Neurological disorders caused by inherited ion-channel mutations

Dimitri M Kullmann and Michael G Hanna

Several neurological diseases—including neuromuscular disorders, movement disorders, migraine, and epilepsy—are caused by inherited mutations of ion channels. The list of these "channelopathies" is expanding rapidly, as is the phenotypic range associated with each channel. At present the best understood channelopathies are those that affect muscle-fibre excitability. These channelopathies produce a range of disorders which include: periodic paralysis, myotonias, malignant hyperthermia, and congenital myasthenic syndromes. By contrast, the mechanisms of diseases caused by mutations of ion channels that are expressed in neurons are less well understood. However, as for the muscle channelopathies, a striking feature is that many neuronal channelopathies cause paroxysmal symptoms. This review summarises the clinical features of the known neurological channelopathies, within the context of the functions of the individual ion channels.

Several neurological diseases caused by inherited mutations of ion channels of muscle, neuron, and glia have been discovered in the past decade. These diseases have come to be known as the "channelopathies".[1,2] Ion channels can also be affected by autoimmunity and possibly by acquired disorders of RNA processing,[3] but those acquired channelopathies (autoimmune and transcriptional, respectively) are not discussed here. A recurring theme in the genetic channelopathies is that mutations of several different ion channels can cause remarkably similar phenotypes (locus heterogeneity). Conversely, distinct mutations of the same gene can cause variable phenotypes (allelic heterogeneity). Thus, classification of channelopathies presents difficulties, whether it is based on gene or by phenotype.

The molecular biology, structure, and function of ion channels have been comprehensively reviewed elsewhere,[4] as have the basic principles of studying the consequences of abnormal ion-channel function for cellular excitability.[5] Some of the earlier muscle channelopathies were identified on the basis of electrophysiological recordings from isolated muscle fibres, which pointed to disturbances of ion-channel function, followed by examination of candidate genes.[6,7] The neuronal channelopathies, however, have mainly been identified on the basis of genetic linkage studies, although homology among related channels has also pointed to likely candidate genes.[8]

Muscle channelopathies

At present, the best understood channelopathies are those that affect muscle-fibre excitability (table 1).[9]

DMK and MGH are at the Institute of Neurology, University College London, and the National Hospital for Neurology and Neurosurgery, London, UK.

Correspondence: Prof Dimitri M Kullmann, Institute of Neurology, Queen Square, London WC1N 3BG, UK. Tel +44 (0)20 78373611 ext 4100; fax +44 (0)20 72785616; email d.kullmann@ion.ucl.ac.uk

Table 1. Ion channels associated with human inherited muscle diseases

Type of channel	Gene	Channel	Disease
Voltage-gated channels			
Na$^+$ channels	*SCN4A*	α subunit of Na$_v$1.4 (skeletal muscle)	Hyperkalaemic periodic paralysis
			Hypokalaemic periodic paralysis
			Paramyotonia congenita
			Other myotonic disorders
K$^+$ channels	*KCNJ2*	α subunit of Kir2.1 inward rectifier (skeletal and smooth muscle)	Andersen's syndrome
	KCNE3	Accessory subunit MiRP2 (assembles with K$_v$3.4)	Hypokalaemic periodic paralysis
Ca^{2+} channels	*CACNA1S*	α subunit of Ca$_v$1.1 (skeletal muscle dihydropyridine-sensitive channel)	Hypokalaemic periodic paralysis
			Malignant hyperthermia
	RYR1	Ryanodine receptor (sarcoplasmic channel)	Malignant hyperthermia
			Central core disease
Cl$^-$ channels	*CLCN1*	ClC1 (skeletal muscle chloride channel)	Myotonia congenita (dominant and recessive)
Ligand-gated channels			
Nicotinic ACh receptors	*CHRNA1*	α$_1$ subunit (skeletal muscle)	Congenital myasthenic syndromes
	CHRNB1	β$_1$ subunit (skeletal muscle)	Congenital myasthenic syndromes
	CHRND	δ subunit (skeletal muscle)	Congenital myasthenic syndromes
	CHRNE	ε subunit (skeletal muscle)	Congenital myasthenic syndromes

Sodium-channel mutations: periodic paralysis, paramyotonia, and other disorders

Among the first channels to be linked to human disease was the major voltage-dependent sodium channel of skeletal muscle, $Na_v1.4$, mutations of which account for most cases of the autosomal dominant disease hyperkalaemic periodic paralysis.[10,11] Patients generally present in childhood with attacks of limb-muscle weakness lasting minutes to hours, often precipitated by rest after exercise. Consciousness is preserved, and ocular and respiratory muscles tend to be spared. Hyperkalaemia is frequently missed by physicians, and, indeed, many patients diagnosed as having normokalaemic periodic paralysis also carry $Na_v1.4$ mutations. With age, the frequency and severity of the attacks diminish, although a progressive vacuolar myopathy can intervene. Hyperkalaemic periodic paralysis is associated with a characteristic decrement in the amplitude of compound muscle action potentials after a period of sustained exercise.[12] Patients generally benefit from treatment to prevent hyperkalaemia (thiazide diuretics, β-adrenoceptor agonists),[13] or from inhibitors of carbonic anhydrase (acetazolamide, dichlorphenamide), which may act through several mechanisms including acidification of the channel microenvironment.[14]

