Muscle channelopathies and critical points in functional and genetic studies

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Muscle channelopathies are caused by mutations in ion channel genes, by antibodies directed against ion channel proteins, or by changes of cell homeostasis leading to aberrant splicing of ion channel RNA or to disturbances of modification and localization of channel proteins. As ion channels constitute one of the only protein families that allow functional examination on the molecular level, expression studies of putative mutations have become standard in confirming that the mutations cause disease. Functional changes may not necessarily prove disease causality of a putative mutation but could be brought about by a polymorphism instead. These problems are addressed, and a more critical evaluation of the underlying genetic data is proposed.

Introduction

Skeletal muscle was the first tissue in which hereditary diseases caused by ion channel defects, the myotonias and periodic paralyses, were described (1). It is now recognized that malignant hyperthermia (MH), central core disease, and the congenital myasthenic syndromes (CMSs) as well as the antibody-mediated myasthenia gravis should be included in the classification of muscle channelopathies (2, 3). Aberrant ion channel splicing due to mutations in other genes leads to muscle channelopathies, such as in the myotonic dystrophies (4, 5); and diseases caused by defects in proteins associated with trafficking, targeting, and clustering of ion channels are also ion channelopathies, e.g., disturbed clustering of acetylcholine receptors (AChRs) at the neuromuscular junction by rapsyn mutations (6). This review focuses on hereditary ion channelopathies and discusses mutation patterns, functional consequences, and possible interpretations of the findings. A brief overview of muscle physiology is provided to review the significance of the channels for muscle function.

Muscle physiology. Motoneuron activity is transferred to skeletal muscle at the neuromuscular junction, generating an endplate potential that depends on acetylcholine (ACh) release from the nerve terminal and its reaction with the subsynaptic nicotinic AChR, a pentameric ligand-gated ion channel (7). Normally, an endplate potential is large enough to induce a sarcolemmal action potential that propagates from the endplate to the tendon and along the transverse tubular system. This membrane region projects deeply into the cell to ensure even distribution of the impulse (Figure 1). The upstroke of the action potential is mediated by opening of the voltage-gated $Na_v1.4$ Na^+ channels (encoded by *SCN4A*), which elicit a Na^+ inward current with rapid activation kinetics. Repolarization of the membrane by fast Na^+ channel inactivation is supported by opening of delayed rectifier K^+ channels that mediate an outward K^+ current. Buffering of afterpotentials is achieved by a high Cl^- conductance near the resting potential, resulting from the homodimeric Cl^- channel ClC-1, encoded by *CLCN1* (2). At specialized junctions in the transverse tubular system, the signal is transmitted from the tubular membrane to the sarcoplasmic reticulum (SR), causing the release of Ca^{2+} ions into the myoplasm, which activate the contractile apparatus (8). This process is called excitation-contraction coupling. Two Ca^{2+} channel complexes are chiefly involved in this process, the voltage-gated pentameric Cav1.1 Ca^{2+} channel (also called the dihydropyridine receptor) located in the transverse tubular system, encoded by *CACNA1S*, and the homotetrameric ryanodine receptor, ryanodine receptor type 1 (RyR1), of the SR (9).

Muscle channelopathies due to altered membrane excitability

Most muscle channelopathies have similar clinical presentation: typically the symptoms occur as episodic attacks lasting from minutes to days that show spontaneous and complete remission, onset in the first or second decade of life, and — for unknown reasons — amelioration at the age of 40 or 50. Frequently, the attacks can be provoked by exercise, rest following physical activity, hormones, mental stress, or certain types of food and drugs.

CMSs: hypo- or hyperexcitable neuromuscular junctions

CMSs are a heterogeneous group of inherited disorders characterized by defective transmission of neuromuscular excitation resulting in muscle fatigue (10). Weakness is usually evident at birth or within the first year or 2 of life and is characterized by feeding difficulties, ptosis, impaired eye movements, and delayed motor milestones. In some cases, strength improves during adolescence and does not exhibit a progressive course. Reflexes are usually brisk, and muscle wasting does not occur. CMSs can lead to congenital arthrogryposis multiplex involving reduced fetal movement and multiple joint contractures in the neonate (11). Electromyography in CMS patients reveals a characteristic decrement of compound action potential amplitude on repetitive stimulation, and single-fiber recordings show an increased variability in the synaptic transmission time ("jitter") and transmission blocks (12).

Presynaptic, synaptic, and postsynaptic loss-of-function proteins. CMSs result from defects in presynaptic, synaptic, and postsynaptic proteins. Presynaptic defects reduce ACh release and resynthesis due to mutations in the choline acetyltransferase. Synaptic CMSs are caused by acetylcholinesterase (AChE) deficiency (13) due to mutations in the collagenic tail subunit (ColQ) that mediates AChE

Nonstandard abbreviations used: ACh, acetylcholine; AChR, ACh receptor; CMS, congenital myasthenic syndrome; MH, malignant hyperthermia; PP, periodic paralysis; RyR1, ryanodine receptor type 1; SR, sarcoplasmic reticulum.

