Human muscle voltage-gated ion channels and hereditary disease
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Insights in the field of ion channels were made possible by the Nobel-prize-winning patch-clamp technique that enables characterization of channel function, and have greatly been inspired by associated diseases pointing to regions of functional significance. These so-called ion channelopathies have common clinical features, recurrent patterns of mutations, and almost predictable mechanisms of pathogenesis. In skeletal muscle, disorders are associated with mutations in Na+, K+, Ca2+, and Cl− channels that lead to hypoexcitability (causing periodic paralysis) and to hyperexcitability (causing myotonia or susceptibility to malignant hyperthermia).

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Abbreviations
CCD central core disease
HyperPP hyperkalemic periodic paralysis
HypoPP hypokalemic periodic paralysis
MH malignant hyperthermia
PAM K+-aggravated myotonia
PC paramyotonia congenita
SR sarcoplasmic reticulum
TTS transverse tubular system

Introduction
Membrane excitability, which is critical for muscle function, is regulated by voltage-gated ion channels. It is therefore not surprising that ion channels are involved in the pathogenesis of diseases of skeletal muscle. Pioneering work on excised muscle tissue of patients with hereditary episodic weakness demonstrated the underlying defect to be a persistent Na+ inward current, depolarizing the membrane and causing inexcitability and weakness [1]. Cloning and analysis of the gene that encodes the voltage-gated Na+ channel of skeletal muscle revealed the first mutations associated with impaired ion channel function and confirmed hyperkalemic periodic paralysis (hyperPP) to be a Na+ channel disorder [2]. Since then, over twenty diseases now termed as channelopathies have been described (for a review see [3••]). Best understood are the disorders of skeletal muscle that serve as a paradigm for episodic disorders of the brain and heart such as migraine, epilepsy, and cardiac arrhythmia.

Muscle physiology
Motoneuron activity is transferred to skeletal muscle in the neuromuscular junction, generating an action potential in the muscle that propagates along the surface membrane including the transverse tubular system (TTS), a membrane region projecting deep into the cell to ensure even distribution of the impulse. The upstroke of the action potential is mediated by opening of the voltage-gated Na+ channels (encoded by the SCN4A gene, and its accessory β-subunit encoded by SCN1B) that elicit a Na+ inward current with rapid activation kinetics. Repolarization of the membrane by rapid Na+ channel inactivation is additionally supported by opening of K+ channels (encoded by KCNC4 and its accessory subunit encoded by KCNE3) that mediate an outward K+ current. Buffering of after-potentials is achieved by a high Cl− conductance near the resting potential resulting from the homodimeric Cl− channel encoded by CLCN1.

At specialized junctions in the TTS, the signal is transmitted from the outer membrane to the inside of the muscle cell causing the release of Ca2+ ions from the sarcoplasmic reticulum (SR), which in turn activates the contractile apparatus, a process called excitation–contraction coupling. Two main Ca2+ channel complexes are involved in this process, the voltage-gated pentameric dihydropyridine receptor located in the TTS (encoded by the CACNA1S gene and accessory subunits encoded by CACNA2D1, CACNG1 and CACNB1) and the homotetrameric ryanodine receptor of the SR (encoded by the RYR1 gene). The voltage-gated Ca2+ channel is activated by membrane depolarization and, by this, activates the ryanodine receptor by direct protein–protein interaction, which in turn releases Ca2+ into the cytosol (for a review see [4•]).

Channel structure
The basic motif of the main cation channel subunit, the α subunit, is a tetrameric association of a series of six transmembrane α-helical segments, numbered S1–S6, connected by both intracellular and extracellular loops, the interlinkers (Figures 1 and 2). The α subunit contains the ion-conducting pore and therefore determines the main characteristics of the cation channel complex that conveys ion selectivity, voltage sensitivity, pharmacology and binding characteristics for endogenous and exogenous ligands. Whereas for Ca2+ and Na+ channels the α subunit consists of a momomer, K+ channels form homo- or heteromultimers because each α subunit consists only of one domain with six transmembrane helices. Accessory subunits (β, γ, or δ) do not share a common structure, some having several transmembrane segments and others being entirely intracellular or extracellular. Functionally, they may influence channel expression, trafficking, and gating.

Voltage-sensitive cation channels have at least one open state and at least two closed states, one from which the channel can directly be activated (the resting state) and one from which it cannot (the inactivated state). This implies that there are at least two gates regulating the opening of the pore, an activation and an inactivation gate. Both functions are usually mediated by the α subunit. Whereas
activation is a voltage-dependent process, inactivation and the recovery from the inactivated state are time dependent.