The phenotypic range of $Na_v1.4$ mutations extends beyond hyperkalaemic and normokalaemic periodic paralysis. Potassium-aggravated myotonia, paramyotonia congenita, and myotonia fluctuans are all autosomal dominant disorders characterised by impaired muscle-fibre repolarisation.[9] Patients present with muscle stiffness and variable degrees of weakness that can fluctuate in severity. By contrast with chloride-channel-associated myotonia (see below), paramyotonia does not improve with exercise. Some cases of hypokalaemic periodic paralysis, commonly associated with calcium-channel mutations (see below) are also associated with $Na_v1.4$ mutations.[15,16]

How can we explain the phenotypic variability associated with sodium-channel mutations? Among substrates for genetic diseases, ion channels are marked out by the fact that their function can be studied with high precision by electrophysiological methods in vitro. Voltage-clamp techniques can be used to document the behaviour of populations of ion channels in individual cells, either in tissue removed from patients or in heterologous expression in various experimental preparations. When combined with patch-clamp techniques, the functional consequence of mutations can even be studied at the level of individual channels.
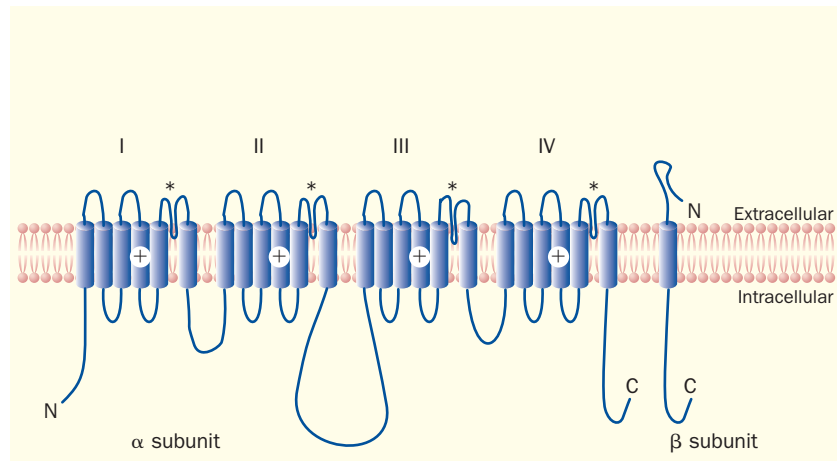

Figure 1. Transmembrane structure of $Na_v1.4$, the principal muscle sodium channel. The α subunit contains four homologous domains (I–IV), each of which consists of six helical transmembrane segments. The fourth segment of each domain (+) contains positively charged amino acids, which sense the transmembrane voltage. The loops between the fifth and sixth segments (*) come together to line the central pore of the ion channel and determine its ionic selectivity. A β subunit with a single transmembrane segment is associated with the α subunit.

Sodium-channel physiology

In common with other voltage-gated sodium and calcium channels, $Na_v1.4$ is made up of a principal pore-forming and voltage-sensing subunit (the α subunit), together with several accessory subunits.[17] The mutations in hyperkalaemic periodic paralysis and other muscle sodium-channel diseases affect this α subunit. The α subunit is made up of a four-fold repeated domain (figure 1). Each of these domains resembles a single subunit of a voltage-gated potassium channel, to which sodium and calcium channels are evolutionarily related. It contains six transmembrane segments linked by intracellular or extracellular loops. The fourth transmembrane segment (S4) of each domain contains several positively charged amino acids, which sense the voltage gradient across the membrane. Another critical area is the extracellular loop between the fifth and sixth segments, which dives into the membrane. The corresponding S5–6 loops from the four domains come together to line the pore of the ion channel. The amino acids of this loop determine the ionic selectivity of the channel, allowing sodium but not other ions to permeate.

After membrane depolarisation, sodium channels open rapidly because of a conformational change imparted by the voltage-sensing S4 segments. The inward sodium flux through the channels accounts for the rapid upstroke of the action potential. They then close rapidly even if the depolarisation persists, a process known as fast inactivation. In an intact muscle fibre, inactivation helps to curtail the action potential. The channels only come out of this inactivated state after membrane repolarisation.

Several mutations of $Na_v1.1$ occur in the cytoplasmic linker between domains III and IV (figure 1). This region is thought to act as a hinged lid, which occludes the channel pore on fast inactivation. Other mutations affect amino acids on the cytoplasmic side of the channel that act as receptors for

this lid, or in the voltage sensor of domain IV. Several mutations associated with myotonia that have been examined functionally impair the rate of inactivation or shift the voltage dependence of inactivation so that a larger depolarisation is required for inactivation. Some of these changes were actually observed in myotubes cultured from patients, before the mutations were identified.[18] The consequence of these alterations is that channels can continue to flicker between open and shut states in the face of continued depolarisation and therefore contribute a persistent sodium current. In a muscle fibre expressing mutant sodium channels, this persistent current is thought to impair repolarisation, and muscle fibres can therefore fire repeatedly, giving rise to myotonia. Myotonia associated with some sodium-channel mutations is exacerbated by rises in extracellular potassium, presumably because this stimulus also contributes to the depolarisation of the membrane. Nevertheless, attacks of paralysis are not a constant part of the phenotype in the myotonic disorders, possibly because channels can eventually inactivate through a second, slower mechanism that occurs on a timescale of seconds. Failure of this slower mechanism may account for many cases of hyperkalaemic periodic paralysis.[19] Muscle fibres become depolarised and inexcitable because of a positive feedback loop: persistent sodium influx promotes depolarisation, which promotes further potassium efflux, resulting in an attack of periodic paralysis. The paralysis only resolves when the extracellular potassium is decreased by active transport and renal clearance.

