Neurological diseases caused by ion-channel mutations

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During the past decade, mutations in several ion-channel genes have been shown to cause inherited neurological diseases. This is not surprising given the large number of different ion channels and their prominent role in signal processing. Biophysical studies of mutant ion channels *in vitro* allow detailed investigations of the basic mechanism underlying these 'channelopathies'. A full understanding of these diseases, however, requires knowing the roles these channels play in their cellular and systemic context. Differences in this context often cause different phenotypes in humans and mice. The situation is further complicated by the developmental effects and other secondary effects that might result from ion-channel mutations. Recent studies have described the different thresholds to which ion-channel function must be decreased in order to cause disease.

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Abbreviations

BFNC	benign familial neonatal convulsions
DFNA/B	autosomal dominant/recessive deafness
EA	episodic ataxia
FHM	familial hemiplegic migraine
GEFS	generalised epilepsy and febrile seizures
LQT	long QT syndrome
SCA	spinocerebellar ataxia

Introduction

Ion channels form permeation pathways for the passive diffusion of ions across biological membranes. They have much higher transport rates than do transporters (permeases) or active pumps, giving rise to the sizeable currents that are characteristic of electric signal processing in nerve and muscle. For ion channels to generate a current, electrochemical gradients need to be established for the permeant ions. This is accomplished by an interplay between active pumps, co-transporters, and constitutively open ion channels. Mutations in these transporter genes can influence electrical signalling indirectly, as exemplified by mutations in a Na^+/H^+ exchanger that lead to epilepsy in mice [1] and by mutations in various different transporters and channels that lead to deafness [2,3,4••,5–7].

Given the large number of ion-channel genes expressed in the nervous system, the channelopathies currently known probably represent the tip of an iceberg. For many ion channels, however, a total loss of function may result in early lethality; therefore, only the more subtle changes in function may lead to the diseases that are observable in

humans. This is probably the case for important Na^+-channel isoforms, such as those dominating excitation in skeletal muscle or heart, and may also be the case for the two channel subunits that assemble to form M-type K^+-channels, which are key regulators of neuronal excitability [8••,9•]. This concept is supported by the observation that many channelopathies are paroxysmal (i.e. cause transient convulsions): mutations leading to a constant disability might be incompatible with life, or may significantly decrease the frequency of the mutation within the human population. In contrast to the severe symptoms associated with the loss of function of certain key ion channels, the large number of ion-channel isoforms may lead to a functional redundancy under most circumstances. Indeed, disruption of some K^+-channel subunits in mice results only in mild phenotypes [10,11].

Neurological diseases may be caused by mutations in many classes of ion channels, including voltage-gated potassium, sodium, calcium and chloride channels, as well as ligand-gated cation and anion channels (see Table 1). This short review will focus on diseases of the central nervous system (CNS) and will only briefly mention the well-understood channelopathies of heart and skeletal muscle (see [12] for an excellent review). We will first focus on individual ion-channel classes. We will then discuss epilepsy, which can be caused by mutations in different ion channel genes, as well as in several other genes.

Diseases caused by mutations in KCNQ K+-channels

Although there are about 80 different K^+-channel genes in the nematode *C. elegans* [13] and probably many more in humans, mutations in about ten K^+-channel genes only are known to cause human disease. Quite remarkably, these include all four known members of the KCNQ subfamily of K^+-channels [4••,14–17]. Investigation of diseases related to these channels allows insight into the various functions of K^+-channels, and provides an interesting paradigm for the different thresholds separating normal current magnitudes from those causing disease (see Table 2).

The first KCNQ potassium channel, KCNQ1 or KvLQT1, was isolated by positional cloning using families affected by the long QT syndrome [14]. This often fatal cardiac arrhythmia is characterised by a prolonged QT interval in the electrocardiogram. Other forms of this syndrome are caused by mutations in SCN5A (a cardiac Na^+-channel), HERG (another K^+-channel expressed in heart), or KCNE1 (a K^+-channel β subunit also called minK or IsK). Recently, the related protein KCNE2 (or MiRP1) was shown to associate with HERG channels. Mutations in its gene were identified in cardiac arrhythmia patients [18]. KCNE1, a small protein with a single transmembrane domain, associates with

Table 1

Ion channels mutated in human neuromuscular diseases.