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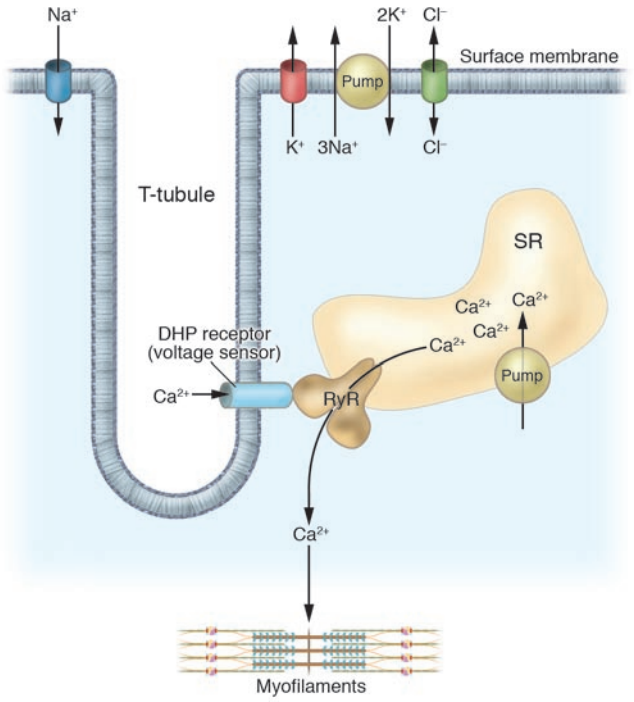

Figure 1
Excitation-contraction coupling of skeletal muscle. A muscle fiber is excited via the nerve by an endplate potential and generates an action potential, which spreads out along the surface membrane and the transverse tubular system into the deeper parts of the muscle fiber. The dihydropyridine (DHP) receptor senses the membrane depolarization, alters its conformation, and activates the ryanodine receptor, which releases Ca^{2+} from the SR, a Ca^{2+} store. Ca^{2+} binds to troponin and activates the so-called contractile machinery.

insertion into the synaptic basal lamina (10). Postsynaptic CMSs are caused by dominant or recessive mutations in 1 of the nicotinic AChR subunits (14) (Figure 2), or in proteins anchoring AChRs into the membrane, such as the rapsyn mutations (Table 1). Loss-of-function mutations of AChR subunits lead to compensatory expression of fetal δ subunits, yielding AChR complexes that differ functionally from the adult type.

Kinetic gain- and loss-of-function nicotinic AChR mutations. Rarely, postsynaptic CMSs are caused by mutations at different sites and different functional domains that alter the kinetic channel properties. These kinetic mutations result in the slow- or fast-channel syndromes. The low-affinity fast-channel syndrome is caused by loss-of-function mutations that have effects similar to those of AChR deficiency but is much rarer. Mutations at different sites lead to fewer and shorter channel activations. In contrast to all the CMSs described above, the slow-channel syndrome presents in childhood, adolescence, or adult life with upper-limb predominance and contractures, does not respond to anticholinesterase, and is progressive. CMS patients with a slow-channel syndrome show increased synaptic response to ACh with characteristic repetitive discharges in response to a single supramaximal stimulus. The syndrome results from gain-of-function mutations in the ion-conducting pore M2 (Figure 2). The leaky AChRs exert an excitotoxic effect and cause endplate myopathy via focal caspase activation (15–18).

Myotonia: plasmalemmal hyperexcitability due to mutant Na^+ or Cl^- channels

Muscle stiffness, termed myotonia, is ameliorated by exercise — the "warm-up phenomenon" — and can be associated with transient weakness during strenuous muscle activity. On the contrary, paradoxical myotonia (also called paramyotonia) worsens with cold and after exercise. Both myotonia and paramyotonia derive from uncontrolled repetitive action potentials of the sarcolemma following an initial voluntary activation. This may be noted as a myotonic burst in the electromyogram. The involuntary electrical activity prevents the muscle from immediate relaxation after contraction, and the patients subsequently experience this as muscle stiffness.

Chloride channel myotonias: Thomsen and Becker. Dominant Thomsen myotonia and recessive Becker myotonia are caused by missense and nonsense mutations in the homodimeric Cl^- channel encoded by *CLCN1* (19) (Figure 3). Functionally, the dominant mutants exert a dominant-negative effect on the dimeric channel complex as shown by coexpression studies, meaning that mutant/mutant and mutant/WT complexes are dysfunctional (20). The most common feature of the resulting Cl^- currents is a shift of the activation threshold toward more positive membrane potentials almost out of the physiological range (21–23). As a consequence of this, the Cl^- conductance is drastically reduced in the vicinity of the resting membrane potential. The recessive mutants that do not functionally hinder the associated subunit supply the explanation of why 2 mutant alleles are required to reduce Cl^- conductance sufficiently for myotonia to develop in Becker myotonia.

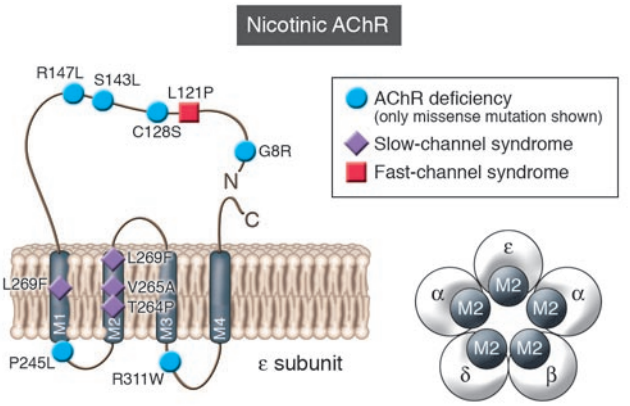

Figure 2
Muscle endplate nicotinic AChR. The nicotinic AChR of skeletal muscle is a pentameric channel complex consisting of 2 α subunits and 1 β, 1 γ, and 1 δ subunit in fetal and denervated muscle, and 2 α subunits and 1 β, 1 δ, and 1 ε subunit in adult muscle. All subunits have a similar structure with 4 transmembrane segments, M1 to M4. They form a channel complex with each subunit contributing equally to the ion-conducting central pore formed by the M2 segments. The pore is permeable to cations. The binding site for ACh is located in the long extracellular loop of the α subunit. The 3 main conformational states of the ligand-gated channels are closed, open, and desensitized. Binding of the transmitter opens the channel from the closed state, and, during constant presence of the transmitter, desensitization occurs. Only after removal of the transmitter, the channel can recover from desensitization and subsequently will be available for another opening. Mutations associated with subtypes of CMSs are indicated by conventional 1-letter abbreviations for the replaced amino acids.