Both alterations in channel behaviour described above could be crudely thought of as representing a "gain of function", a common mechanism seen in dominantly inherited diseases, in that the mutant channels mediate an increased sodium flux. Nevertheless, this concept fails to capture the complexity of the role of sodium channels in controlling the excitability of muscle fibres, and only through understanding of the detailed consequences of the alterations can the distinct phenotypes be explained.

Calcium-channel mutations: hypokalaemic periodic paralysis and other disorders

The account given above does not explain why some $Na_v1.4$ mutations are associated with hypokalaemia.[15,16] Hypokalaemic periodic paralysis is more typically associated with mutations of the calcium channel $Ca_v1.1$.[20,21] This is also an autosomal dominant disorder, and it is commoner than the hyperkalaemic variant described above. It generally presents with paralysis triggered by carbohydrate ingestion and is similar to hyperkalaemic periodic paralysis, although the duration of paralytic attacks is much longer (typically 12–24 h). The post-exercise decrease in compound muscle action potential does not distinguish between hypokalaemic and hyperkalaemic periodic paralysis. In the absence of a family history, the neurologist should consider thyrotoxic periodic paralysis and look for other causes of electrolyte disturbance. In common with the hyperkalaemic variant, hypokalaemic periodic paralysis also frequently responds to acetazolamide, although agents that lower the serum potassium concentration exacerbate it.

The pore-forming and voltage-sensing α subunit of $Ca_v1.1$ has the same general topology as the sodium channel, with four repeated domains, each containing six transmembrane segments.[22] However, different amino acids form the selectivity filter of the S5–6 pore loop, explaining the preference for calcium over sodium ions. Inactivation is much slower.

Few mutations have been identified in hypokalaemic periodic paralysis, in the α subunits of either calcium or sodium channels.[16] Muscle fibres tend to be depolarised relative to the normal range,[23] but the mechanisms underlying this process are poorly understood. A reduction in calcium-channel current density has also been reported,[24] but why this should give rise to attacks of profound muscle weakness in association with hypokalaemia is far from clear.

Potassium-channel mutations: periodic paralysis

Mutations of another channel, the inwardly rectifying potassium channel Kir2.1, can cause attacks of periodic paralysis with high, low, or normal serum potassium.[25] These attacks occur as part of the autosomal-dominant disorder Andersen's syndrome (which also includes cardiac arrhythmia and craniofacial abnormalities). The mechanisms underlying this disease are poorly understood, although preliminary data point to loss of function; that is, a reduction in potassium current density. A further cause of periodic paralysis is mutations of the accessory subunit MiRP2.[26] This subunit associates with voltage-gated potassium channels expressed in muscle, and the wild-type form increases the potassium current flowing through these channels. Two identified mutations have been reported to decrease this current.[26]

Chloride-channel mutations: myotonias

A large number of mutations of CLC1, the principal chloride channel expressed in muscle, are associated with myotonia congenita. This disorder has conventionally been named Thomsen's or Becker's disease, depending on whether it is dominantly or recessively inherited. However, an individual mutation can sometimes behave dominantly or recessively in different families, although this behaviour may reflect the presence of an additional unrecognised mutation in some members (compound heterozygosity). Moreover, the disease is commonly milder in female patients, for reasons that remain obscure.

Myotonia congenita presents in the first or second decade of life, in most cases. Some patients develop muscle hypertrophy, possibly resulting from excessive spontaneous muscle-fibre activity. However, muscle weakness, sometimes accompanied by atrophy, is also recognised. Many patients report an amelioration of stiffness with repeated contraction, the so-called "warm-up" phenomenon. Characteristic myotonic discharges are seen on electromyography. These features do not distinguish the disease from myotonic dystrophy or proximal myotonic myopathy. If patients require treatment, several drugs acting on sodium channels (mexiletine, tocainide, phenytoin) have all been reported to be useful. Of these, mexiletine is the most effective in our experience.

The topology and function of chloride channels are quite distinct from those of sodium and calcium channels.[27] The muscle chloride channel is probably homodimeric,[28] although there is uncertainty about whether it has a single pore or two separate pores. Muscle fibres have a moderately high resting chloride conductance, which normally increases further on membrane depolarisation. Because the chloride reversal potential is relatively negative, the channel contributes to membrane repolarisation. The CLC1 mutations that have been examined lead to a reduction in current, often accompanied by a shift in the activation threshold to more depolarised values.[29,30] Abnormalities in chloride conductance were originally predicted from studies in myotonic goats.[31] This impairment in repolarisation is thought to give rise to repetitive action potentials in situ, the hallmark of myotonia.[32] Whether a mutation behaves in a dominant or recessive manner depends on the severity of its effect on kinetics and current density, and this feature can be probed by coexpressing wild-type channels in vitro.[30]