Ion channel subunit	Gene	Disease	Refererence
Na+-channel α	SCN4A	Paramyotonia congenita	[58–60]
		Hyperkalemic periodic paralysis	
		Hypokalemic periodic paralysis	
	SCN5A	Long QT type 3 (cardiac arrhythmia)	[61,62]
Na+-channel β	SCN1B	Generalized epilepsy with febrile seizures type 1	[32]
K+-channel α	KCNA1	Episodic ataxia type 1	[23]
	KCNQ1	Long QT type 3 (cardiac arrhythmia)	[14]
		Jervell-Lange-Nielson (cardiac arrhythmia and deafness)	
	KCNQ2/3	Benign familial neonatal convulsions	[15–17]
	KCNQ4	Hereditary hearing loss (DFNA2)	[4••]
	HERG	Long QT type 2 (cardiac arrhythmia)	[63]
K+-channel β	KCNE1	Long QT type 5 (cardiac arrhythmia)	[64]
		Jervell-Lange-Nielson (cardiac arrhythmia and deafness)	
	KCNE2	Cardiac arrhythmia	[18]
Ca2+-channel α	CACNA1A	Episodic ataxia type 2	[34,39]
		Familial hemiplegic migraine	
		Spinocerebellar ataxia type 6	
	CACNA1S	Hypokalemic periodic paralysis	[46]
	CACNA1F	Congenital stationary night blindness type 2	[47,48]
	RYR1	Malignant hyperthermia	[65,66]
		Central core disease	
ClC Cl⁻ channels	CLCN1	Myotonia congenita (dominant and recessive)	[50]
Glycine receptor channel	GLRA1	Hyperekplexia	[51]
ACh receptor channel	CHRNA1	Congenital myasthenia	[67]
	CHRNA4	Autosomal dominant nocturnal frontal lobe epilepsy	[53]
Connexins	GJB1 (Cx32)	Charcot-Marie-Tooth disease	[68,69]
	GJB2 (Cx26)	Hereditary hearing loss (DFNA3 and DFNB1)	[70,71]
	GJB3 (Cx31)	Hereditary hearing loss (DFNA2)	[2]
	GJB6 (Cx30)	Hereditary hearing loss (DFNA3)	[7]

Although the table lists only ion channel defects associated with neuromuscular disorders, many more channelopathies are known, for example those associated with renal ion channels (Bartter's syndrome) and ion channels of secretory epithelia (cystic fibrosis). The table has a wider focus than has the main text – readers are referred to the listed references for further information.

KCNQ1 (which is the pore-forming subunit and has six transmembrane domains) to form the slowly activating K+-current I_{Ks} found in the myocard. Depending on the severity of the functional defect, mutations in these subunits lead to different diseases: dominant-negative mutations, which decrease the activity of the tetrameric channel down to approximately 10%, are sufficient to cause cardiac arrhythmias in heterozygous patients. A total loss of function, as is found in patients homozygous for recessive mutations, additionally results in congenital deafness. This is due to a defect in K+-secretion into the scala media of the inner ear. Thus, the repolarization of cardiac action potentials is more sensitive to a decrease in channel function than is the transepithelial transport in the cochlea [19].

KCNQ2 and KCNQ3 are neuron-specific isoforms that are co-expressed in broad regions of the nervous system. They assemble to form heteromeric channels that have properties of the M-type K+ current [8••,9•,20]. This highly regulated current is active near the threshold of action potential firing and is an important determinant of neuronal excitability.