Table 1
Hereditary muscle channelopathies

Gene	Locus	Channel protein	Disease	Heredity	Effect
SCN4A	17q23.1-25.3	$Na_v1.4$ Na^+ channel α subunit	Hyperkalemic periodic paralysis, paramyotonia congenita, K^+-aggravated myotonia	Dominant	Gain
			Hypokalemic periodic paralysis type 2	Dominant	Loss
CACNA1S	1q31-32	L-type Ca^{2+} channel α1 subunit, DHP receptor	Hypokalemic periodic paralysis type 1	Dominant	Unclear
			MH type 5	Dominant	Unclear
RyR1	19q13.1	RyR1, Ca^{2+} release channel	MH	Dominant	Gain
			Central core disease	Dominant or recessive	Gain
KCNJ2	17q23-24	Kir2.1 K^+ channel α subunit	Andersen syndrome	Dominant	Loss
KCNQ2	20q13.3	Kv7.2 K^+ channel α subunit	Neuromyotonia with benign neonatal familial convulsions	Dominant	Loss
CLCN1	7q32-qter	ClC-1 voltage-gated Cl^- channel	Thomsen myotonia	Dominant	Loss
			Becker myotonia	Recessive	Loss
		Altered splicing of ClC-1	Myotonic dystrophy type 1	Dominant	Loss
			Myotonic dystrophy type 2	Dominant	Loss
CHRNA1	2q24-32	nAChR α1 subunit	CMS	Dominant or recessive	Gain or loss
CHRNB1	17p12-11	nAChR β1 subunit	CMS	Dominant or recessive	Gain or loss
CHRND	2q33-34	nAChR δ subunit	CMS	Dominant or recessive	Gain or loss
CHRNE	17p13-p12	nAChR ε1 subunit	CMS	Dominant or recessive	Gain or loss
RAPSN	11p11	Rapsyn, nAChR-associated	CMS	Recessive	Loss

DHP, dihydropyridine; nAChR, nicotinic AChR.

Chloride channel myotonia in myotonic dystrophies
Myotonic dystrophy, the most common inherited muscle disorder in adults, is a progressive multisystemic disease characterized by muscle wasting, myotonia, subcapsular cataracts, cardiac conduction defects, gonadal atrophy, hearing deficiencies, and cognitive deficits. There are 2 clinically distinguished types: type 1, with the classical phenotype caused by an expansion of an unstable CTG trinucleotide repeat in the 3′ untranslated region of the myotonic dystrophy protein kinase (*DMPK*) gene on chromosome 19q13.3 (24), and type 2, with a more proximal pattern of weakness (25) caused by an expansion of a CCTG tetranucleotide repeat in intron 1 of the *ZNF9* gene coding for a zinc finger protein (26). The pathogenesis of the myotonia is based on an alternative splicing of the ClC-1 RNA, leading to loss of function of the channel protein (4, 5).

Sodium channel myotonia and paramyotonia
In Na^+ channel myotonia and paramyotonia, there is a gating defect of the Na^+ channels that destabilizes the inactivated state such that channel inactivation may be slowed or incomplete (27, 28). This results in an increased tendency of the muscle fibers to depolarize, which generates repetitive action potentials (myotonia). The mutant channels confer a dominant gain of function on the channel as well as on cell excitability (Figure 4).

One hot spot for the paramyotonia mutations is a special voltage-sensing transmembrane region that couples channel inactivation to channel activation (29); another hot spot is an intracellular protein loop containing the inactivation particle (30). The K^+-aggravated myotonia mutations are found in intracellular regions of the protein, potentially interfering with the channel inactivation process (28, 31). Corresponding to the severity of the disruption of the inactivation gate structure on the protein level, there are 3 clinical severities to be distinguished: (a) myotonia fluctuans, where patients may not be aware of their disorder; (b) myotonia responsive to acetazolamide with a Thomsen-like clinical phenotype; and (c) myotonia permanens, where continuous electrical myotonia leads to a generalized muscle hypertrophy including facial and neck muscles, suggestive of facial dysmorphia. In all 3 types, body exertion or administration of depolarizing agents may result in a severe or even life-threatening myotonic crisis (32).

Periodic paralysis: plasmalemmal hypoexcitability due to mutant Na^+ or Ca^{2+} channels
Symptoms occur episodically with varying intervals of normal muscle function and excitation because ion channel defects are usually well compensated and an additional trigger is often required for muscle inexcitability. Two dominant episodic types of weakness with or without myotonia are distinguished by the serum K^+ level during the attacks of tetraplegia: hyperkalemic and hypokalemic periodic paralysis (PP). Intake of K^+ and intake of glucose have opposite effects in the 2 disorders: while K^+ triggers a hyperkalemic attack and glucose is a remedy, glucose provokes hypokalemic attacks, which are ameliorated by K^+ intake. Because of additional release of K^+ from hyperkalemic PP muscle and uptake of K^+ into hypokalemic PP muscle, serum K^+ disturbance can be so severe during a paralytic attack that cardiac complications arise. During an attack, death can also occur due to respiratory insufficiency (2).