Other voltage-gated channelopathies of muscle

Another rare muscle channelopathy is malignant hyperthermia, which is generally uncovered after general anaesthesia. This disorder is potentially fatal and typically follows exposure of susceptible individuals to volatile anaesthetics (eg, halothane) and depolarising muscle relaxants (eg, suxamethonium).[33] In its full-blown form, patients develop muscle rigidity, rhabdomyolysis, tachycardia, hyperthermia, acidosis, hyperkalaemia, and shock. The mortality rate has fallen from 70% to 10% with the advent of dantrolene therapy.[34] This autosomal dominant disorder is a striking example of phenotypic convergence (or locus heterogeneity): malignant hyperthermia can result from mutations of either $Ca_v1.1$ (the channel associated with hypokalaemic periodic paralysis, see above)[35] or its structurally distinct partner, the ryanodine receptor, which mediates calcium efflux from the sarcoplasm.[36] The development of DNA-based diagnosis has implications for the screening of susceptible individuals for this potentially lethal disorder.[37] Ryanodine-receptor mutations are also associated with a rare progressive myopathy, central core disease.[38,39]

Muscle nicotinic acetylcholine-receptor mutations: congenital myasthenic syndromes

One of the most extensively studied, and best understood, muscle channelopathies affects a ligand-gated channel—the muscle nicotinic acetylcholine receptor (figure 2). Skeletal muscle is depolarised when this receptor is activated by acetylcholine released from motor endplates. Many mutations have been identified in congenital myasthenic syndromes.[40,41] (This must be distinguished from neonatal myasthenia, caused by passive transfer of antibodies from a mother with myasthenia gravis.) Congenital myasthenic syndromes (CMS) are heterogeneous, and only some are caused by mutations of acetylcholine receptors ("postsynaptic" CMS). Other forms are caused by presynaptic acetylcholine synthesis/packaging or release disorders ("presynaptic"

CMS), or arise from acetylcholinesterase deficiency ("synaptic" CMS).

Depending on the severity and subtype of the disorder, patients with acetylcholine-receptor mutations present in infancy or later with weakness and fatiguability affecting ocular, pharyngeal, respiratory, or limb muscles. Neonatal arthrogryposis is also recognised, implying abnormalities of intrauterine movements.[42,43] Different mutations are inherited dominantly or recessively. Electromyography reveals the characteristic decremental response on repetitive stimulation, and increased latency jitter and block with single-fibre recordings, also seen in myasthenia gravis. However, some cases associated with a slow-channel phenotype (see below) can also show characteristic repetitive discharges in response to a single supramaximal stimulus.

A more detailed insight into the pathophysiology comes from examination in vitro of the responses in muscle fibres to spontaneous release of acetylcholine from vesicles. Three distinct patterns are recognised. In the fast-channel variant, miniature end-plate potentials are shorter in duration than normal.[44] In the commoner slow-channel variant, they are longer than normal. These changes result from distinct abnormalities in channel gating, which either accelerate or retard the opening and closing of the channels. Because gating and ligand-binding are intimately related, slow-channel mutations are also associated with an increase in acetylcholine affinity.[45] Although, at first sight, an increase in affinity should further increase neuromuscular transmission, receptors can also enter a desensitised closed state if the ligand does not dissociate. Some slow-channel syndromes are also associated with a progressive myopathy, which possibly reflects an excitotoxic effect of increased cation influx into muscle fibres.[46] A third form results from a deficiency in numbers of acetylcholine receptors without
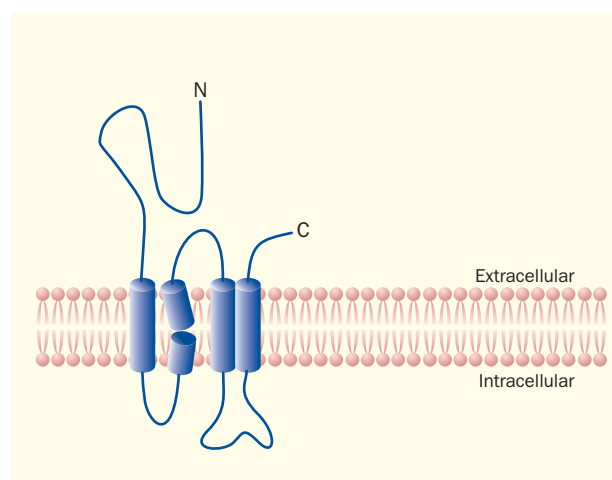

Figure 2. Transmembrane structure of a member of the nicotinic-receptor family. Five subunits come together to form a functional channel. The large extracellular amino terminus contains the ligand-binding domain. The second transmembrane segment lines the central pore of the ion channel and also gates ion flux, possibly through a hinge-like movement. Different mutations disrupt channel biogenesis, ligand binding, transduction from binding to opening, kinetics, and ion permeation.

kinetic alterations.[47] This form is associated with a decrease in the amplitude of spontaneous end-plate potentials. The slow-channel disease arguably results from a gain of function, in that the total current flowing through the receptor is prolonged. (And conversely, the fast-channel and receptor-deficiency forms represent loss of function.)

The histological features of congenital myasthenic syndromes caused by acetylcholine-receptor mutations are generally similar to those seen in acquired myasthenia gravis, with loss of junctional folds and widening of the synaptic cleft. Some patients affected by the fast-channel variant respond to anticholinesterases and 3,4-diaminopyridine, whereas those with the slow-channel syndrome may benefit from quinidine. In contrast to acquired myasthenia gravis, immunosuppression is ineffective.