Mutations in either subunit can cause benign familial neonatal convulsions (BFNC) [15–17], a transient, generalised epilepsy of infancy. In contrast to the KCNQ1 mutations that result in the long QT syndrome, a slight loss of KCNQ2/KCNQ3 function is sufficient to cause epilepsy [9•]. Expression studies in *Xenopus* oocytes indicate that the mutations that cause BFNC lead to a 20–30% loss of K+ channel function in patients [9•]. One may assume that a more severe loss of function could lead to more severe symptoms, possibly resulting in early lethality. Indeed, none of the KCNQ2 or KCNQ3 mutations identified so far exerts a dominant-negative effect on wild-type subunits.

Mutations in KCNQ4 cause a form of slowly progressive dominant deafness (DFNA2) [4••]. Functional analysis indicated a dominant-negative effect on co-expressed wild-type subunits [4••]. This is also the case for other KCNQ4 missense mutations (T Friedrich, T Jentsch, unpublished data) that were reported later [21]. However, one mutation found in DFNA2 [21] leads to an early frameshift, predicting a lack of a dominant-negative effect.

Table 2

Residual channel function in potassium channelopathies.

Gene	Site of expression	Mode of inheritance	Disease	Remaining function
KCNQ1	Heart, cochlea Other tissue	Dominant	LQT1 (Romano-Ward)	~10% Dominant negative on one allele
		Recessive	LQT1 (Jervell-Lange-Nielson)	None Loss of both alleles
KCNQ2	Brain	Dominant	BFNC	~75% (in the heteromer)
KCNQ3				Loss of function on one allele
KCNQ4	Cochlea, brain Other tissues	Dominant	DFNA2	10–15% Dominant negative on one allele or haploinsufficiency
KCNA1	Brain, PNS	Dominant	EA-1	10–15% Dominant negative on one allele or haploinsufficiency

The level to which K+-currents need to be decreased in order to cause disease differs broadly between the different K+-channel diseases. Since these potassium channels are tetramers, certain mutations can have strong dominant negative effects. For instance, the incorporation of a single mutated subunit into the tetrameric channel may totally abolish channel function. In this case, only channels consisting entirely of wild-type subunits will yield current. In heterozygous patients carrying such a dominant-negative mutation on one allele, the abundance of functional channels consisting entirely of wild-type subunits will be only 1/16 of normal. Such strong dominant negative mutations (which result, for example, from missense mutations in the pore) are found in the dominant long QT syndrome or in DFNA2. Interestingly, this level of channel activity is still sufficient for inner ear function; here, the current flowing through KCNQ1/KCNE1 has to be reduced further (by a loss of genes encoding the channels on both alleles) to cause deafness. By contrast, normal levels of KCNQ2/KCNQ3 heteromers need to be decreased only slightly to cause the neonatal epilepsy BFNC. It should be remembered, however, that all results reported in this table were obtained in heterologous expression systems and not in native tissue. PNS, peripheral nervous system.

Interestingly, the deafness in these patients does not seem to be much less severe. It is currently unclear how mutations in KCNQ4 cause deafness. It has been suggested that a partial loss of KCNQ4 function leads to the degeneration of sensory outer hair cells. This is the only cochlear cell type that expresses KCNQ4 [4••].

Mutations of the KCNA1 K+-channel
Among the numerous other K+-channels found in the CNS, KCNA1 (Kv1.1) is the only other one known to be mutated in human CNS disease. It is highly expressed in cerebellar basket cells and in myelinated peripheral nerves, and can form heteromers with Kv1.2 [22]. This delayed-rectifier channel contributes to the repolarization of action potentials. Mutations in *KCNA1* affect the motor system and cause a dominant form of episodic ataxia (EA-1) that is accompanied by myokymia (spontaneous discharges of peripheral motoneurons) [23]. In some families, epileptic seizures have also been observed [24]. These, however, did not affect all patients carrying the mutation, indicating a low penetrance. Seizures were also observed in a mouse model with a total knock-out of the *kcna1* gene [25].