Sodium channel PP with myotonia. Most $Na_v1.4$ mutations that cause hyperkalemic PP are situated at inner parts of the transmembrane segments or in intracellular protein loops (Figure 4) and affect structures that form the docking site for the fast-inactivation particle (33, 34). Thereby, they impair fast channel inactivation and lead to a persistent Na^+ current (35). At the beginning of an attack, the sustained inward current is associated with a mild membrane depolarization and leads to myotonia. The progressing attack is characterized by membrane inexcitability and muscle weakness, since the penetrated Na^+ ions go along with a more

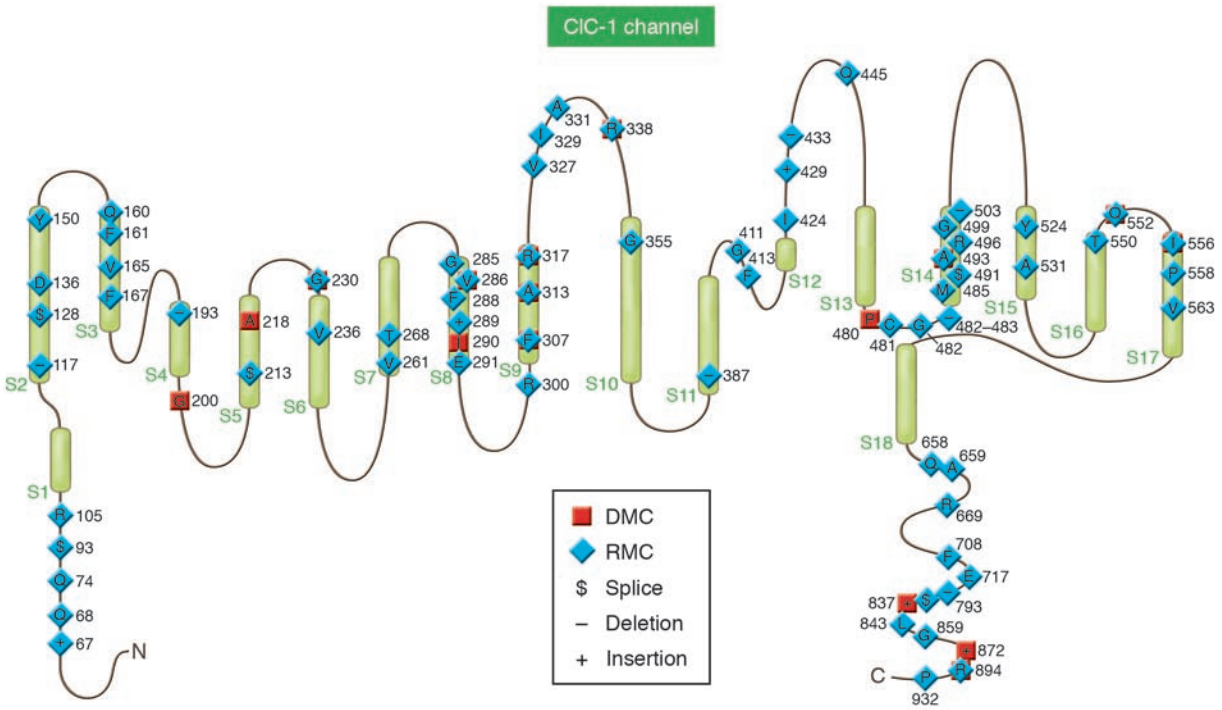

Figure 3
ClC-1, the major chloride channel of skeletal muscle. A membrane topology model of the ClC-1 monomer is shown. X-ray measurements and cryo-electron microscopy have elucidated the structure of the channel (104, 105) and confirmed the conclusions derived from electrophysiological results. The functional channel is an antiparallel assembled homodimer. It possesses 2 independent ion-conducting pores, each with a fast-opening mechanism of its own, 2 selectivity filters, and 2 voltage sensors (106). The channel is functional without any other subunits. Symbols are used for the mutations leading to either dominant myotonia congenita (DMC) or recessive myotonia congenita (RMC). The amino acids at which substitutions occur are indicated by 1-letter abbreviations and numbered according to the protein sequence. Adapted with permission from *Nature* (104).

severe sustained membrane depolarization that inactivates most Na^+ channels. Depending on the location of the underlying mutation, symptoms typical of hyperkalemic PP, K^+-aggravated myotonia, and paramyotonia congenita can overlap in a given patient (36). Sodium channel inhibitors such as mexiletine and flecainide are highly effective in preventing sodium channel myotonia and weakness in paramyotonia patients but not in patients with hyperkalemic PP. Particularly, mutant sodium channels that exhibit an enhanced closed-state inactivation offer a pharmacogenetic strategy for mutation-specific treatment (37).