The acetylcholine receptor (in common with many other ligand-gated channels) consists of five subunits, each of which has a large extracellular amino-terminus, which binds the agonist, and four transmembrane domains (figure 2).[4] The second of these domains lines the pore, and forms the selectivity filter (it allows only small cations to permeate). It also undergoes a conformational change on ligand binding. Two acetylcholine molecules must bind to the pentamer before the channel can open fully, and the channel closes when the ligand dissociates, or through desensitisation (a process akin to inactivation) if the ligand is not cleared. Five distinct genes code for muscle-receptor subunits: α_1, β_1, γ, δ, and ϵ. Adult nicotinic receptors have the stoichiometry $(\alpha_1)_2\beta_1\delta\epsilon$, but in the fetus the γ subunit takes the place of the ϵ subunit. Mutations have been identified in all the subunits other than γ.[40,41] Overall, the congenital myasthenic syndromes provide some of the clearest examples of phenotype–genotype correlations: the severity of the disorder, its electromyographic features, and associated myopathy can, to some extent, be accounted for by the site and nature of the mutation and its consequences for channel function.

Neuronal channelopathies

Potassium-channel mutations: episodic ataxia type 1

By comparison with the muscle channelopathies, the mechanisms of diseases caused by mutations of ion channels that are expressed in neurons are less well understood. As before, a striking feature is that many channelopathies cause paroxysmal symptoms (table 2). One such disease is a form of autosomal dominant paroxysmal cerebellar ataxia, episodic ataxia type 1, due to

Table 2. Neuronal and glial ion channels associated with human inherited neurological diseases

Type of channel	Gene	Channel	Disease
Voltage-gated			
Na+ channels	SCN1A	α subunit of Na$_v$1.1 (somatic sodium channel)	Generalised epilepsy with febrile seizures plus / Severe myoclonic epilepsy of infancy
	SCN2A	α subunit of Na$_v$1.2 (axonal sodium channel)	Generalised epilepsy with febrile seizures plus
	SCN1B	β_1 subunit of sodium channels	Generalised epilepsy with febrile seizures plus
K+ channels	KCNA1	α subunit of K$_v$1.1 (axonal/ presynaptic delayed rectifier)	Episodic ataxia type 1
	KCNQ2	M-type potassium channel subunit (with KCNQ3)	Benign familial neonatal convulsions
	KCNQ3	M-type potassium channel subunit (with KCNQ2)	Benign familial neonatal convulsions
Ca2+ channels	CACNA1A	α subunit of Ca$_v$2.1 (P/Q-type channel in cerebellar neurons and presynaptic terminals)	Familial hemiplegic migraine / Episodic ataxia type 2 / Spinocerebellar ataxia type 6
Ligand-gated			
Nicotinic ACh receptors	CHRNA4	β_2 subunit of nicotinic receptors (with α_4)	Autosomal dominant nocturnal frontal-lobe epilepsy
	CHRNB2	α_4 subunit of nicotinic receptors (with β_2)	Autosomal dominant nocturnal frontal-lobe epilepsy
Glycine receptors	GLRA1	α_1 subunit (spinal-cord inhibitory synapses)	Familial hyperekplexia
GABA$_A$ receptors	GABRG2	γ_2 subunit (brain inhibitory synapses)	Generalised epilepsy and febrile seizures plus
Glial channels			
Gap-junction proteins	GJB1	Connexin 32 (paranodal myelin)	X-linked Charcot-Marie-Tooth disease

Channelopathies causing deafness or blindness are not listed

mutations of the pore-forming subunit of the voltage-gated potassium channel K$_v$1.1.[48] Patients generally present with brief attacks of cerebellar incoordination that last for up to a few minutes and are triggered by stress or exertion. Between attacks, many patients have continuous motor-unit activity, with characteristic myokymia on electromyography, although this feature is commonly subclinical. Since the original discovery of mutations in the *KCNA1* gene, which codes for the α subunit of K$_v$1.1, further kindreds have been identified, with a broader range of phenotypes including neuromyotonia alone or with seizures.[49,50] There are striking differences in severity and response to drugs used to treat the disorder (mainly carbamazepine and acetazolamide) among kindreds bearing different mutations.

K$_v$1.1 is one of a large family of voltage-gated potassium channels. These channels are normally closed at resting membrane potentials and open rapidly upon depolarisation, accounting for a large part of the repolarisation of action potentials. Kv1.1 is the human orthologue of Shaker, the first potassium channel gene to be cloned in *Drosophila melanogaster*. The channel contains four pore-forming α subunits, each of which has the six transmembrane structure of one of the four repeated domains of sodium channels (figure 3). Although K$_v$1.1 describes a homomeric channel composed of four identical subunits, native channels
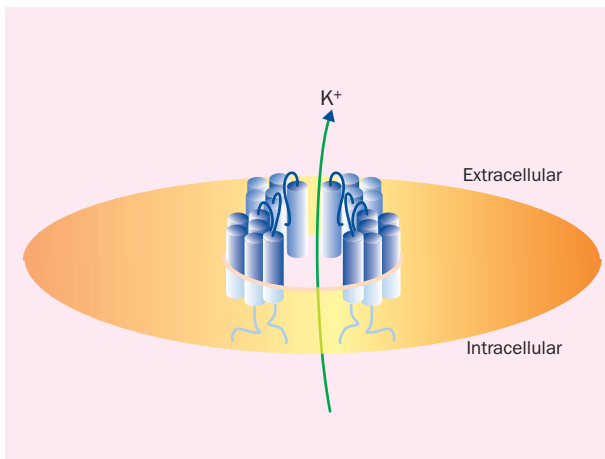
Figure 3. Voltage-dependent potassium channels such as $K_v1.1$ are composed of four α subunits, each of which resembles a single domain of an α subunit of a sodium or calcium channel. The fourth segment of each subunit contains the voltage sensor, and the loops between the fifth and sixth segments line the central pore of the ion channel. Additional cytoplasmic β subunits (not shown) have an important role in mediating inactivation. Because the channel is heteromultimeric, mutant and wild-type subunits can interact to affect channel trafficking, targeting, and kinetics.