Functional studies have revealed various effects of the KCNA1 mutations that have been identified in patients with EA-1. Some mutations cause a shift of the voltage dependence of activation to positive voltages, while others change the kinetics of gating and inactivation [26–28]. In the cases where this was addressed experimentally, similar but less pronounced changes in the biophysical properties were also found for wild-type/mutant heteromeric channels that would be formed in heterozygous patients with this dominant disease. This influence on the heteromer can be regarded as a dominant-negative effect of the mutated subunits. Similar studies were performed on Kv1.1/Kv1.2 heteromers carrying just one mutant Kv1.1 subunit [29•]. However, non-functional and non-interacting KCNA1 subunits may also be found in EA-1, suggesting that the loss of function associated with haploinsufficiency suffices to cause this syndrome [27].

Mutations of voltage-gated Na+-channels
Voltage-gated Na+-channels are required to generate the electrical excitation in neurons, heart and skeletal muscle fibres, which express tissue-specific isoforms. These channels are heteromers of a pore-forming α-subunit and a modulatory β1-subunit, with an additional β2-subunit in neuronal channels. Mutations in the α-subunit of the skeletal muscle isoform (SCN4A) were identified in paramyotonia congenita and hyperkalemic periodic paralyis, and mutations in the heart isoform (SCN5A) are seen in a form of the long QT syndrome (reviewed in [12]). These missense mutations lead to additional, 'late' Na+-currents by affecting the inactivation process, which in turn leads to a slight depolarization of the membrane resulting in hyperexcitability. Because a small fraction of non-inactivating mutant channels suffices to produce this effect, a dominant mode of inheritance is observed. One of the neuronal isoforms (scn8a) is mutated in the *med* and *jolting* mouse models [30,31].

Recently, a mutation in a neuronal β-subunit (SCN1B) was found in a large family with generalised epilepsy and febrile seizures (GEFS+) [32]. Functional analysis demonstrated a loss of function of this accessory subunit, resulting in a slower inactivation of the Na+-channel complex. In a manner somewhat similar to gain of function mutations in SCN4A and SCN5A, the resulting late Na+-currents may lead to a hyperexcitability of neurons, which, in turn, will result in seizures.

Mutations of voltage gated Ca²⁺-channels

Voltage-dependent Ca²⁺-channels are composed of a single pore-forming α-subunit (of which different isoforms exist) and several accessory subunits (for a review, see [33]). Three different human neurological diseases are attributable to mutations in the *CACNA1A* gene. (The reader should note that the Ca²⁺-channel α-subunits have recently been renamed. Thus, CACNL1A3 is now CACNA1S, and CACNL1A4 is CACNA1A.) This gene encodes one of several neuronal α-subunit isoforms. In addition to other regions of the brain, the gene is predominantly expressed in Purkinje and granule cells of the cerebellum.

Truncations, which presumably lead to a total loss of channel function, cause episodic ataxia type *2* (EA-2) [34,35]. Several missense mutations have been identified in patients with familial hemiplegic migraine (FHM), a particularly severe form of migraine [34]. Electrophysiological analysis of the mutant channels found in FHM in heterologous expression systems revealed that they still function as Ca²⁺-channels, but have altered properties [36,37]. However, the picture is complex, as both a gain of function (e.g. a shift of the voltage-dependence to less depolarised potentials) and a loss of function (e.g. lower single-channel conductance) was described. It is currently unclear how these different changes in channel function lead to migraine.

Interestingly, another *CACNA1A* mutation (G583A) has recently been identified in a family presenting with both migraine and ataxia [38]. One may speculate that a more severe loss of channel function than that causing FHM may explain the phenotype. Unfortunately, the biophysical effect of this mutation has not yet been studied.