The voltage sensitivity of cation channels is conveyed by the S4 segments, which are thought to move outward upon depolarization and channel opening [5,6], compatible with the first protein cryo-electron-microscopic study on single channel proteins [7••]. During channel closing, not all voltage sensors move back at once, generating a variety of closed states and explaining the distribution of voltage sensor mutations to phenotypes in Na+ channels [8••]. The ion conducting pore is thought to be lined by the S5–S6 interlinkers [9], which contain the selectivity filter [10]. Whereas the localization of the activation gate may well be within the pore, the inactivation gate has been shown to be located in different regions in the Na+ and K+ channels [11,12].

In contrast to the cation channels, not much about the structure/function relationship of Cl− channels is known. They form homodimers and heterodimers [13,14]. The main CLCN1-encoded Cl− channel of skeletal muscle conducts over the whole physiological voltage range, showing inward rectification in the negative potential range. Hydrophobicity blots have suggested 13 putative transmembrane helical segments [15], but by a combination of glycosylation and electrophysiological experiments on mutant proteins, it became clear that both the amino terminus and carboxyl terminus must be located intracellularly, requiring an even number of transmembrane segments. Additionally, the S8–S9 interlinker was shown to be extracellular by location of a glycosylation site. Two different possibilities of configuration arose from these results: firstly, a model that places S4 extracellularly and the hydrophobic core of S9–S12 crosses the membrane several times (Figure 4; [16]); or, alternatively, a model that places S4 associated to the pore region and therefore transmembraneously [17].

**Channelopathies**

Clinically, skeletal muscle ion channelopathies appear as recurring episodes of muscle stiffness or weakness typically triggered by circumstances such as cold, exercise, oral K+ load, or drugs. Muscle stiffness, termed myotonia,
ameliorates with exercise and can be associated with transient weakness during quick movements lasting only a few seconds. Conversely, paradoxical myotonia or paramyotonia worsens with exercise and cold and is followed by long spells of limb weakness lasting from hours to days. Depending on mode of transmission and K⁺ sensitivity, four forms of myotonia and paramyotonia may be distinguished: dominant K⁺-aggravated myotonia (PAM); dominant K⁺-insensitive myotonia congenita; paramyotonia congenita (PC) and recessive generalised myotonia congenita (Table 1). Myotonia is the clinical phenotype brought about by uncontrolled repetitive firing of action potentials leading to involuntary muscle contraction. The contrary, lack of action potentials or inexcitability, results in muscle weakness. Two dominant episodic types of weakness with or without myotonia are distinguished by the serum K⁺ level during the attacks of tetraplegia: hypokalemic periodic paralysis (HypoPP) and HyperPP (Table 1). Myotonia is the clinical phenotype brought about by uncontrolled repetitive firing of action potentials leading to involuntary muscle contraction. The contrary, lack of action potentials or inexcitability, results in muscle weakness. Two dominant episodic types of weakness with or without myotonia are distinguished by the serum K⁺ level during the attacks of tetraplegia: hypokalemic periodic paralysis (HypoPP) and HyperPP (Table 1). Finally, susceptibility to malignant hyperthermia, a dominant pharmacogenetic predisposition to react unfavorably upon administration of depolarizing muscle relaxants and volatile anesthetics during anesthesia, is also a voltage-gated ion channelopathy. An acute, potentially lethal crisis is characterized by muscle hypermetabolism, rhabdomyolysis, body temperature elevation, muscle rigidity, and cardiac arrhythmia (Table 1). MH is pathogenetically based on an uncontrollable intracellular Ca²⁺ release via the ryanodine receptor.

Na⁺ channel disorders
For PAM, PC, and HyperPP, the Na⁺ channel adynamia paramyotonia complex underlying pathogenesis mechanism is the same: a gating defect of the Na⁺ channel destabilizing the inactivated state (i.e. channel inactivation may be slowed or incomplete). This results in an increased tendency of the muscle fibers to depolarize. Whereas Na⁺ influx at slight depolarization itself generates action potentials and myotonia, stronger depolarizations lead to general inactivation of both mutant and the wild-type Na⁺ channels (in a dominant disorder, both a mutant and a wild-type allele are present) and, thus, weakness. Because the mutant channels exert an effect on cell excitability, the mutations produce a dominant change or gain of function.