are thought to consist of combinations of different members of the K_v1 family (especially $K_v1.2$ and $K_v1.4$).[51] Four cytoplasmic accessory β subunits are also associated, with the result that many channel stoichiometries potentially exist.

$K_v1.1$ is expressed widely in the CNS, especially in the axons of principal (glutamatergic) and inhibitory (GABAergic) neurons. It is especially abundant in membrane specialisations surrounding the initial segments of the axons of Purkinje cells.[52] Impaired repolarisation of GABAergic axons in this region may contribute to cerebellar ataxia.[53] $K_v1.1$ is also expressed in the juxtaparanodal regions of motor axons, a location that helps to explain the occurrence of spontaneous motor-unit firing.[54]

As hinted above, many of the features of episodic ataxia type 1 could result from loss of function of $K_v1.1$. This idea is supported by detailed electrophysiological studies of mutated channels.[49,50,55–59] However, the consequences of distinct mutations vary substantially, from small increases in the voltage threshold or time taken for channel activation, to complete absence of potassium flux. These differences seem to contribute to the allelic heterogeneity, in that mutations associated with a more profound impairment of channel function are seen in families with severe or drug-resistant disease, or with additional features such as epilepsy. To explore this correlation further the mutated channel has to be coexpressed not only with its wild-type counterpart—to reproduce the heterozygous situation in affected patients—but also with other members of the K_v1 family and accessory subunits. This is difficult because of the large number of possible channel stoichiometries. Nevertheless, this approach has revealed that some mutants exert a dominant negative effect on the wild-type allele. A simple explanation for the paroxysmal nature of episodic ataxia type 1 remains elusive. Unlike hyperkalaemic periodic paralysis, attacks of cerebellar

incoordination are not obviously associated with electrolyte disturbances in episodic ataxia type 1.

Calcium-channel mutations: FHM, EA2, SCA6

In common with the muscle channelopathies, those affecting the CNS also show evidence of locus heterogeneity. Another form of episodic ataxia (type 2; EA2) results from mutation of the $Ca_v2.1$, the pore-forming subunit of P/Q-type calcium channels.[60] Attacks are longer than in episodic ataxia type 1, and are associated with severe nausea and vomiting. Interictal myokymia is not a feature, but patients frequently develop nystagmus and slowly progressive cerebellar degeneration. The disorder is also inherited in a dominant manner.

The channelopathies affecting $Ca_v2.1$ also show striking allelic heterogeneity. Other dominantly inherited mutations are associated with familial hemiplegic migraine (FHM) and with a pure progressive cerebellar ataxia—spinocerebellar ataxia type 6 (SCA6).[61] Although these disorders appear very distinct, close examination of affected families shows some overlap in features.[62] The full phenotypic range of $Ca_v2.1$ mutations is probably not known, although an interesting recent finding is that some patients may be at risk of coma and cerebral oedema.[63]

$Ca_v2.1$ is expressed abundantly in the cell bodies and dendrites of cerebellar Purkinje and granule cells, which may help to explain the episodic ataxia and progressive cerebellar degeneration. $Ca_v2.1$ also accounts for much of the calcium influx at presynaptic nerve terminals at many central and peripheral synapses, where it helps trigger neurotransmitter release.[22] Because the cerebellum is not obviously implicated in hemiplegia or migraine, disorders of neurotransmitter release may be at the root of these features. Interestingly, abnormalities of neurotransmission at the neuromuscular junction have been reported in some patients with EA2.[64]

Distinct types of mutations appear to underlie the different disorders: mis-sense mutations occur in familial hemiplegic migraine, whereas episodic ataxia type 2 tends to be associated with premature stop codons and splice-site mutations, predicted to result in truncated peptides.[60] As for SCA6, this disease is associated with a small expansion of a polyglutamine sequence in the carboxyl terminus.[61] Mis-sense mutations have, however, also been identified in families with episodic or pure progressive ataxia.[65,66] Attempts to explain the phenotypes on the basis of the functional consequences of the mutations have met with mixed success; different mutations seen in familial hemiplegic migraine have been reported to cause various alterations in channel density and kinetics,[67–69] and inconsistent results have been reported for the polyglutamine expansion associated with SCA6.[70–72] As for premature truncations of $Ca_v2.1$, these are expected to result in a non-functional peptide.[73] Surprisingly, one premature stop codon identified in a sporadic patient with absence epilepsy in addition to episodic ataxia was found to exert a dominant negative effect on the wild-type allele.[74] This finding is unexpected because multiple $Ca_v2.1$ α subunits are not known to interact. This mutation is especially interesting because several spontaneous mutations of this subunit and

accessory ones in mice are associated with the same combination of ataxia and absence seizures, as is targeted deletion of the $Ca_V2.1$ α subunit. The role of this channel in sporadic absence epilepsy remains to be elucidated.[75]