The third human disease attributable to a *CACNA1A* mutation is spinocerebellar ataxia type 6 (SCA-6). This is caused by an expansion of CAG repeats within the open reading frame [39]. It is likely that SCA-6, like other triplet repeat diseases, is caused by a 'toxic' effect of the expanded polyglutamine stretch at the carboxy-terminus of the channel encoded by the CAG repeat. Indeed, it was shown recently that this expansion results in intracellular aggregation of the mutated protein both in cultured cells and in the cerebellum of patients [40•]. These aggregates eventually lead to apoptosis in cultured cells. This may account for the neurodegeneration seen *in vivo*.

A missense and a truncating mutation in the *cacna1a* gene lead to absence seizures and ataxia in *tottering* and *leaner*

mice, respectively [41]. Thus, the murine phenotype, which exhibits epileptic seizures even before the onset of ataxia, is not a mirror-image of the human diseases. Several other genetic forms of mouse epilepsy have been shown to be caused by mutations of the accessory subunits of Ca²⁺-channels [42]. The *lethargic* mouse, which also displays seizures and ataxia, has a mutation in the β4 subunit [43]. This disrupts its association with the α1-subunit, and may result in the formation of different α–β pairs by favouring the association of α1 with the β1, β2 and β3 subunits [44]. The absence epilepsy in *stargazer* mice is attributable to a mutation in the γ-subunit [45]. This subunit is not present in all Ca²⁺-channels *in vivo*, and its functional role is currently unclear [33].

To date, no human neurological disease is known to be caused by a mutation in an accessory subunit of a Ca²⁺-channel. However, there are other diseases that result from mutations in α-subunits. Mutations in *CACNA1S* cause hypokalemic periodic paralysis, a muscle disease [46]. Mutations in *CACNA1F*, which encodes the retina-specific α-subunit α1F, cause incomplete X-linked congenital stationary night blindness (CSNB2) [47,48]. This disorder apparently results from the complete loss of function of the L-type channel, which is involved in retinal neurotransmission.

Ion channels, neuronal excitability and epilepsy

Epilepsy is one of the most common neurological disorders and affects roughly 1% of the population. It is characterized by synchronised, pathological electrical activity of large groups of neurons: this electrical hyperactivity leads to epileptic seizures. In some forms of epilepsy, abnormal electrical brain activity can also be recorded in symptom-free intervals between seizures.

Epilepsy has a large genetic component, which has been estimated to be about 50%. Most forms of genetic epilepsy are probably polygenic. Progress in identifying the underlying genes has been limited to rare, monogenic forms of epilepsy, which are easier to investigate. Since the electrical hyperactivity that causes epilepsy is directly created by currents flowing through ion channels, the genes encoding such channels constitute promising candidate genes for the cause of epilepsy. Therefore, it is not unexpected that mutations in ion-channel genes underlie some forms of epilepsy in humans and mice. In fact, given the large number of ion channel genes, it is surprising that only four such genes have so far been implicated in human epilepsy .

Considering only loss-of-function mutations, K+-channel and Cl⁻-channel mutations are the best candidates for the cause of epilepsy, as these channels normally dampen the excitability of neurons. Thus, their elimination could lead to a cell-autonomous hyperexcitability. However, given the complex circuitry of the CNS, the loss of channels that directly excite (i.e. depolarise) neurons (such as Na+-channels, and glutamate- and acetylcholine-receptor channels) could also lead to a hyperexcitability of downstream neurons, if they are normally inhibited by the affected upstream neurons.

It is surprising that mutations in only a few types of K$^+$-channel subunit are known to cause human epilepsy. These are the subunits KCNQ2 and KCNQ3, which together form heteromeric channels [15–17]. Additionally, in some families KCNA1 mutations cause epilepsy as well as ataxia [24]. Results from investigations of properties such as the kinetics and the drug-sensitivity of currents through KCNQ2/KCNQ3 heteromer channels have, satisfyingly, corroborated the suggestion that these heteromers provide the molecular basis for M-type potassium currents [8$^{\bullet\bullet}$]. These currents have been studied for 20 years and are known to be important regulators of neuronal excitability. They are negatively regulated by several neurotransmitters: this regulation includes inhibition by muscarinic M1 receptors, a feature now known to be shared by KCNQ1 through KCNQ4 [49]. The M-current is slowly activated by depolarization, and the channels through which it flows are already open at the slightly depolarised voltages near the threshold for action potential generation. The regulation of M-currents by several neurotransmitters thereby allows for a sensitive control of repetitive action-potential firing. The high sensitivity of this control probably explains the fact that a small (20–30%) loss of KCNQ2/KCNQ3 current suffices to cause neonatal epilepsy [9$^{\bullet}$]. The observation that seizures normally disappear after several weeks points to the complex and developmentally regulated interplay between different ionic conductances and neuronal circuits, making it difficult to predict the effects of single ion-channel defects.