Corresponding to the phenotype and the type of inactivation defect, the mutations are located mainly in the voltage-sensing S4 segment of domain IV that is suggested to couple the inactivation to the activation process [18], in the III–IV interlinker known to contain the inactivation gate (PC and PAM; [19]), and at several disseminated intracellularly faced positions potentially involved in generating an acceptor for the inactivation particle or steric
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hinderence of the binding of the two (PAM and HyperPP; [20,21]; Figure 1). This allows the reader to draw two conclusions, both of which are correct. First, there could be an overlapping of the phenotypes of the three disorders. Second, more severe membrane depolarization found in HyperPP may be caused by incomplete inactivation and a persistent current, whereas more slight depolarizations of shorter duration in PC and PAM may be caused by slowing of the inactivation time course (Figure 3).

Local anaesthetics and class I antiarrhythmic drugs, such as mexiletine and lidocaine derivatives, are antymyotonic

Table 1
An overview of the voltage-gated ion channelopathies of skeletal muscle.

<table>
<thead>
<tr>
<th>Disease entity</th>
<th>chr.</th>
<th>Gene and gene product</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myotonia congenita</td>
<td>7q35</td>
<td>CLCN1 Cl– channel ClC1</td>
<td>ar (Becker), ad (Thomsen); generalized myotonia, warm-up phenomenon, muscle hypertrophy, transient weakness (Becker)</td>
</tr>
<tr>
<td>Paramyotonia congenita</td>
<td>17q23</td>
<td>SCN4A Na+ channel α subunit</td>
<td>ad; paradoxical myotonia, cold-induced muscle stiffness followed by weakness/paralysis</td>
</tr>
<tr>
<td>K+-aggravated myotonia</td>
<td>17q23</td>
<td>SCN4A Na+ channel α subunit</td>
<td>ad; generalized myotonia of variable severity, aggravation by K+ administration, no weakness</td>
</tr>
<tr>
<td>HyperPP</td>
<td>17q23</td>
<td>SCN4A Na+ channel α subunit</td>
<td>ad; episodic attacks of mainly generalized weakness, hyperkalemia during episode, triggering by rest after body exertion or K+ intake, additional myotonia but not paramyotonia</td>
</tr>
<tr>
<td>HypoPP</td>
<td>1q32</td>
<td>CACNA1S Ca2+ channel α1 subunit</td>
<td>ad; episodic attacks of mainly generalized weakness, hypokalemia during episode, triggering by carbohydrate-rich food or exercise, amelioration by K+ intake, no myotonia</td>
</tr>
<tr>
<td></td>
<td>17q23</td>
<td>SCN4A Na+ channel α subunit</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11q13-14</td>
<td>KCNE3 K+ channel β subunit</td>
<td></td>
</tr>
<tr>
<td>Malignant hyperthermia</td>
<td>19q12-q13</td>
<td>RYR1 Ryanodine receptor Ca2+ channel</td>
<td>ad; triggering by volatile anesthetics or depolarizing muscle relaxants, generalized muscle rigidity, hyperthermia, metabolic acidosis, rhabdomyolysis</td>
</tr>
<tr>
<td></td>
<td>1q32</td>
<td>CACNA1S Ca2+ channel α1 subunit</td>
<td></td>
</tr>
<tr>
<td>Central core disease</td>
<td>19q12-q13</td>
<td>RYR1 Ryanodine receptor Ca2+ channel</td>
<td>ad; congenital myopathy with characteristic histological central cores, muscle hypotonia, proximal weakness, CK elevation, and susceptibility to malignant hyperthermia</td>
</tr>
</tbody>
</table>

ad, autosomal dominant; ar, autosomal recessive; chr., chromosomal location of causative gene; CK, creatine kinase; vg, voltage gated.

Figure 3
Two examples of faulty inactivation of mutant sodium channels of skeletal muscle associated with potassium-aggravated myotonia. Patch-clamp recordings from normal (WT) and mutated (Gly1306Val and Val1589Met) channels expressed in human embryonic kidney cells. (a) Families of sodium currents recorded at various test potentials in the whole-cell mode show slowed decay and failure to return completely to baseline. Slowed inactivation is more pronounced with Gly1306Val, persistent inward sodium current is larger for Val1589Met. (b) Traces of five single-channel recordings each obtained by clamping the membrane potential to ~20 mV. Mutant channels show re-openings that are the reason for the ‘macroscopic’ current alterations shown in the upper panels. Modified from [19].
agents because they stabilize the inactivated state and lead to the phenomenon called use-dependent block. Because the spontaneous attacks of weakness typical for HyperPP are not influenced by mexiletine — because no repetitive action potentials occur that can be attenuated by a use-dependent blocker — diuretics such as hydrochlorothiazide and acetazolamide can be administered. These drugs decrease frequency and severity of paralytic episodes by lowering serum K+ and other so-far unexplained favourable properties (e.g. influencing myoplasmic pH and plasmalemmal K+ channels [22]).

