutations found were predicted to lead to premature truncation of the protein; however, more recently, missense mutations and intronic mutations (that would be predicted to cause aberrant splicing) have also been correlated with this disease.

Functional effects of one missense mutation (F1491S, located in III56) have been studied by means of heterologous expression of mutant human \( \alpha_{1\text{A-2}} \) (with \( \beta_3 \) and \( \alpha_\delta \)) in HEK293 cells. Although immunostaining indicated that the mutant channel protein was expressed, calcium channel activity was abolished. Another study examined the effects of the R1279X and A1593D/delY1594 mutations on human \( \text{Ca}_v\cdot2.1 \) (coexpressed with \( \beta_1 \) and \( \alpha_\delta \)) in tsA-201 cells and Xenopus oocytes. The R1279X-mutated channel produced no current when expressed either in tsA-201 cells or Xenopus oocytes. The A1593D/delY1594 mutant did not mediate current in tsA-201 cells, but did mediate current when expressed in Xenopus oocytes. Compared to control \( \text{Ca}_v\cdot2.1 \) currents, the currents from the mutated channel exhibited a depolarizing shift in the voltage-dependence of activation, an increase in inactivation during 3-s test pulses, and a slowing of recovery from inactivation. This latter study thus demonstrates the need to employ more than one type of expression system before concluding that channels are non-functional. In addition, mutations need not lead to a complete loss of function to produce the symptoms of EA-2; dramatic reduction in current can be sufficient to cause these symptoms.

**Episodic and progressive ataxia** was originally categorized as a distinct clinical entity although its attributes overlap those of EA-2 and SCA6. Distinguishing features include earlier onset (5–15 years of age), more prominent cerebellar atrophy and, unlike EA-2, insensitivity to treatment with acetazolamide. Some family members exhibit severe progressive ataxia without episodic features, while others have a combination of progressive and episodic ataxia. The disease has been associated with a missense mutation (G293R; Fig. 3).
of a conserved amino acid in the S5–S6 linker (pore region) of repeat I.\textsuperscript{61} Heterologously expressed G293R-mutated Ca\textsubscript{v}2.1 (coexpressed with β\textsubscript{1}\textsubscript{a} and α\textsubscript{2,δ} in Xenopus oocytes) displayed a decrease in current density, a depolarizing shift in the voltage-dependence of activation, and enhanced inactivation compared to control Ca\textsubscript{v}2.1 currents. The mutant channels exhibited a decrease in mean open time.\textsuperscript{60}

Primary generalized epilepsy and episodic and progressive ataxia has been associated with a Ca\textsubscript{v}2.1 mutation in an 11-year-old patient.\textsuperscript{62} The patient had generalized tonic-clonic seizures and frequent absence attacks. The episodic ataxia was similar to that in EA-2 patients. Molecular screening of Ca\textsubscript{v}2.1 identified a heterozygous point mutation (R1820X; Fig. 3) that would produce a premature stop codon and complete loss of the C-terminal region of the channel protein. Expression studies in Xenopus oocytes determined that the mutant Ca\textsubscript{v}2.1 was non-functional (coexpressed with β\textsubscript{3} and α\textsubscript{2,δ}). When coexpressed with wild-type Ca\textsubscript{v}2.1, the mutant Ca\textsubscript{v}2.1 exhibited a robust dominant negative interaction with expression of the wild-type subunit.\textsuperscript{62} A second study investigated a family in which five patients exhibited absence epilepsy in combination with cerebellar ataxia.\textsuperscript{63} A novel point mutation (E147K) in domain I-S2 region of the Ca\textsubscript{v}2.1 calcium channel was identified that segregated with the epileptic/ataxic phenotype. Functional analyses demonstrated that this channel mutation resulted in a decrease in calcium current amplitude.\textsuperscript{63}

Autosomal dominant spinocerebellar ataxia 6 (SCA6) is a dominantly inherited disorder characterized by ataxia, nystagmus, dysarthria, and neuronal loss in the cerebellum (Purkinje and granule cells) and the dentate and inferior olivary nuclei. Symptoms initially appear at 40–50 years of age and become progressively more severe. Cerebella of SCA6 patients exhibit a severe loss of Purkinje cells, especially in the vermis, and various morphological changes in Purkinje cells and their dendritic arbors.\textsuperscript{64}

Molecular analyses of patients with SCA6 demonstrated an expanded CAG repeat in exon 47 of the Ca\textsubscript{v}2.1 gene. Normal individuals have 4–16 CAGs and affected individuals have 21–27, which is much smaller than the trinucleotide repeat expansions associated with many other neurodegenerative disorders.\textsuperscript{29} In addition, the repeat is more stable and anticipation (progressive expansion from generation to generation) is much milder than observed with other trinucleotide repeat disorders. Great clinical variability has been described in SCA6 symptoms. Similar length CAG expansions have been observed in individuals having symptoms of both SCA6 and EA-2\textsuperscript{65} or only of EA-2,\textsuperscript{49} which may indicate that the two diseases actually represent a continuum of symptoms. Repeat length effects have been found; however, other unknown factors must be important for phenotypic expression of SCA6. In support of this concept is that two sisters homozygous for SCA6 with identical CAG expansion length (25 repeats) and age of onset (27 years old) presented clinically with marked differences in disease progression and severity.\textsuperscript{66}

One patient had rapidly progressing ataxia, moderate dysarthria, mild dysphagia, nystagmus and moderate cerebellar atrophy. The other sister presented with milder and far slower progressing ataxia, slightly slurred speech and moderate cerebellar atrophy, but no other clinical symptoms.

The CAG expansion in SCA6 introduces polyglutamines at a position approximately 100 amino acids from the carboxyl terminal (Fig. 3). Increased repeat size is associated with both greater disease severity and earlier onset. The pathological consequences of many diseases involving trinucleotide repeats are thought to be related to altered stability of the mutant protein, toxic metabolic breakdown products of the glutamines, or the interaction of the CAG repeats with proteins required for processing RNA. Alternatively, the polyglutamine expansion causing SCA6 might alter the biophysical properties, expression or protein-protein interactions, of Ca\textsubscript{v}2.1.