So far, no human epilepsy is known to be caused by Cl$^-$-channel mutations. In skeletal muscle, the ClC-1 Cl$^-$-channel plays a prominent role in dampening electrical excitation: mutations in its gene are known to cause myotonia [50]. In contrast, voltage-gated Cl$^-$-channels are probably less important in the brain. However, mutations in the glycine-receptor Cl$^-$-channel lead to startle disease (hyperekplexia) [51]. While disruption of some GABA$_A$ receptor Cl$^-$-channel subunits in mice causes severe epilepsy in addition to other symptoms [52], no GABA$_A$ receptor mutations have been identified in human epilepsy.

Mutations in the α$_4$-subunit of the nicotinic acetylcholine receptor cause another rare form of idiopathic epilepsy — autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) [53,54]. Only a few families with mutations in this receptor have been identified. These mutations do not lead to a total loss of function, but rather alter the properties of the channel in different ways [55–57]. It is currently unclear how, and in which neurons, the mutated receptors initiate epileptic activity. The same is true for GEFS$^+$. Here, however, the functional loss of a β-subunit of voltage-gated Na$^+$-channels suggests a cell-autonomous gain in excitability because the channels now inactivate at a slower rate [32].

Conclusions

The number of ion channels implicated in human disease has increased substantially over the past few years, and this trend

will surely continue. Although ion channel diseases often provide a direct pathophysiological explanation, the situation is not always that simple. For instance, none of the Ca^{2+}-channel genes involved in epilepsy in mice have been implicated in human epilepsy [42]. This highlights the complexity of the CNS and the difficulties in predicting effects of ion channel mutations in an extended neuronal network. Further, one should not forget that ion channels (and electrical activity in general) can have significant effects on brain development. Thus, it would be too simplistic to try to predict the effect of an ion channel mutation just by taking into account its role in the fully developed neuronal network (which is a daunting task in itself). In contrast to channelopathies of the skeletal muscle and the heart, it will take a long time to fully understand the cause and the effect of the CNS channelopathies.

Update

Escayg and colleagues [72$^{\bullet}$] have identified two different mutations in the voltage-sensor domains of a neuronal Na$^+$-channel α-subunit in patients with a rare form of generalized epilepsy. This type of epilepsy has previously been shown to be caused by mutations in the β-subunit of the very same Na$^+$-channel [32], and it was known that a second locus for this inherited disease existed on chromosome 2q23-24. This work now finally shows that alterations in either subunit of this channel may cause epilepsy.

Mutations in Ca^{2+}-channel accessory subunits have been known to cause neuronal disease phenotypes in mice, yet so far no pathogenic mutations had been found in humans. After screening 90 pedigrees with familial epilepsy or ataxia, the authors identified two different mutations, one truncation and one missense mutation in the β$_4$-subunit gene in three kindreds [73]. The effects of the mutated β subunit on Ca^{2+}-channel function remain elusive, but the mutations found are strong candidates for the cause of epilepsy and ataxia in the affected pedigrees.

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deafness which abolishes channel activity and exerts a strong dominant-negative effect on wild-type subunits. KCNQ4 can associate with KCNQ3 and has some properties of M-type currents. In the cochlea, it is expressed in sensory outer hair cells and may be required for K-efflux.

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