**A disorder of Na\(^+\), Ca\(^{2+}\), and K\(^+\) channels**

In contrast to the gain-of-function changes associated with HyperPP, HypoPP is associated with a loss-of-function defect of Na\(^+\), Ca\(^{2+}\), and K\(^+\) ion channels [23,24*,25,26,27**]. In Na\(^+\) and Ca\(^{2+}\) channels, the mutations are located solely in the voltage-sensing S4 segments of domain 2 (Na\(^+\)) or domains 2 and 4 (Ca\(^{2+}\)) (Figures 1 and 2). In the latter, the reported mutation is situated in the accessory β subunit. Functionally, the inactivated state is stabilised in the Na\(^+\) channel mutants [24*,28], whereas the channel availability is reduced for the Ca\(^{2+}\) channel mutants [29,30*]. It is still a mystery, however, how the loss of function of these two cation channels can produce the long-lasting depolarization associated with the weakness [31], but it does imply that a concomitant myotonia is not to be expected, as is the case. In contrast, for the very rare K\(^+\) channel variant of hypoPP, a reduced current density has been demonstrated that produces a slight membrane depolarization when heterologously expressed in a muscle cell line [27**]. Therapeutically, long-term low-dose intake of acetazolamide (as for HyperPP) is recommended to avoid attacks of weakness in HypoPP. During acute paralysis phases though, oral administration of K\(^+\) to depolarise the membrane, has proved to relieve symptoms.

**Cl\(^-\) channel disorders**

Whereas dominant K\(^+\)-insensitive myotonia congenita (DMC) is clinically almost indistinguishable from PAM, recessive generalized myotonia congenita (RGM) is much more severe (i.e. earlier onset and muscle hypertrophy due to involuntary body building by the myotonia). Both DMC and RGM are caused by mutations in the Cl\(^-\) channel ([32]; Figure 4). All mutations in this anion channel bring about loss-of-function changes leading to reduced Cl\(^-\) conductance and, thus, instability of the membrane resting potential resulting from lack of buffering of the after potentials [33]. As expected, the dominant mutants exert a dominant negative effect on the dimeric channel complex as shown by co-expression studies. That is, even mutant/wild-type complexes are dysfunctional [34]; this is not the case for the recessive mutants, which do not functionally hinder

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**Figure 4**

A revision of the original membrane topology model that was based on hydrophobicity analysis [15] of the skeletal muscle chloride channel monomer CIC-1, which is functional as a homodimeric channel complex (modified from [34]). The different symbols used for the known mutations leading to dominant Thomsen-type myotonia, recessive Becker-type myotonia, and animal models (recessive myotonic mice and dominant myotonic goat) are explained on the bottom-left. Conventional one-letter abbreviations were used for replaced amino acids located at positions given by the respective numbers of the human protein.
the co-associated subunit. These reports have led to a double-barrel model of the Cl⁻ channel with two independent ion-conducting pores each with a fast gate of its own that is affected by the recessive mutations, but with a common slow gate shared with the co-associated subunit that is affected by the dominant mutations [35]. Intriguingly, this structure has been confirmed by cryo-electronmicroscopy on two-dimensional protein crystals [36••]. Because of the disseminated distribution of the mutations, structure/function relationships for the Cl⁻ channel are not as clear as for the cation channels (Figure 4).

Many patients with myotonia congenita can manage their disease without medication. Should treatment be necessary, myotonic stiffness responds well to drugs that reduce the increased excitability of the cell membrane by interfering with the Na⁺ channels (i.e. local anaesthetics, antifibrillar and antiarrhythmic drugs, and related agents). These drugs suppress myotonic runs by decreasing the number of available Na⁺ channels, and have no known effect on Cl⁻ channels. Of the many drugs tested that can be administered orally, mexiletine is the drug of choice.