One functional study expressed rabbit Ca\textsubscript{v}2.1 (BI-1) with 4, 24, 30 and 40 CAG repeats in BHK cells (coexpressed with α\textsubscript{2,δ} and β\textsubscript{1}).\textsuperscript{67} Currents from cells expressing α\textsubscript{1A}-30 CAG or -40 CAG showed a significant (8 mV) hyperpolarizing shift in the voltage-dependence of inactivation. This would considerably decrease the number of available channels at resting potential.\textsuperscript{67} In another study, heterologous expression of rabbit-human chimeric Ca\textsubscript{v}2.1 in Xenopus oocytes showed β-subunit specific alterations in electrophysiological properties of the mutant channels.\textsuperscript{68} When coexpressed with α\textsubscript{2,δ} and β\textsubscript{2}, the mutated Ca\textsubscript{v}2.1 exhibited a loss of regulation by G-proteins, a hyperpolarizing shift in the voltage-dependence of activation and a slowed rate of inactivation, although no changes in activation or inactivation occurred upon coexpression with α\textsubscript{2,δ} and β\textsubscript{3} or β\textsubscript{2,3}. Similarly, after coexpression in HEK293 cells of human Ca\textsubscript{v}2.1 (homologous to the construct used in ref. 68) together with α\textsubscript{2,δ} and β\textsubscript{1C}, increased surface expression was observed with only small alterations in voltage-dependence of activation and no changes in inactivation.\textsuperscript{69}

Although the CAG repeat is in frame for some C-terminal splice isoforms of the α\textsubscript{1A} subunit, it is out of frame for others.\textsuperscript{29} Thus, understanding the consequences of the CAG repeat expansion requires information on the differential expression of the C-terminal splice isoforms. Immunological studies have shown that the exon 47-encoded polyglutamine tract is abundantly expressed in cell bodies and dendrites of cerebellar Purkinje cells, the cells most affected by this disease.\textsuperscript{68} It appears that it will also be important to have additional information regarding the differential expression of isoforms with alternative splicing of regions other than the C-terminal tail. For example, the presence or absence of the NP insertion in repeat IV\textsuperscript{26} (see Fig. 2) affected the functional consequences of polyglutamine expansions in channels heterologously expressed in HEK293 cells (coexpressed with α\textsubscript{2,δ} and β\textsubscript{1}).\textsuperscript{70} The Ca\textsubscript{v}2.1(-NP) isoforms with 13, 24 and 28 polyglutamines exhibited a hyperpolarizing shift in the voltage-dependence of inactivation, which was proportional to the length of the polyglutamine repeat. However, for Ca\textsubscript{v}2.1(+NP), 13 polyglutamines had no effect on inactivation and 28 polyglutamines caused a depolarizing shift of 5 mV. Thus, in SCA6 patients, altered function of Ca\textsubscript{v}2.1(-NP), which is highly expressed in Purkinje cells, may cause Purkinje cell degeneration due to a decrease in Ca\textsuperscript{2+} influx. Yet, other types of neurons that express both Ca\textsubscript{v}2.1(-NP) and Ca\textsubscript{v}2.1(+NP) (e.g., hippocampal neurons) may survive because a depolarizing shift in activation for Ca\textsubscript{v}2.1(+NP) might compensate for the alterations caused by Ca\textsubscript{v}2.1(-NP).\textsuperscript{70}

The effects of SCA6 expanded repeats have been studied in a knock-in mouse model that expresses human Ca\textsubscript{v}2.1 with 28 polyglutamine repeats (disease range).\textsuperscript{71} Mice homozygous for the SCA6 knock-in exhibit severe ataxia and dystonia and die three weeks after birth. However, in the knock-in mouse, SCA6 channel biophysical properties and current density are not changed, in contrast to the alterations found in heterologous expression studies.
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Figure 4. Mutations in CaV2.1 associated with murine disorders and in auxiliary subunits associated with human and murine channelopathies. Schematic representation of the CaV2.1, αδβ and γ subunit membrane topology and locations of mutations. The circles represent mutations in CaV2.1 causing mouse disease phenotypes: rolling Nagoya (R1262G),85 rocker,86 rolling Nagoya4,75 and leaner.74,75 The leaner mutation predicts a novel sequence starting at 1922 or 1968 depending upon the isoform expressed. The mutations depicted in the β subunit are associated with the following diseases: stars show location of the human diseases epilepsy and episodic ataxia (C104F) and juvenile myoclonic epilepsy (R482X).90 The circle in the β subunit shows location of splice site insertion of 4 bp in the lethargic mouse.82 The mouse mutant stargazer has been associated with a disruption in the γ subunit gene resulting in the total absence of the γ subunit protein.95 The ducky mutation in the αδ subunit gene94,95 results in two different transcripts: one transcript with exons 1–3 and a novel sequence (this mutant protein is depicted by the red line in the schematic), and a second transcript with exons 2–39 that is not translated. The allelic disease phenotypes: cortex (tgla) is a recessively inherited neurological disease of mice characterized by seizures (absence and focal motor) and mild ataxia.72 There is minor diffuse loss of cerebellar granule and Purkinje cells, an increased density of noradrenergic fibers from the locus coeruleus, and abnormal persistence of tyrosine hydroxylase expression in Purkinje cells.73 Tottering results from a single nucleotide change that substitutes a leucine for proline (P601L; Fig. 4) in the IIS5–S6 linker of CaV2.1.74,75 In BHK cells heterologously expressing the τg mutated channel, gating was unchanged but current density was reduced by 56%; calcium channel currents were also reduced in cerebellar Purkinje cells of τg/τg mice.70 The reduction in Purkinje cells may have been a consequence of reduced channel open probability because (i) levels of cerebellar CaV2.1 mRNA were normal in τg/τg mice75 and (ii) the mutation did not affect the amplitude of single channel currents in the BHK cells.76 Defects in transmitter release in the neocortex have been observed in tottering mice.77 The Ca2⁺ transients in presynaptic terminals of hippocampal Schaffer collaterals exhibited a diminished contribution from P/Q-type currents and an increased contribution from N-type currents.78 Concomitantly, neurotransmitter release at these synapses shifted from a predominant reliance on P/Q-type channels to an almost exclusive reliance on N-type channels.