Na^+ and Ca^{2+} channel PP without myotonia. In contrast to the gain-of-function changes associated with hyperkalemic PP, hypokalemic PP is associated with a loss-of-function defect of 2 different ion channel types: Cav1.1 (hypokalemic PP type 1) and $Na_v1.4$ (clinically indistinguishable hypokalemic PP type 2) (38–40). The mutations are located exclusively in the voltage-sensing S4 segment of domain 2 of $Na_v1.4$ and domain 2 or 4 of Cav1.1 (Figure 4). Functionally, the inactivated state is stabilized in the Na^+ channel mutants (39, 41, 42), while the channel availability is reduced for the Ca^{2+} channel mutants (43, 44). It is still unclear how the loss-of-function mutations of these 2 cation channels can produce the long-lasting depolarization that leads to the weakness seen in patients (45, 46). The attacks of weakness drastically reduce the patients' ability to perform activities of daily living. For many, loss of their jobs and social relationships is more distressing than the physical handicap.

K^+ channel PP with cardiac arrhythmia. Patients with Andersen syndrome may experience a life-threatening ventricular arrhythmia independent of their PP, and long QT syndrome is the primary cardiac manifestation (47, 48). The syndrome is characterized by the highly variable clinical triad of PP, ventricular ectopy, and potential dysmorphic features (49, 50). The paralytic attack may be hyperkalemic or hypokalemic, and, accordingly, the response to oral K^+ is unpredictable. Mutations of the Kir2.1 K^+ channel, an inward rectifier expressed in skeletal and cardiac muscle, are causative of the disorder (51). Kir2.1 channels are essential for maintaining the highly negative resting membrane potential of muscle fibers and accelerating the repolarization phase of the cardiac action potential. The mutations mediate loss of channel function by haploinsufficiency or by dominant-negative effects on the WT allele (52) and may lead to long-lasting depolarization and membrane inexcitability.

Muscle channelopathies due to an altered excitation-contraction coupling

Muscle contractures, i.e., electrically silent contractions due to intracellular Ca^{2+} exceeding the mechanical threshold, as well as flaccid weakness are characteristic features of disturbed muscle excitation-contraction coupling. Two allelic forms are well studied: MH and central core disease.

Malignant hyperthermia

Susceptibility to MH is an autosomal dominant predisposition to respond abnormally when exposed to volatile anesthetics, depolarizing muscle relaxants, or extreme physical activity in hot environ-

