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.
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 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+,1.4 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 CIC-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 Ca2+ ions into the myoplasm, which activate the contractile apparatus (8). This process is called excitation-contraction coupling. Two Ca2+ channel complexes are chiefly involved in this process, the voltage-gated pentameric Cav1.1 Ca2+ 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 collagen tail subunit (ColQ) that mediates AChE binding to the postsynaptic AChR (14). Postsynaptic CMSs are caused by mutations in the chloride channel CLCN1 (15) that mediates Cl− conductance at the postsynaptic neuromuscular junction.

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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adolescence, or adult life with upper-limb predominance and con
deficiency but is much rarer. Mutations at different sites lead to
response to a single supramaximal stimulus. The syndrome results
from gain-of-function mutations in the ion-conducting pore M2
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 proper-
ties. These kinetic mutations result in the slow- or fast-channel syn-
dromes. 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 con-
tractures, does not respond to anticholinesterase, and is progress-
ive. 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, para-
doxical myotonia (also called paramyotonia) worsens with cold and
after exercise. Both myotonia and paramyotonia derive from uncon-
trolled 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 Thoms-
sen myotonia and recessive Becker myotonia are caused by miss-
sense 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 suf-
ciently for myotonia to develop in Becker myotonia.

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 depolar-
ization, alters its conformation, and activates the ryanodine receptor,
which releases Ca2+ from the SR, a Ca2+ store. Ca2+ binds to troponin
and activates the so-called contractile machinery.

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 struc-
ture 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 ε 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 pres-
ence of the transmitter, desensitization occurs. Only after removal of
the transmitter, the channel can recover from desensitization and sub-
sequently will be available for another opening. Mutations associated
with subtypes of CMSs are indicated by conventional 1-letter abbrevia-
tions for the replaced amino acids.

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**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 CIC-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 dysmoria. 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 Ca2+ 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,1.4 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

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**Table 1**

**Hereditary muscle channelopathies**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Channel protein</th>
<th>Disease</th>
<th>Heredity</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN4A</td>
<td>17q23.1-25.3</td>
<td>Na,1.4 Na+ channel α subunit</td>
<td>Hyperkalemic periodic paralysis, paramyotonia</td>
<td>Dominant</td>
<td>Gain</td>
</tr>
<tr>
<td>CACNA1S</td>
<td>1q31-32</td>
<td>L-type Ca2+ channel α1 subunit, DHP receptor</td>
<td>Hypokalemic periodic paralysis type 2</td>
<td>Dominant</td>
<td>Loss</td>
</tr>
<tr>
<td>RyR1</td>
<td>19q13.1</td>
<td>RyR1, Ca2+ release channel</td>
<td>MH type 5</td>
<td>Dominant</td>
<td>Unclear</td>
</tr>
<tr>
<td>KCNJ1</td>
<td>17q23-24</td>
<td>Kir2.1 K+ channel α subunit</td>
<td>Central core disease</td>
<td>Dominant or recessive</td>
<td>Gain</td>
</tr>
<tr>
<td>KCNJ2</td>
<td>20q13.3</td>
<td>Kv7.2 K+ channel α subunit</td>
<td>Andersen syndrome</td>
<td>Dominant</td>
<td>Loss</td>
</tr>
<tr>
<td>CLCN1</td>
<td>7q32-pter</td>
<td>CIC-1 voltage-gated Cl- channel</td>
<td>Neuromyotonia with benign neonatal familial convulsions</td>
<td>Dominant</td>
<td>Loss</td>
</tr>
<tr>
<td>ZNF9</td>
<td>17p13-p12</td>
<td>Altered splicing of CIC-1</td>
<td>Thomsen myotonia</td>
<td>Dominant or recessive</td>
<td>Gain or loss</td>
</tr>
<tr>
<td>CHRNA1</td>
<td>17p12-11</td>
<td>nAChR α1 subunit</td>
<td>Becker myotonia</td>
<td>Dominant</td>
<td>Loss</td>
</tr>
<tr>
<td>CHRNB1</td>
<td>17p13-p12</td>
<td>nAChR β1 subunit</td>
<td>Myotonic dystrophy type 1</td>
<td>Dominant</td>
<td>Loss</td>
</tr>
<tr>
<td>CHRND</td>
<td>2q33-34</td>
<td>nAChR δ subunit</td>
<td>Myotonic dystrophy type 2</td>
<td>Dominant or recessive</td>
<td>Gain or loss</td>
</tr>
<tr>
<td>CHRNE</td>
<td>11p11</td>
<td>Rapsyn, nAChR-associated</td>
<td>CMS</td>
<td>Recessive</td>
<td>Loss</td>
</tr>
</tbody>
</table>

DHP, dihydropyridine; nAChR, nicotinic AChR.
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).
ments (53). During exposure to triggering agents, a pathologically high increase in myoplasmic Ca$^{2+}$ concentration leads to increased muscle contraction, 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 non-muscle 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 a1 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 may secondarily modify the channels chemically, which may 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.1.4, 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.1.4 was expressed in cells without endogenous β subunits. Therefore, posttranslational modifications and association of sodium channels with other membrane proteins such as 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.1.4, 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.1.4 was expressed in cells without endogenous β subunits. Therefore, posttranslational modifications and association of sodium channels with other membrane proteins such as...
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 Na1.4 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 Na1.5 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 Ca2+ 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 Na1.1 and Na1.2 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 Ca2+ channel mutations, is similar: the mutations lead to either reduced or increased Ca2+ 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).

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.
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 known number of 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 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 CIC-1 (103) concerns an amino acid that is highly conserved in several members of the CIC-channel family, such as CIC-2, CIC-7, CIC-Ka, and CIC-Kb. In contrast, several of the known disease-causing CIC-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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