Leaner (τgln) is another recessively inherited murine disorder. The mutant phenotype includes severe ataxia72 and a substantial loss of cerebellar Purkinje and granule cells.79 The surviving Purkinje cells exhibit aberrant morphology80 and aberrant tyrosine hydroxylase (TH) expression.81 The τgln mutation has been identified as a single nucleotide substitution in a splice donor consensus sequence of the CaV2.1 gene, which results either in a short or long isoform. The short isoform has altered sequence that begins at amino acid 1922 (Fig. 4; numbered according to the mouse CaV2.1) and terminates 57 amino acids later, and the long isoform has altered sequence that begins at amino acid 1968 and terminates 90 amino acids later.74,75 In Purkinje cells from τgln mice, whole cell calcium current was reduced in amplitude ~65%,76,82,83 The reduction was specifically in the ω-Aga-IVA-sensitive component of current, and the remaining ω-Aga-IVA-sensitive current had unchanged gating properties.72 Dove et al.83 suggest that the reduced current density results from decreased open probability. Interestingly, Wakamori et al.76 found that the behavior of the short and long τgln isoforms differed. In comparison with wild-type CaV2.1 expressed in BHK cells, current density was unchanged for the long isoform although both activation and inactivation were shifted in the depolarizing direction (5–10 mV), whereas current density was reduced for the short isoform without any other changes. Physiological effects of the leaner mutation include defective neurotransmitter release at neocortical synapses.77 In addition, marked alterations in rapid Ca2⁺ buffering have been observed in leaner Purkinje cells. These neurons exhibited a diminished Ca2⁺ buffering ability, attributed to reduced Ca2⁺ uptake by the endoplasmic reticulum and a decrease in Ca2⁺-binding proteins.84 Rolling Nagoya (τgno) is recessive, and affected mice have some phenotypic characteristics shared with their allelic counterparts tottering and leaner, and other characteristics that are distinct. Rolling Nagoya mice exhibit poor motor coordination of hind limbs and sometimes stiffness of hindlimbs and tail, but they lack apparent seizures. The severity of the ataxia is between that of rolling Nagoya and leaner mice. No significant numbers of apoptotic cells are observed in τgno cerebella, with normal cell density and no obvious decrease in size of the granule cell layer. Calbindin immunostaining of mutant cerebella is normal (another indication that there is no significant loss of Purkinje cells), but TH expression in tau neurons fails to show the normal, postnatal decrease.81 Rolling Nagoya results from a single nucleotide change that substitutes a glycine for arginine 1262, which is located in the IIS4 segment85
resulting in the locomotor deficits in sensitivity and reduced activity of CaV2.1 channels would significantly accelerate of inactivation compared to control wild-type calcium current with a small increase in current amplitude and a small rable alterations in electrophysiological properties.85 Mutant Purkinje activity, displaying only abortive Na+ spike activity. The altered voltage phosphorylation sites that could affect channel function.90

Rocker (krt) mutant mice exhibit ataxic, unstable gait accompanied by intention tremor, typical of cerebellar dysfunction. The cytoarchitecture and gross morphology of the brain is normal; however, dendritic abnormalities have been found in mature cerebellar cortex. The Purkinje cell dendrites have a reduction in branching and show a “weeping willow” appearance of the secondary branches. This mutant phenotype is recessive and has been associated with a point mutation (T1310K) located in the IIIS5–IIIS6 linker of CaV2.186 (Fig. 4). Functional studies of this mutation in CaV2.1 have yet to be published.

CaV2.1-null mice. Two groups have used gene-knockout to examine the consequences of complete absence of CaV2.1. There is consensus that mice homozygous null for CaV2.1 are viable at birth but develop a rapidly progressive ataxia and dystonia before usually dying at approximately 3–4 weeks of age.87,88 However, the electrophysiological correlates of these pathological changes are less clear based on analyses of cerebellar granule cells. Specifically, results from the two studies differed as to whether changes occurred in the density of L-, N- and R-type Ca2+ currents in CaV2.1-knockouts and as to whether P/Q-type Ca2+ current density was reduced in heterozygotes compared to homozygous wild-types. One group88 reported that occasional homozygous null mice survived past weaning and that the cerebella in these animals (at 15 weeks) exhibited a striped pattern of Purkinje cell loss and a graded loss of granule cells, which was more severe in the anterior lobes. This neuronal loss was not observed before postnatal day 40. Thus, the neurodegeneration was similar to that seen in leaner cerebella.

Mutations in auxiliary subunits of calcium channels. In addition to the α1 subunit, most high-voltage activated calcium channels contain the auxiliary subunits alpha2-delta (αδ1-3) and beta (β1-4), and possibly gamma (γ1-8) as well The β subunits are critical for surface expression of these calcium channels and also modulate the kinetics and voltage dependence; the other subunits also influence expression and properties of these channels, but to a lesser degree.89 Channelopathies caused by mutations in each of these auxiliary subunits are summarized in Figure 4 and described below.

Mutations in the β4 subunit. Juvenile myoclonic epilepsy. Juvenile myoclonic epilepsy has been found in the screening of a small pedigree to cosegregate with a single copy of the mutation R482X in the human β4 subunit90 (Fig. 4). This mutation would result in a protein with a prematurely truncated C-terminus (thus, eliminating part of an interaction domain for the α1 subunit). Analysis by coexpression with CaV2.1 in Xenopus oocytes revealed that rat R482X-β4 produced calcium current with a small increase in current amplitude and a small acceleration of inactivation compared to control wild-type β4. In addition, the R482X mutation would cause the deletion of two consensus phosphorylation sites that could affect channel function.90

Generalized epilepsy and praxis-induced seizures and episodic ataxia. Generalized epilepsy and praxis-induced seizures and episodic ataxia have been associated with a single missense mutation (C104F) in the human β4 subunit90 (Fig. 4). An allele of β4 bearing the C104F mutation was identified in two families which exhibited different clinical symptoms: one family with generalized epilepsy and seizures induced by playing complex strategic games and a second family with episodic ataxia. When coexpressed in Xenopus oocytes with the CaV2.1 subunit, the C104F-β4 subunit produced a calcium current with a small increase in current amplitude but no obvious alterations in channel kinetics compared to control wild-type β4. Escayg et al.90 suggested that this amino acid substitution might affect channel clustering or targeting by disrupting a domain conserved among β isoforms.