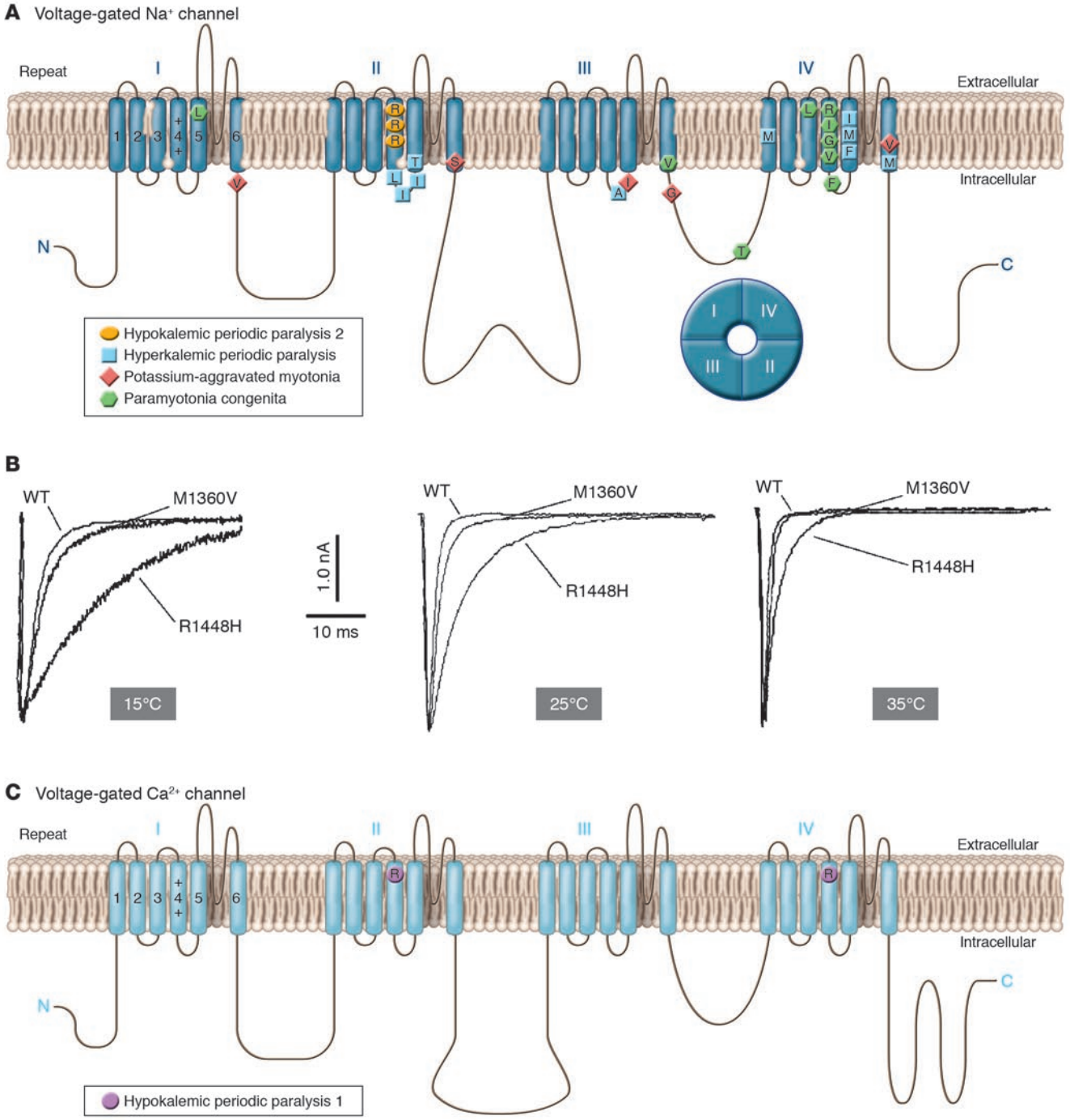

Figure 4

Voltage-gated Na^+ and Ca^{2+} channels: structure and function. The α subunit consists of 4 highly homologous domains, I–IV, with 6 transmembrane segments each (S1–S6). The S5–S6 loops and the S6 transmembrane segments form the ion-selective pore, and the S4 segments contain positively charged residues that confer voltage dependence to the protein. The S4 segments are thought to move outward upon depolarization, thereby inducing channel opening. The repeats are connected by intracellular loops; in the Na^+ channel (**A**), the III–IV linker contains the supposed inactivation particle, whereas the slowly activating and inactivating L-type Ca^{2+} channel does not possess a fast-inactivation gate (**C**). When inserted in the membrane, the 4 repeats of the protein fold to generate a central pore. Mutations associated with the various diseases are indicated. (**B**) Activation, inactivation, and recovery from the fast-inactivated to the resting state are voltage- and time-dependent processes. Compared is the fast inactivation of WT and 2 mutant skeletal muscle Na^+ channels expressed in human embryonic kidney cells: R1448H, a cold-sensitive mutation causing paramyotonia congenita, and M1360V, a temperature-insensitive mutation causing hyperkalemic periodic paralysis. The whole-cell current responses to a depolarization from –100 mV to 0 mV were superimposed at 25°C and 35°C. Adapted with permission from the *Journal of Physiology* (107).

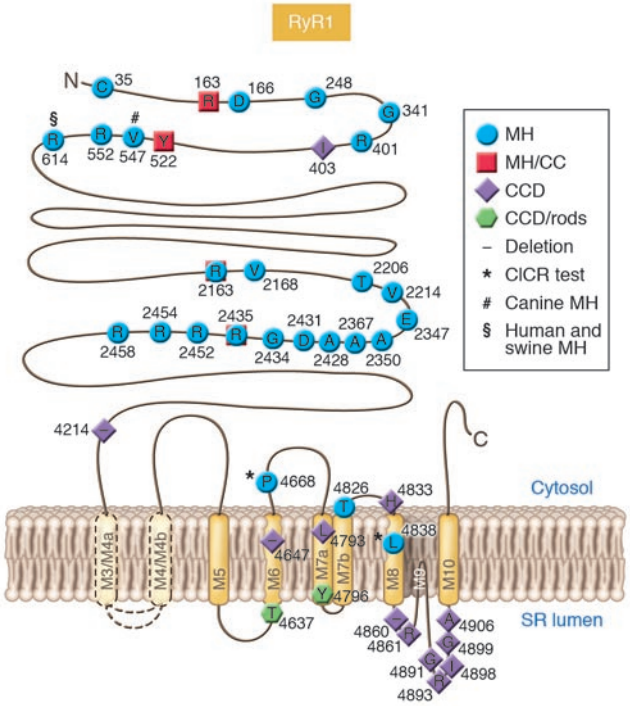

Figure 5
Skeletal muscle RyR1. RyR1 forms a homotetrameric protein complex that is situated in the SR membrane and functions as a Ca^{2+} release channel. The cytosolic part, the "foot," bridges the gap between the transverse tubular system and the SR. It contains binding sites for various activating ligands, like Ca^{2+} (μM), ATP (nM), calmodulin (nM), caffeine (mM), and ryanodine (nM), and inactivating ligands, like dantrolene (>10 μM), Ca^{2+} (>10 μM), ryanodine (>100 μM), and Mg^{2+} (μM). The transmembrane segments, M3–M10, are numbered according to both the earlier model of Zorzato et al. (108) and the recently modified model of MacLennan and colleagues (109). The first 2 cylinders, with dashed lines, indicate the tentative nature of the composition of the first predicted helical hairpin loop (M3–M4 or M4a–M4b). The long M7 sequence is designated as M7a and M7b. The proposed selectivity filter between M8 and M10 is designated as M9 even though it is clearly not a transmembrane sequence. Mutations causing susceptibility to MH and/or central core disease are indicated. Susceptibility to MH was defined by use of the in vitro contracture test or, in a single case, by the Japanese Ca-induced Ca release test (CICR test). MH/CC, MH with some central cores; CCD, central core disease; CCD/rods, CCD with nemaline rods.

ments (53). During exposure to triggering agents, a pathologically high increase in myoplasmic Ca^{2+} concentration leads to increased muscle metabolism and heat production, resulting in muscle contractures, hyperthermia associated with metabolic acidosis, hyperkalemia, and hypoxia. The metabolic alterations usually progress rapidly, and, without immediate treatment, up to 70% of the patients die. Early administration of dantrolene, an inhibitor of Ca^{2+} release from the SR, has successfully aborted numerous fulminant crises and has reduced the mortality rate to less than 10%.