**A pharmacogenetic disorder of Ca²⁺ channels**

Individuals with susceptibility to malignant hyperthermia (MH) do not present with any muscular symptoms outside of the operating room. An associated allelic myopathy, central core disease (CCD), though, causes decreased muscle tone and permanent weakness (Table 1). To date, more than 20 disease-causing point mutations in the gene encoding the Ca²⁺ release channel of the SR (encoded by RYR1) have been identified for MH susceptibility, and are situated in the cytosolic part of the protein ([37]; Figure 5). In contrast, those associated with CCD myopathy are located in the pore lumen and internal SR loops [38].

Hypersensitivity of RYR1 to anesthetic triggering agents is pathogenetically causative in functional tests of both excised muscle, isolated native proteins, and heterologously expressed full-length ryanodine receptors. In heterologous non-muscle expression systems, MH mutant Ca²⁺ channels show gain of function by increased sensitivity to triggering agents such as halothane [39]; the cells expressing the mutants have higher resting Ca²⁺ levels, smaller endoplasmic reticulum Ca²⁺ stores, and reduced maximal Ca²⁺ release compared with the wild type cells [40].
Conversely, CCD mutants show a decreased Ca$^{2+}$ release via the ryanodine receptor, pointing to a loss of function due to change in the ion-conducting pore region [41••]. As expected, during an anesthetic MH crisis, dantrolene, an inhibitor of intracellular Ca$^{2+}$ release, has been very effective in reducing the mortality rate from 70% to 10%. This drug is not administered to CCD patients as it would increase their weakness.

A second gene for MH susceptibility is the voltage-gated Ca$^{2+}$ channel, also causative for HypoPP. Mutations are located in the III–IV interlinker [42], the significance of which is unknown because the decisive region of interaction between this Ca$^{2+}$ channel and the ryanodine receptor, has been demonstrated to lie in the II–III interlinker ([43]; Figure 2). Functional studies are still lacking but a facilitated interaction between the two proteins in the sense of a gain of function may be expected. As suggested by the regional distribution of the mutations in this Ca$^{2+}$ channel, no phenotypic overlap is found between HypoPP and MH susceptibility.

**Conclusion**

Current research on ion-channel disorders employs short-cuts derived from the fact that these channelopathies share the common features of attack-like symptoms of hyperexcitability or underexcitability provoked by typical factors, and additional progressive components related to cell degeneration. Genetic studies may therefore be accelerated by a candidate gene approach, and functional studies by expression cloning of ion channel subunits. Pathogenesis mechanisms associated with the mode of transmission predict functional consequences: recessive mutations lead to loss of function, whereas dominant mutations lead to change or gain of function (dominant positive effect) or loss of function (dominant negative effect in multimeric proteins or haplo-insufficiency). Over 400 ion channel genes are encoded by the human genome. This suggest that the identification of several more channelopathies can be expected, which can serve as a model for understanding disease pathogenesis of the more frequent non-hereditary idiopathic variants.

**Acknowledgements**

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


This paper identifies the skeletal muscle sodium channel as subunit being responsible for type 2 HypoPP functionally. Slowing of and decreased amplitudes of action potentials, sodium current reduction and enhanced channel inactivation suggest that HypoPP-2 is the first sodium channel disease caused by loss of function.


This paper shows that the Shaw-like voltage-gated potassium channel Kv3.4 (α subunit) and the β subunit MiRP2 (encoded by KCNE3), form a complex channel that sets the resting membrane potential of skeletal muscle. A KCNE3 mutation predicting Arg83His substitution was identified in two periodic paralysis families that reduces current density and therefore the resting potential in heterologously expressed cells.


This paper reports the formation of two-dimensional crystals of a CIC channel protein reconstituted into phospholipid bilayer membranes. Cryo-electronmicroscopic analysis of these crystals yields a projection structure at 6.5 Å resolution, which shows off-axis water-filled pores within the dimeric channel complex.


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41. Avila G, O'Brien JJ, Dirksen RT: Excitation-contraction uncoupling by a human central core disease mutation in the ryanodine receptor. *Proc Natl Acad Sci USA* 2001, 98:4215-4220. Shows that homoxogenous expression of the CDC-causing I4897T mutation in dyspedic myotubes results in a complete uncoupling of sarcosomal excitation from voltage-gated SR Ca2+ release without significantly altering resting cytosolic Ca2+ levels. SR Ca2+ content, or RyR1-mediated enhancement of dihydropyridine receptor channel activity.