Lethargic (lh) is a recessive, murine disorder with behavioral traits overlapping those of human and murine CaV2.1 diseases (ataxia and absence seizures), but without apparent neuronal degeneration.90 The causative mutation is an insertion of four nucleotides into a splice donor site of the β4 gene.92 This results in two mutant isoforms of β4, both of which are lacking the consensus sequence required for binding to α1 subunits (Fig. 4). McEnery et al.93 found that the forebrain and cerebellum of lh mice lack immunodetectable β4 and have increased expression of β1B. This upregulation of other β subunits could lead to rescue or compensation of function in cells expressing the mutated β4 subunit. This mechanism may account for the observation that neither calcium channel subtypes nor calcium-dependent transmitter release was significantly altered at hippocampal synapses in lethargic mice.78

Mutations in the α2δ subunit. Ducky (du). Ducky is a recessive murine disorder and is characterized by an ataxic, wide-based gait and paroxysmal dyskinesia. The mice are reduced in size and fail to survive beyond 35 days. In du mice, dysgenesis occurs in the medulla, spinal cord and cerebellum, with the Purkinje cells exhibiting thickened dendrites and reduced dendritic arbors having a “weeping willow” morphology.94 The du mouse has been utilized as a murine model for epilepsy due to its phenotype of spike-wave seizures and ataxia. The du locus was identified as the α2δ2–Ca channel subunit gene;95 du1 includes exons 1–3 and a novel sequence, and du2 includes exons 2–39 and is not translated into protein94 (Fig. 4). Another ducky strain, du2 results from a 2-bp deletion within exon 9 causing premature truncation.95

Calcium channel currents in du/du cerebellar Purkinje cells were reduced by 35% compared to currents in wild-type cells.95 When heterologously expressed in COS-7 cells, wild-type α2δ-2 caused no changes in biophysical parameters except for an increased surface expression of CaV2.1 + β4.94 Surface expression in COS-7 cells (i.e., peak current) was reduced by 51% when wild-type α2δ-2 was replaced by its du-mutant,94 without large changes in other properties at the macroscopic or single channel level. Thus, the du mutation appears to cause a reduction of the number of functional CaV2.1 channels in the membrane.

Mutations in the γ2 subunit. Stargazer (stg). Stargazer is another recessive murine disorder and is characterized by ataxia, head-tossing and absence seizures.96 In stargazer, the γ2 gene has an insertion of an ETn retrotransposon in intron 2 causing premature transcriptional termination (Fig. 4) and total absence of γ2 protein.97 Although classified as a calcium channel subunit, γ2 also interacts with
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AMPA receptors and synaptic PDZ proteins; moreover, cerebellar granule cells in stargazer mice lack functional AMPA receptors and voltage-gated calcium currents seem relatively unaffected.\(^\text{98}\)

However, heterologous expression indicated that \(\gamma_2\) may have small effects on channels containing Ca\(V_2.1\) or Ca\(V_2.2\).\(^\text{97,99}\) Thus, it is unclear whether altered properties of calcium currents contribute to the stargazer phenotype.

Genetic variants of low-voltage-activated (T-type) calcium channels. T-type channels are important for low threshold calcium spikes, pacemaker activity and neuronal oscillations, and are especially significant for burst firing behavior in thalamic neurons.\(^\text{100}\)

 Genetic variants in the low-voltage activated channel Ca\(V_3.2\) have been associated with childhood absence epilepsy and other forms of idiopathic generalized epilepsy\(^\text{101-103}\) (Fig. 5). Functional differences between these variants have been analyzed by heterologous expression;\(^\text{103-108}\) however, the alterations in function are often small and do not collectively provide a clear explanation for the mechanism of pathology. Perhaps most commonly, the disease-associated variants display a hyperpolarizing shift in the voltage-dependence of activation or a decrease in steady-state inactivation, which would result in increased channel activity.\(^\text{104}\) Conversely, both increases and decreases of Ca\(V_3.2\) channel activity could alter the timing of neuronal firing sufficiently to contribute to seizures.\(^\text{103}\) However, the majority of the channel variations associated with epilepsy do not significantly affect Ca\(V_3.2\) biophysical properties.\(^\text{107,108}\) These "biophysically silent" variations may disrupt function by modifying interactions with channel associated proteins.\(^\text{109}\)

Alternatively, the sequence variations could interfere with splicing events as has been suggested for several mutations associated with idiopathic generalized epilepsy.\(^\text{110}\) Several polymorphisms clustered in the intracellular loop between repeats I and II (I–II loop) have been reported to increase surface expression of Ca\(V_3.2\) channels.\(^\text{105}\)

In summary, it appears that there are not yet clear-cut linkages established between genetic alterations of T-type channels and epileptogenesis.

**Calcium Channelopathies of Muscle**

Calcium channel defects have been linked to several human skeletal muscle disorders such as: hypokalemic periodic paralysis (HypoPP), malignant hyperthermia (MH) and central core disease (CCD). Recently, calcium channel defects have also been associated with two cardiac diseases, arrhythmogenic right ventricular cardiomyopathy type-2 (ARVD2) and familial polymorphic ventricular tachycardia (FPVT). Causative mutations have been localized to calcium channel genes involved in excitation-contraction (E-C) coupling. Two key proteins involved in the process of E-C coupling are the dihydropyridine receptor (DHPR; an L-type calcium channel) in the plasmalemma and the ryanodine receptor (RyR) in the sarcoplasmic reticulum (SR). The DHPR expressed in muscle is a voltage-gated calcium channel containing the Ca\(V_{1.1}\) subunit (in skeletal muscle) or the Ca\(V_1.2\) subunit (in cardiac muscle). The RyR protein (RyR1 isoform in skeletal and RyR2 in cardiac muscle) is a calcium release channel composed of four identical subunits. In skeletal muscle, a depolarization-induced conformational change of the DHPR\(^\text{111,112}\) results in the opening of the skeletal RyR channel in the SR. This activation may involve a direct molecular interaction between these two junctional components since E-C coupling in skeletal muscle does not require the entry of extracellular calcium. In cardiac muscle, opening of the DHPR channel causes calcium entry into the cell; the Ca\(^{2+}\) ions then activate the immediately adjacent RyRs by Ca-induced calcium release.