In most families, mutations can be found in the gene encoding the skeletal muscle ryanodine receptor, RyR1 (Figure 1 and Figure 5). This Ca^{2+} channel is not voltage-dependent on its own but exists under the control of Cav1.1. MH mutations are usually situated in the cytosolic part of the protein and show gain-of-function effects: they increase RyR1 sensitivity to caffeine and other activators, as shown in functional tests of excised muscle, isolated native proteins, and ryanodine receptors expressed in muscle and nonmuscle cells (54). For another MH locus on chromosome 1q31-32, an R1086H disease-causing mutation was identified in the skeletal muscle L-type calcium channel α1 subunit (55, 56). The mutation is located in an intracellular loop of the protein, whose functional significance for EC coupling is under debate (57). Although mutations in the same gene cause hypokalemic PP type 1, this disorder is not thought to be associated with MH susceptibility (58, 59).

Central core disease

RyR1 mutations in the SR-luminal region cause central core disease, a congenital myopathy clinically characterized by muscle hypotrophy and weakness and a floppy-infant syndrome, often alongside other skeletal abnormalities such as hip displacement and scoliosis (60). Pathognomonic is the abundance of central cores devoid of oxidative enzyme activity along the predominant type 1 muscle fibers. Some mutations decrease the open probability of the RyR1 channel so that it loses the ability to release Ca^{2+} in response to the altered conformation of the dihydropyridine receptor that is induced by depolarization of the plasma membrane (61). However, RyR1 retains the ability to influence the open probability of the dihydropyridine receptor, with which it interacts. Other mutations increase the open probability of the RyR1 channel, leading to depleted SR Ca^{2+} stores and weakness. Both dominant and rare recessive mutations have been described, the latter transiently presenting as multi-minicore disease (62).

In vitro functional studies of channel mutants

As illustrated above, functional expression of mutations has contributed to the understanding of the molecular pathogenesis of several muscle channelopathies. However, there are undeniable problems of interpreting changes in function brought about by mutants in in vitro expression systems. The overexpression of introduced DNA in a heterologous cell system may lead to an unphysiological localization of the encoded protein. This can lead to a false conclusion regarding channel significance (compare ref. 63 with refs. 64–67). Secondly, the cells chosen for functional expression may have endogenous channel subunits that can potentially interact with or be upregulated by the introduced DNA. These can generate currents that may be falsely assumed to appear due to the introduced DNA (compare ref. 68 with refs. 69, 70). Thirdly, heterologous expression systems may secondarily modify the channels chemically, which may lead to misinterpretation of the functional significance of the channel subunits of $Na_v1.4$, for example, which exhibits more rapid inactivation kinetics when expressed in human embryonic cell lines than in *Xenopus* oocytes. Originally, this finding was attributed to the lack of expression of the accessory β subunit in the oocytes; however, the rapid kinetics of the channel were also found when $Na_v1.4$ was expressed in cells without endogenous β subunits. Therefore, posttranslational modifications and association of sodium channels with other membrane proteins such

as cytoskeletal components are now considered responsible for the differences in kinetics (2).

The function of ion channels is highly dependent on the expression system used. The functional significance implied by these experiments may not necessarily be valid for the physiological situation in vivo. Additionally, a functional change may not necessarily prove that a naturally occurring amino acid substitution causes a disease. The functional change could be brought about by a polymorphism instead. In spite of inherent difficulties in obtaining such findings, several of these "functional polymorphisms" have been described. For example, S906T in the skeletal muscle $Na_v1.4$ sodium channel was found to segregate perfectly with PP in several large pedigrees and to alter entry into and recovery from slow channel inactivation. However, it occurs in 5% of the population without association with any disease (71). In the PP families mentioned, S906T turned out just to be linked to another change that is causing PP and that was identified much later.

Functional polymorphisms in cardiac Kv7.1 and Kv11.1 K^+ channels occur in approximately 11–30% of the population (72–74), and those in cardiac $Na_v1.5$ sodium channels have been detected in approximately 20% of the population (75, 76). These polymorphisms have been suggested to mediate susceptibility to life-threatening arrhythmia caused by elongated QT intervals in the ECG, even though the prevalence of the polymorphisms is several hundred times higher than that of the long QT syndrome.

The question arises of whether it is justified to consider up to 30% of the population to be at risk for long QT syndrome and what consequences such a high long QT prevalence should have. Given the variability with which humans are prone to polymorphisms, it is not surprising that these polymorphisms must be associated with changes in function that can generate disease-susceptible, disease-protective, or otherwise disadvantageous or advantageous features (such as intelligence or attractiveness). Therefore, it is not at all clear how to interpret changes of channel function brought about by naturally occurring amino acid changes when studying in vitro expression systems.

In contrast to polymorphisms that may lead to functional changes, mutations in ion channels may cause changes of function that are irrelevant to disease. For example, familial hypokalemic PP type 1 still baffles scientists even though the genetic cause was identified 10 years ago (38) and the functional defects of the mutant Ca^{2+} channel have been described in various expression systems (43, 44, 77, 78). Nevertheless, how a channel that is primarily involved in muscle excitation-contraction coupling can elicit the long-lasting membrane depolarization shown to be the cause of the paralysis (45, 46) is still not understood. Another striking example is that opposite changes of function that have been described for putative mutations of the same channel can cause the same clinical phenotype. This unexpected observation contradicts the idea that a singular pathogenetic mechanism can be deduced from similar functional defects observed in vitro. For example, several missense mutations in the voltage-gated neuronal Na^+ channels $Na_v1.1$ and $Na_v1.2$ are thought to cause a dominant monogenic form of epilepsy. When expressed in mammalian cells or in transgenic mice, some of the mutations enhanced channel inactivation and reduced membrane excitability, while others destabilized the inactivated channel state and increased cell excitability (79–82). The situation for familial hemiplegic migraine, a rare subtype of migraine with aura caused by Cav2.1 Ca^{2+} channel mutations, is similar: the mutations lead to either reduced or increased Ca^{2+} influx into the cytoplasm so that both gain and loss of channel mechanisms can cause the same phenotype (83–87).