Sodium channel and GABA_A-receptor mutations: generalised epilepsy with febrile seizures plus

The principle of genetic heterogeneity is most striking in the case of another epileptic syndrome consisting of febrile and afebrile seizures persisting beyond childhood: generalised epilepsy with febrile seizures plus (GEFS+). This disorder is associated with mutations of two different sodium-channel α subunits ($Na_V1.1$ and $Na_V1.2$),[76,77] the accessory sodium-channel subunit β_1,[78] and with the GABA_A-receptor subunit γ_2.[79,80] These mutations are all inherited in a dominant manner, and a major breakthrough was the recognition that they could give rise to very variable phenotypes even within affected families. The range of seizure types includes not only febrile and afebrile generalised tonic-clonic seizures that arise in childhood, but also myoclonic, absence, and atonic seizures. Some affected individuals are affected by a severe drug-resistant form associated with intellectual impairment, myoclonic-astatic epilepsy. The variability in manifestation contrasts with the relatively uniform phenotypes of many other channelopathies, and it is unclear to what extent it reflects environmental factors or modifying genes.

The neuronal sodium channels $Na_V1.1$ and $Na_V1.2$ are critical for the initiation and propagation of action potentials. Although they are widely distributed throughout the CNS, they show a differential localisation in the dendritic and axonal compartments of many neurons.[17] β_1 is one of two accessory subunits and it assembles with either of the α subunits. It accelerates the inactivation of the sodium channels. In a striking parallel with the muscle sodium-channel disorders, several GEFS+ mutations are predicted to result in an impairment of inactivation, resulting in a prolongation of the sodium flux after depolarisation (a gain of function). This result occurs either because of a defect of fast inactivation intrinsic to the α subunit, or because the β subunit is non-functional.[78] Thus, GEFS+ in these cases can be tentatively attributed to an increase in neuronal excitability. Whether this increase results from repetitive action-potential initiation in the axon or from improved dendritic boosting of synaptic depolarisation remains to be determined.

Interestingly, the genetic heterogenity shown by GEFS+ crosses the boundary between voltage-gated and ligand-gated channels. GABA_A receptors are the main mediator of fast inhibitory transmission in the brain. They have a similar pentameric structure to nicotinic acetylcholine receptors, with the main functional difference being that they are selectively permeable to chloride and bicarbonate anions. Although many different subunits exist, most neuronal GABA_A receptors fall into a few common stoichiometries. The γ_2 subunit is commonly associated with one or more α and β subunits, and it has an important role in localising GABA_A receptors to inhibitory synapses.[81] One of the mutations identified in GEFS+ reduces the maximum current when coexpressed with α and β subunits.[79] Thus, the epileptic phenotype is most simply explained by a reduction in fast inhibition. The other mutation, however, has been reported to have no effect on current amplitude, but to reduce the potentiating effect of benzodiazepines, a result that the authors interpret as implying that endogenous benzodiazepine agonists normally increase GABAergic inhibition.[82]

The account of GEFS+ given above agrees with the principle that generalised epilepsy can result from excessive neuronal excitation, or insufficient synaptic inhibition. However, this view is challenged by a recent report that heterozygous non-sense mutations of $Na_V1.1$ frequently occur in a severe sporadic form of epilepsy.[83] Severe myoclonic epilepsy of infancy is characterised by drug resistance and a poor prognosis. Tonic, clonic, and tonic-clonic seizures occur, frequently associated with febrile illnesses. Other seizure types occur later, including myoclonic, absence, and partial seizures, akin to a severe form of GEFS+. In addition, patients are affected by ataxia and by developmental, speech, and motor arrest. Life expectancy is severely curtailed, which possibly explains why the mutations are sporadic. The mutations that have been identified are predicted to cause major truncations of $Na_V1.1$ and are thus expected to cause a reduction in sodium-channel function, by contrast with the pattern seen in GEFS+.

Neuronal nicotinic-receptor mutations: autosomal dominant nocturnal frontal-lobe epilepsy

Two other distinct epileptic channelopathy phenotypes are recognised. Autosomal dominant nocturnal frontal-lobe epilepsy is due to mutations of either of two neuronal nicotinic acetylcholine receptor subunits: α_4 and β_2.[84–86] Interestingly, these two subunits coassemble to form one of the common heteromeric receptors in the brain.[87] Most patients present in childhood or adolescence, many with violent seizures occurring during sleep, sometimes with preserved consciousness.[88] The disorder is commonly misdiagnosed as a parasomnia, and electroencephalography abnormalities can be overlooked. Carbamazepine is generally effective. Although the disease is inherited in a dominant manner, penetrance is incomplete.

The mechanisms of this disorder are poorly understood. Several different functional consequences for acetylcholine-receptor function have been reported for distinct mutations, without an obvious common theme.[85,89–91] Furthermore, the normal function of $\alpha_4\beta_2$ nicotinic receptors is unclear. Many CNS nicotinic receptors are presynaptic, and their activation seems to increase the release of other neurotransmitters.[92] However, the circumstances under which they themselves are activated remain to be elucidated.