**Muscular dysgenesis (mdg) mice and dyspedic (dys) mice.** Muscular dysgenesis (mdg) mice and dyspedic (dys) mice lack functional DHPR or RyR1, respectively. Cultured muscle cells from these animals can be used for expression of cDNAs and thus allow analysis of the effects of specific DHPR and RyR mutations in a near native environment. Mdg mice have a single nucleotide deletion in the gene encoding Ca\(V_{1.1}\) (skeletal DHPR) resulting in a truncated channel protein\(^\text{113}\) (Fig. 6). Dyspedic mice contain a targeted disruption of the gene encoding RyR1.\(^\text{114,115}\) In dyspedic myotubes, there is a loss of E-C

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**Figure 5.** Variants in the Ca\(V_{3.2}\) subunit associated with epilepsy susceptibility. Locations of the variants are depicted in the schematic of this low-voltage activated calcium channel. Superscript numbers indicate the references in which the mutations were identified and described.
Figure 6. Mutations in Ca$_{\alpha}$1.1 subunit causing hypokalemic periodic paralysis and malignant hyperthermia. The different mutation symbols signify different clinical diseases: circle = hypokalemic periodic paralysis, square = malignant hyperthermia, triangle = muscular dysgenesis mouse. Superscript numbers indicate the references in which the mutations were identified and described.

Skeletal muscle channelopathies. Hypokalemic periodic paralysis (HypoPP) is a dominantly-inherited human disorder presenting as episodic weakness of the trunk and limbs that can last hours to days and is generally accompanied by a reduction in serum K$^+$ levels. This disease usually becomes apparent during childhood or early adulthood and has reduced penetrance in females. Rest can provoke attacks after exercise and factors that lower serum K$^+$ levels (carbohydrate ingestion or insulin injection); other provoking factors include emotion, stress, cold exposure and alcohol ingestion. HypoPP has been associated with three missense mutations in the voltage-sensing (S4) segments of Ca$_{\alpha}$1.1 (Fig. 6): R528H located in IIS4, 120 and R1239H/G in IVS4.121,122

A small number of studies have been carried out on cultured myotubes derived from muscle biopsies of HypoPP patients carrying the R528H mutation. One study (based on three patients) found a small hyperpolarizing shift (~6 mV) in the voltage-dependence of both activation and inactivation of the L-type calcium current (i.e., the calcium current attributable to Ca$_{\alpha}$1.1), but found no differences in depolarization-induced release of calcium from the SR.123 A second study (based on a single patient) found that the L-type current had a slightly reduced density and greatly (2-fold) slowed activation.124

Analyses by means of heterologous expression have not revealed any consistent functional changes in L-type calcium channels with introduced HypoPP mutations. Compared to wild-type Ca$_{\alpha}$1.1, expression in L-cells of rabbit R528H-Ca$_{\alpha}$1.1 produced current with a 70% decrease in density but no significant differences in gating.125 Compared to wild-type Ca$_{\alpha}$1.2, expression in HEK293 cells of R650H Ca$_{\alpha}$1.2 (homologous to R528H in Ca$_{\alpha}$1.1) produced current with a 38% reduction in amplitude and a slight (5 mV) hyperpolarizing shift of both activation and inactivation.126 In a preliminary report, Gonzales et al.127 described no significant differences in voltage-dependence or kinetics of either L-type current or calcium release in dysgenic myotubes after heterologous expression of rabbit Ca$_{\alpha}$1.1 containing the R528H, R1239H or R1239G mutations. Similarly, Jurkat-Rott et al.125 found no differences in calcium currents or calcium release between wild-type and R528H rabbit Ca$_{\alpha}$1.1 expressed in immortalized dysgenic muscle cells.

Even if heterologous expression had demonstrated consistent effects on calcium currents or E-C coupling, it would be difficult to explain the etiology of HypoPP. Specifically, when bathed in low K$^+$ solution, biopsied intercostal muscle fibers of HypoPP patients were depolarized by 20 mV (compared to a 10 mV hyperpolarization in normal muscle).128 If a similar depolarization were to occur in vivo, it would inactivate voltage-gated sodium channels and thus induce muscle paralysis.

Because it is not obvious how altered calcium currents or E-C coupling would produce persistent depolarization triggered by low K$^+$, indirect mechanisms have been suggested (reviewed in ref. 129). For example, a decrease in Ca$^{2+}$ entry could change activation of a Ca$^{2+}$-sensitive channel with direct control over resting potential (e.g., Ca$^{2+}$-activated K$^+$ channel). Alternatively, the expression or subcellular distribution of channels important for controlling membrane potential might be altered by small but long-term changes in cytoplasmic calcium levels.

Malignant hyperthermia (MH) and central core disease (CCD) are dominantly inherited muscle disorders that arise from altered handling of intracellular calcium (reviewed in refs. 130–132). Thus, it is most convenient when discussing these diseases to combine the information about etiology and symptomatology. A number of mutations in RyR1 have been associated with these diseases, but mutations in other proteins can also be causal (see below). No matter what the causative mutation, RyRs represent an important trigger point for the pathophysiology, since RyRs play a key role in controlling intracellular calcium. In addition to being regulated via the DHPR, RyRs are activated by elevations in intracellular calcium (“calcium-induced-calcium-release” or “CICR”). In general, mutations in RyR1 can produce three different types of channel defects: (i) “leaky channels” that increase basal SR Ca$^{2+}$ leak causing elevated myoplasmic Ca$^{2+}$ and depletion of SR Ca$^{2+}$ stores, (ii) “EC uncoupled” channels that have reduced capacity of releasing Ca$^{2+}$ following voltage or ligand stimulation although the calcium stores are full, (iii) partial or nearly-complete ablation of channel expression.
RyR1 protein carrying mutations associated with malignant hyperthermia cause the calcium release channel to be “overactive” so that massive calcium release can be triggered by halogenated anesthetics (which decrease the threshold for CICR) or by depolarizing skeletal muscle relaxants (which trigger low amounts of calcium release via the normal E-C coupling pathway). Pharmacological agents used during surgery can thus trigger an episode of MH. These episodes are characterized by skeletal muscle rigidity, tachycardia, unstable and rising blood pressure, and elevated body temperature. If not treated immediately, patients may die within minutes from ventricular fibrillation, within hours from pulmonary edema or coagulopathy, or within days from post-anoxic neurological damage and cerebral edema or obstructive renal failure, resulting from release of muscle proteins into the circulation (reviewed in ref. 133).