Epidemiology and genetic linkage studies of channel mutants

Frequency of the putative mutation in a control population

Because of the shortcomings of the interpretation of functional studies, the genetic screening of large and adequately matched control populations for absence of the putative mutations is important to prove disease causality. Two reports have proposed the typing of 150–200 controls (300–400 chromosomes) for putative mutations with a prevalence of 1% by power analysis (88, 89). A more general algorithm that recommends exclusion of the putative mutation in ethnically matched control chromosomes has recently been proposed (90). For a proposed maximally tolerable error of 1% and a mutation present on 1% of tested patient chromosomes, the equation advises to test 460 control chromosomes (230 control individuals) (Figure 6).

Therefore, the common laboratory practice of excluding a novel mutation in approximately 100 healthy controls is insufficient. An example is an R83H substitution in a K^+ channel β subunit, MiRP2, suggested to cause PP because it showed a loss of function in vitro and was found in 2 of 100 of such patients but in none of 120 unaffected controls (91). In later studies, the substitution was identified in 1 of 104 and 1 of 138 patients, but also in 8 of 506 and 3 of 321 controls (90, 92). When these results are taken together, the substitution is present in 1.17% of patients

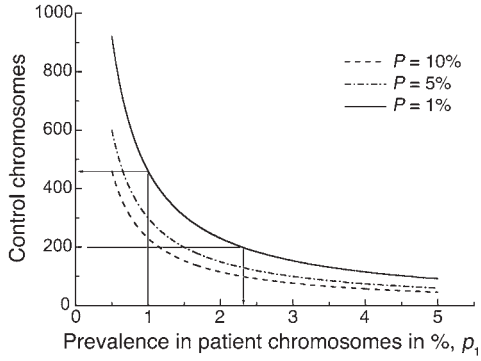

Figure 6

Proposed number of control chromosomes. A statistical algorithm helps to calculate the number of controls required to minimize the error. Let the prevalence of a mutation in patient chromosomes be p_1 and the prevalence in control chromosomes be p_0. Then the probability of an arbitrary control chromosome not carrying the mutation is $(1 - p_0)$. Because the world control population is large, the probability P of arbitrarily choosing n chromosomes thereof without the mutation may be approximated by $P = (1 - p_0)^n$. The null hypothesis would be that the mutation frequency is equal in patient and control chromosomes, i.e., $p_0 = p_1$ and $P = (1 - p_1)^n$. The number of control chromosomes to be tested can be calculated by resolution of the equation for the number $n = \ln(P)/\ln(1 - p_1)$. When the error probability P is set at 1%, the number of required control chromosomes is $n = -4.6/\ln(1 - p_1)$ and $n = 460$ for the example of $p_1 = 1\%$. The curve demonstrates that 100 control individuals (200 chromosomes) would be adequate for a p_1 of 2.5%, a prevalence that is much higher than that of the most frequent monogenic disorder. Adapted with permission from *Neurology* (90).

and in 1.16% of healthy controls, which does not support disease causality. Even though the difference between defining a putative mutation as truly disease-causing and defining it as a functional polymorphism may seem only marginal on a scientific level, this difference has drastic consequences for an affected carrier whose diagnosis is made or confirmed by the finding and who is being medically treated. This problem will increasingly need to be addressed in future studies when the number of known mutations and putatively associated phenotypes continues to increase.

Genetic linkage studies within families

Genetic linkage analyses were very successful in finding the gene loci in MH and also in hypokalemic PP (39, 93–95). In contrast to these genome-wide analyses in which large pedigrees were studied, a number of relatively small MH families for which linkage to the ryanodine receptor gene on chromosome 19 was excluded were tested in a candidate-gene approach. The first alternative locus was assigned to chromosome 17q11.2-q24, which suggested *SCN4A*, the gene encoding the skeletal muscle sodium channel, as candidate gene (96, 97). Apparently some of the families had a muscle Na^+ channelopathy (98, 99), which could explain the anesthesia-related events as exaggerated myotonic reactions. The generalized muscle spasms and resulting systemic alterations are usually triggered by succinylcholine in patients with a Na^+ channelopathy and can resemble MH; as further evidence for the myotonic origin of the crises, susceptibility to MH was excluded in such patients by the European in vitro contracture test (32, 58, 100–102). Suggestions of 4 further MH loci, made each in a single pedigree, still await confirmation.

Phylogenetic analysis of genes for conserved sequences and structural regions

Good conservation of amino acid residues is no guarantee that all changes of such a residue would lead to disease. For example, the polymorphism W118G in ClC-1 (103) concerns an amino acid that is highly conserved in several members of the ClC-channel family, such as ClC-2, ClC-7, ClC-Ka, and ClC-Kb. In contrast, several of the known disease-causing ClC-1 mutations such as F413C or Q552R affect residues are not equally well conserved in these channels.

These examples of questionable interpretation of genetic or functional data emphasize the importance of combining as many of the above criteria as possible to be able to make a reliable decision regarding whether a given variant may be deleterious or not. Genetic animal models or gene expression profiling may clarify these areas in the future.

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