M-current disorder: benign familial neonatal convulsions

Benign familial neonatal convulsions are caused by another autosomal dominant epileptic channelopathy with high penetrance. Brief generalised seizures begin in the first week of life, and they resolve in most cases within 6 weeks.[93] There is a higher than normal risk of epilepsy later in life, but development is otherwise unaffected. The potassium

channel KCNQ2 was discovered by positional cloning in families with this disorder.[94,95] Homology screening subsequently revealed a closely related channel, KCNQ3, which also bears mutations in other affected families.[96] This search also led to an unexpected breakthrough in the understanding of neuronal signalling mechanisms. The function of these channel subunits was unclear until they were coexpressed: KCNQ2 and KCNQ3 coassemble and give rise to a potassium current that is inhibited by muscarinic receptors.[97,98] Mutations of either KCNQ2 or KCNQ3 seem to produce a small reduction in the amplitude of this so-called M current. The normal role of the M current is thought to suppress burst firing of neurons, and failure of this mechanism may account for the lower seizure threshold in carriers of KCNQ2 or KCNQ3 mutations. However, why the seizures tend to resolve spontaneously remains unclear.

Glycine-receptor mutations: familial hyperekplexia

Familial hyperekplexia was one of the first neuronal channelopathies to be discovered. It is caused by mutations of the α_1 subunit of glycine receptors.[99] Together with $GABA_A$ receptors, glycine receptors mediate fast inhibition in the spinal cord and brainstem but not in the forebrain. Distinct mutations are inherited in an autosomal dominant or recessive pattern.[99–102] Patients can present in infancy with muscle stiffness and muscle spasms, but more commonly the disorder manifests as excessive, non-habituating startle responses evoked by sensory or auditory stimuli. Falls can occur. The stiffness and startle responses tend to resolve with age, and clonazepam and sodium valproate are effective in many cases.

Glycine receptors belong to the nicotinic superfamily of ligand-gated channels and share a pentameric structure with acetylcholine and $GABA_A$ receptors.[103] In the adult, the stoichiometry of synaptic glycine receptors is thought to be three α_1 subunits, which bind the agonist, and two β subunits, which act to anchor the receptors at synapses. The mis-sense mutations that have been identified tend to be clustered in the agonist-binding amino-terminus, and in the M1–M2 loop, which is thought to be important for transduction of ligand binding to channel opening. However, a frame-shift mutation has also been reported, which is predicted to disrupt almost the whole channel.[104] Interestingly, this mutation is inherited in a recessive manner, which has several implications. First, complete absence of α_1 is not lethal, unlike the effect of a similar non-sense mutation in mice.[105] Second, because heterozygous carriers are unaffected, only one allele is required for normal inhibition in the spinal cord. Finally, familial hyperekplexia is predicted to result from loss of function. The latter conclusion has been supported by functional analyses of several mutations.[106,107]

Glial channelopathies
Connexin 32: X-linked Charcot-Marie-Tooth disease
Only one glial channelopathy is known to cause a neurological disease (table 2). X-linked Charcot-Marie-Tooth disease is characterised by a progressive motor and sensory neuropathy, with moderately slowed conduction (with velocities intermediate between those seen in the common

Search strategy and selection criteria
Data for this review were identified from surveys of medical and scientific journals and Medline searches. Peer-reviewed papers on the clinical presentation, management, genetics, and functional consequences of ion-channel mutations were included.

demyelinating and axonal forms). Many mutations of the *connexin 32* gene have been identified.[108] Connexins are small proteins that contain two transmembrane segments. They assemble in hexamers to form connexons. Two connexons, in apposed cell membranes, pair up to form a gap junction. These specialisations are thought to represent diffusional paths for small molecules, enabling rapid communication between distinct cells, or, in the case of connexin 32, between concentric layers of myelin sheaths contacting axons. Connexin 32 is expressed in Schwann cells and also in the CNS, where it is thought to be localised to the myelinating oligodendrocytes. Impaired gap-junction function is thought to underlie the pathogenesis of X-linked Charcot-Marie-Tooth disease, possibly because of disruption of cytoplasmic homoeostasis in the innermost layers of myelin.

Conclusions

Many other ion channels are likely to be implicated in neurological diseases in the future.[109] In addition, the phenotypic range associated with individual channels is likely to be broader than currently known. Until now, attention has been focused mainly on families with mendelian inheritance. However, the finding of mutations in patients with sporadic epilepsy[74,83] prompts a more extensive search for channelopathies in the absence of a family history. Another situation that needs to be explored is the possibility that sequence variations of ion-channel genes may be silent when inherited in isolation but cause disease when they occur in combination (compound heterozygosity).

The genetic channelopathies have helped us to understand the pathogenesis of several classes of disease. Many puzzles remain, however. Not least among these are the mechanisms that underlie migraine and hemiplegia in association with $Ca_V2.1$ mutations, and epilepsy with nicotinic-receptor mutations. Some mutations are in the process of being expressed in animal models.[110] In combination with a detailed understanding of molecular and cellular signalling by ion channels, this approach may well give a unique insight into common mechanisms of a wide range of diseases including neuromuscular and movement disorders, epilepsy, and headache.

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