In central core disease (reviewed in ref. 133), individuals typically display a slowly progressive or non-progressive myopathy (usually presenting in infancy) associated with hypotonia and proximal muscle weakness. Histologically, the central regions (cores) of both type I and type II skeletal muscle fibers display disintegration of the contractile apparatus, ranging from blurring and streaming of Z lines to total loss of myofibrillar structure. Many individuals with CCD also are susceptible to MH.

Thus far, 36 identified missense mutations and four deletion mutations in RyR1 have been associated with MH susceptibility and/or CCD\(^{134-165}\) (Fig. 7). While some mutations produce MH alone, other mutations cause both MH and CCD, and still others produce only CCD. The majority of the RyR1 mutations appear to be clustered in the N-terminal amino acids 35–614 (MH/CCD region 1), the centrally located residues 2163–2458 (MH/CCD region 2), and C-terminal residues 4214–4914 (MH/CCD region 3).\(^{166}\) An MH-related porcine disorder (reviewed in ref. 130) is caused by the mutation R615C, which is homologous to the R614C mutation in humans. Swine heterozygous for R615C display increased muscle mass and increased calcium release in response to a variety of agents, whereas animals homozygous for the mutation suffer “porcine stress syndrome” with MH symptoms brought on by physical and emotional stress.\(^{152}\) A canine form of MH, A547V\(^{132}\), is inherited as an autosomal dominant trait and shares most of the clinical manifestations with MH in pigs and humans.\(^{167}\) Mutations in genes other than RyR1 may also be responsible for MH. For example, in a single family, MH segregated with a missense mutation (Fig. 6) in the Ca\(_{v}\).1.1 gene (R1086H).\(^{168}\) Moreover, the demonstration of linkages with MH and several loci distinct from either RyR1 or Ca\(_{v}\).1.1 indicate the possibility of additional gene targets.\(^{131,169}\) The RyR1 mutations causing MH and CCD have been reported to have variable functional consequences. In part, this variability could be due to the study of different disease-causing mutations, which may have different effects. However, the variability most likely also arises from the use of a variety of experimental systems (muscle biopsies, myotube cultures obtained from affected individuals, expression of cDNAs in cell lines or dyspedic myotubes).

Muscle bundles biopsied from MH patients contract in response to low concentrations of caffeine and halothane than muscle from normal individuals. Muscle biopsies from patients with the deletion mutation, delE2347, exhibit unusually large electrically-evoked contractions compared to non-MH muscle.\(^{156}\) Paralleling the contraction results from biopsied muscle, intracellular calcium release in cultured myotubes derived from MH patients showed an increased sensitivity to halothane\(^{170}\) as well as to ryanodine.\(^{171}\) The kinetic properties of the [Ca\(_{2+}\)]\(_i\) rise in cultured human MH (G2435R) muscle differ significantly from controls.\(^{171}\) Calcium release from SR vesicles of MH muscle also exhibits a lower threshold for activation by calcium, an enhanced sensitivity to caffeine, and a reduced sensitivity to inhibitory concentrations of calcium.\(^{172}\) In addition to an increase in Ca\(_{2+}\)-, halothane- and TFP-induced calcium release, a reduction in the rate and capacity of calcium loading of heavy SR vesicles from MH patients has been observed.\(^{173}\) Porcine MH muscle fibers also displayed an increased sensitivity to agonists such as halothane and caffeine\(^{174}\) as well as a 15-mV negative shift in Ca\(_{2+}\) release induced by depolarization.\(^{175}\) Introduction of a number of MH-causing missense mutations into RyR1 (expressed in HEK293 cells) causes an increased sensitivity to low concentrations of caffeine and halothane.\(^{176}\) It has been suggested that the increased sensitivity of RyR1 to activators observed in many studies may result at least in part from elevated, resting intracellular Ca\(_{2+}\).\(^{177}\)

Two mutations associated with a severe and highly penetrant form CCD have been analyzed by means of heterologous expression. The CCD for one of these mutations (Y4796C) is accompanied by MH susceptibility,\(^{153}\) whereas for the other (I4898T) it is not.\(^{148}\)
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Rabbit Y4796C-RyR1 expressed in HEK293 cells exhibited a decreased EC$_{50}$ for activation by caffeine, a reduced amplitude of the caffeine response and an elevated resting [Ca$^{2+}$]$_i$ from which it was concluded that this mutation causes RyR1 to be "leaky".\textsuperscript{153} Expressed in HEK293 cells, rabbit I4898T-RyR1 also resulted in an increased resting [Ca$^{2+}$]$_i$ but loss of responses to caffeine and halothane.\textsuperscript{148} Expression of I4897T-RyR1 in dyspedic myotubes resulted in the complete uncoupling between electrical excitation of the plasma membrane and SR Ca$^{2+}$ release, but no change occurred in either resting myoplasmic [Ca$^{2+}$]$_i$, or in SR Ca$^{2+}$ content.\textsuperscript{178} When I4897T-RyR1 was co-expressed with wild-type RyR1 (mimicking the situation in patients), voltage-gated SR release was depressed by 60%. Based on the studies above, it appears that CCD accompanied by MH may result from hyperactive (and thus leaky) RyRs, whereas CCD without MH can result from hypoactive RyRs.

Three different knock-in mice have been constructed to investigate the mechanism by which RyR1 mutations cause malignant hyperthermia (R163C, Y522S) and/or central core disease (I4895T).\textsuperscript{179-181} Myotubes from Y522S mice have enhanced RyR1 sensitivity to activation by temperature, caffeine and voltage.\textsuperscript{179} However, these Y522S myotubes do not exhibit an increase in basal calcium levels or intracellular calcium store depletion as was previously described for Y522S RyR1 expressed in heterologous systems.\textsuperscript{182-184} Muscle cells from RyR1 R163C mice show an increased resting calcium level, increased sensitivity to activation by depolarization or agonist, and a decreased inhibitory regulation by Mg$^{2+}$.\textsuperscript{180} In the knock-in mouse model of the RyR1 I4895T mutation (which causes severe form of CCD in humans), DHPR and RyR1 localization is normal within the triad junction, but RyR1-mediated calcium release is abolished (both DHPR- and ligand-mediated release).\textsuperscript{181}

**Cardiac Muscle Channelopathies**

Both voltage-gated calcium channels and SR calcium release channels have been studied extensively in relation to arrhythmias and heart failure.

C$_{a_v}$1.2 (L-type Ca channel) mutations (Fig. 8) have been associated with Timothy syndrome, a multisystem disorder characterized by lethal cardiac arrhythmias, congenital heart disease, immune deficiency, intermittent hypoglycemia, cognitive abnormalities and autism. Macroscopic analyses of C$_{a_v}$1.2 channels carrying Timothy syndrome mutations (G402S, G406R) suggested a reduction in current inactivation resulting in sustained depolarization.\textsuperscript{185,186} However, single channel recordings have shown that this apparent decrease in inactivation may have actually been due to a decrease in unitary conductance and an increase in spontaneous mode 2 gating (frequent openings of much longer duration).\textsuperscript{187} Armstrong and colleagues\textsuperscript{187} suggest that aberrant phosphorylation of C$_{a_v}$1.2 contributes to the excitotoxicity associated with Timothy syndrome.

Recently, a clinical cardiac disorder characterized by ST-segment elevation, short QT-intervals and sudden cardiac death has been attributed to missense mutations A39V and G490R in C$_{a_v}$1.2 and S481L in the $\beta$$_{2b}$ subunit. Each of these mutations caused a reduction in the calcium current amplitude when the mutant channels were expressed in CHO cells.\textsuperscript{188}

Mutations in RyR2. Mutations in RyR2 are responsible for inherited cardiac disorders associated with a propensity to malignant ventricular tachyarrhythmias and constitute an important cause of sudden death in both young and adult individuals. More than 70 mutations in RyR2 have been associated with arrhythmogenic right ventricular dysplasia type 2 (ARVD2), and catecholaminergic polymorphic ventricular tachycardia (CPVT) (Fig. 9; reviewed in ref. 189).

ARVD2 is an autosomal dominant disease characterized by partial degeneration of the myocardium of the right ventricle, electrical instability and sudden death. Thus far, six missense mutations in RyR2 have been correlated with ARVD2: R176Q, R420W, L433P, N2386I, Y2392C, T2504M.\textsuperscript{190,191} These mutations are located at highly conserved amino acids in the cytosolic region of the protein and cluster in regions where mutations causing MH or CCD in RyR1 also cluster; R176Q exactly corresponds to the R163C mutation in RyR1 causing MH/CCD. The mutations N2386I and T2504M are located in the domain thought to interact with FKBP12.6, a regulatory subunit of RyR2.\textsuperscript{190} Mice with an RyR2 knock-in for the ARVD2 mutation R176Q have some cardiac phenotypic similarities to affected humans.\textsuperscript{192}

![Figure 8. Mutations in C$_{a_v}$1.2 subunit causing Timothy syndrome or short-QT syndrome. The different mutation symbols signify different clinical diseases: the circles show location of mutations causing Timothy syndrome, and the stars depict location of short-QT mutations. Superscript numbers indicate the references in which the mutations were identified and described.](image-url)
CPVT is an autosomal dominant defect characterized by volleys of bidirectional and polymorphic ventricular tachycardias in response to vigorous exercise, with no structural evidence of myocardial disease. This disease has a relatively early onset and a highly malignant course with estimates of mortality ranging from 30–50% by the age of 20–30 years. Thus far, more than 40 missense mutations in RyR2 have been correlated with this disorder (reviewed in refs. 189, 193–205). Priori and colleagues\(^\text{193}\) described three different missense mutations in the RyR2 gene of three distinct families: P2328S, Q4201R and V4653F. A second study found four additional missense mutations: S2246L, R2474S, N4104K and R4497C.\(^\text{194}\) These mutations are located in regions of the protein that correlate with RyR1 mutation hot spots for MH and/or CCD. Recently, two ‘hot spots’ of disease-causing mutations have been localized in the three-dimensional structure of RyR2 with cryo-EM/single particle reconstruction difference mapping; both the central and NH\(^2\)-terminal ‘hot spots’ map relatively close to one another in regions within the clamp-shaped structure.\(^\text{206,207}\)

The mechanism by which the RyR2 mutations cause arrhythmia has been a topic of debate, and several hypotheses have been suggested (reviewed in refs. 193, 208 and 209). Specifically, it has been debated whether RyR2 mutations induce abnormal calcium transients in the absence of adrenergic stimulation, whether the defect is cause by altered interaction with FKBP12.6, whether these mutations increase the Ca\(^{2+}\) sensitivity of the channel, and whether the mutations alter the ability of the channel to remain closed. In heterologous expression systems and under conditions that simulate adrenergic activation, RyR2 channels carrying CPVT mutations exhibit a gain-of-function defect at the single-channel level consistent with increased basal Ca\(^{2+}\) leak and a resistance to inhibition by Mg\(^{2+}\).\(^\text{208}\)

The arrhythmogenic mechanism of CPVT has been investigated with knock-in mice carrying the R4496C mutation.\(^\text{209}\) R4496C cardiomyocytes exhibited spontaneous, delayed after depolarizations (DADs) (which were not present in wild-type myocytes). Following \(\beta\)-adrenergic stimulation, the knock-in myocytes exhibited further enhancement of triggered action potentials arising from the action potential DADs. The authors\(^\text{209}\) concluded that \(\beta\)-adrenergic stimulation worsened a preexisting defect of the R4496C RyR2 channel. Recently, the RyR2 mutation G2367R has been associated with hypertrophic cardiomyopathy\(^\text{210}\) and the mutations S4565R and R2267H associated with sudden infant death syndrome and altered channel function.\(^\text{211}\)

**Summary**

Mutations of genes encoding calcium channels are rapidly being identified as causing a diverse group of nerve and muscle diseases. Calcium channel mutations in Ca\(_{\alpha_{1.4}}\) and Ca\(_{\alpha_{1.2.1}}\) subunits and \(\beta\), \(\gamma\) and \(\delta\) auxiliary subunits have been associated with a number of neurological diseases, and defects in Ca\(_{\alpha_{1.1}}\) and Ca\(_{\alpha_{1.2}}\) subunits and RyR1 and RyR2 calcium release channels have been associated with several muscle disorders. The majority of the calcium channel mutations are associated with movement disorders (exceptions are night blindness and arrhythmias), perhaps because these are most readily observed. However, it is intriguing to speculate that linkage analysis will eventually establish correlations between calcium channel mutations and subtle behavioral disorders or even personality traits.

![Figure 9. Mutations in RyR2 causing human cardiac disorders. Schematic locations of the mutations are not drawn in the diagram (amino acid locations of the RyR mutations are listed). Whether the mutation has been associated with CPVT, indicated as #), catecholaminergic polymorphic ventricular tachycardia (CPVT, indicated as #), hypertrophic cardiomyopathy (HCM, indicated as +) or sudden infant death syndrome (SIDS, indicated as *) is noted. Superscript numbers indicate the references in which the mutations were identified and described.](image)

The calcium channelopathies differ from those of voltage-gated sodium and potassium channels, in which the disease phenotype is directly attributable to the changes in electrical excitability of nerve and muscle that result from altered biophysical properties of the channels. The only obvious mechanistic correlation for calcium channelopathies is that a given calcium channel mutation has the most profound impact on the organ or brain region in which that channel is most highly expressed (e.g., night blindness, cerebellar disorders, skeletal and cardiac muscle defects). However, there is often no clear link between disease severity and impact on channel function. Indeed, serious diseases can result from mutations that cause trivial alterations of calcium currents analyzed in vitro. In addition, the same disease phenotype can result from hyper- or hypo-activity of a particular calcium channel. Similarly puzzling is that seemingly comparable mutations of Ca\(_{\alpha_{1.2.1}}\) produce dominantly inherited diseases in humans and recessively inherited diseases in mice. Overall, the absence of clear phenotype-genotype correlations may reflect the fact that most analyses thus far have focused on the biophysical properties of channel function, whereas the mutations could well affect subcellular distribution of a channel or its association with other proteins important in signaling cascades. Moreover, genetic background is clearly important in determining the phenotypic consequences of channel mutations as evidenced by identical mutations producing very different clinical symptoms (e.g.,

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**Figure 9. Mutations in RyR2 causing human cardiac disorders. Schematic locations of the mutations are not drawn in the diagram (amino acid locations of the RyR mutations are listed). Whether the mutation has been associated with CPVT, indicated as #), catecholaminergic polymorphic ventricular tachycardia (CPVT, indicated as #), hypertrophic cardiomyopathy (HCM, indicated as +) or sudden infant death syndrome (SIDS, indicated as *) is noted. Superscript numbers indicate the references in which the mutations were identified and described.**

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**Table:**

| Region 1 |
| --- |
| A77V\(^{203}\#\) |
| R178Q\(^{190}\#\) |
| V188M\(^{199}\#\) |
| R420W\(^{191}\#\) |
| L433P\(^{190}\#\) |

| Region 2 |
| --- |
| E1724K\(^{195}\#\) |
| S2246L\(^{196}\#\) |
| A2254V\(^{195}\#\) |
| R2267H\(^{211}\#\) |
| V2306L\(^{204}\#\) |
| E2311D\(^{197}\#\) |
| P2328S\(^{194}\#\) |
| R2474S\(^{195}\#\) |
| G2367R\(^{210}\#\) |
| N2386I\(^{190}\#\) |
| A2387T\(^{205}\#\) |
| A2392C\(^{191}\#\) |
| A2394G\(^{195}\#\) |
| R2401H\(^{200}\#\) |
| R2474S\(^{196}\#\) |
| V2475F\(^{198}\#\) |
| T2504M\(^{190}\#\) |
| L2534V\(^{202}\#\) |

| Region 3 |
| --- |
| L3778R\(^{197}\#\) |
| S3938R\(^{199}\#\) |
| G3964R\(^{199}\#\) |
| F4020L\(^{195}\#\) |
| E4076K\(^{195}\#\) |
| N4104K\(^{196}\#\) |
| H4108N\(^{195}\#\) |
| V4201R\(^{194}\#\) |
| R4497C\(^{196}\#\) |
| M4504\(^{205}\#\) |
| A4510T\(^{194}\#\) |
| S4565R\(^{211}\#\) |
| A4607P\(^{205}\#\) |
| V4653F\(^{194}\#\) |
| G4682S\(^{195}\#\) |
| H4762P\(^{195}\#\) |
| V4771L\(^{197}\#\) |
| L4851C\(^{201}\#\) |
| I4867M\(^{197}\#\) |
| V4880A\(^{205}\#\) |
| N4895D\(^{197}\#\) |
| F4902L\(^{204}\#\) |
| E4950K\(^{197}\#\) |
| R4959Q\(^{204}\#\) |
disparate disease severity and progression in sisters with the same SCA6 mutation\(^6\).

Understanding genotype-phenotype correlation of calcium channelopathies is complicated by the fact that Ca\(^{2+}\) plays such important and diverse roles as an intracellular regulatory molecule. Thus, even small changes in calcium channels may dramatically alter cellular development and homeostasis and thus result in widespread changes in cellular function. An important challenge for the future remains the identification of fundamental mechanisms involved in the calcium channelopathies: electrical behavior, transmitter release, enzyme cascades, channel targeting or gene expression/development. Besides providing a better understanding of pathogenesis, future research on the disease-causing mutations may provide new insight into cell biological roles of calcium channels and into relationships between structure and function.

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