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Genetic neurological channelopathies

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SUMMARY

Ion channels are crucial for the normal function of excitable tissues such as neurons and skeletal muscle. Since the discovery that the paroxysmal muscle disorder periodic paralysis is caused by mutations in genes that encode voltage-gated ion channels, many genetic neurological channelopathies have been defined. These channelopathies include epilepsy syndromes that show a mendelian pattern of inheritance, certain forms of migraine and disorders of cerebellar function, as well as periodic paralysis. The clinical diversity of these disorders relates in part to the tissue-specific expression of the dysfunctional channel, but is probably influenced by other, as yet unidentified, genetic and non-genetic factors. The complementary disciplines of molecular genetics and cellular and *in vitro* electrophysiology have resulted in significant advances in understanding of the basic molecular pathophysiology of some of these disorders. The single-gene neurological channelopathies are generally regarded as a paradigm for understanding common human paroxysmal disorders, such as epilepsy and migraine. This article reviews the clinical and molecular features of some of the single-gene channelopathies that affect muscle and brain. The possible role of ion-channel functional and genetic variation in predisposing individuals to common forms of human epilepsy and migraine are also considered. The implications for accurate genetic diagnosis and therapeutic intervention are highlighted.

KEYWORDS ataxia, genetic channelopathy, migraine, myotonia, paralysis

REVIEW CRITERIA

Pubmed (using Entrez) and MEDLINE searches were undertaken to identify articles published up to October 2005. Searches used keywords including "genetic channelopathy", "neurological channelopathy" and "ion channel". Abstracts were reviewed and prioritized, full papers were reviewed and references obtained as appropriate. Reference to the author's own published genetic channelopathy research was also included where appropriate.

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INTRODUCTION

The normal function of neurological tissues, such as brain, peripheral nerves and skeletal muscle, relies on the complex interplay between key ion channels, which determine membrane excitability. Although originally considered to be incompatible with survival, it is becoming increasingly clear that genetically induced dysfunction of membrane-spanning ion channels is the underlying cause of many single-gene neurological diseases in humans. Collectively, these disorders are known as the genetic neurological channelopathies.[1]

Despite their clinical diversity, the genetic neurological channelopathies exhibit at least three common features. First, they are paroxysmal—that is, patients usually experience episodes of impaired neurological function separated by periods of normality. Some of the commonest neurological disorders seen in humans, including migraine and epilepsy, are paroxysmal, and much interest has focused on the possibility that a predisposition to these common disorders might be mediated by genetic variation in ion-channel genes.[1,2] Second, episodes are triggered by environmental factors, such as temperature and physical or emotional stress. Third, the genetic neurological channelopathies tend to share a common natural history: over time, the frequency of attacks usually declines, but the patients are often left with fixed tissue dysfunction and, therefore, some persisting neurological disability.

Although individually uncommon, the genetic neurological channelopathies are becoming widely recognized as a group, and cases will be encountered by clinicians in most branches of clinical neurology.[2] At least 40 separate single-gene neurological channelopathies have now been identified. The range of clinical presentations is somewhat bewildering, and in part reflects the tissue distribution of the mutated ion channel. Clinical phenotypes include muscle disorders such as periodic paralysis and myotonia (muscle stiffness), disorders of

peripheral nerve excitability such as neuromyotonia, and the aforementioned brain disorders migraine and epilepsy.[2] Detailed genetic and cellular electrophysiological studies *in vitro* and in mouse models have resulted in important advances in understanding of the fundamental pathophysiological basis of some of these single-gene disorders.

In this review, the clinical, genetic and electrophysiological features of selected single-gene neurological channelopathies are considered. The challenges of determining the possible roles of ion-channel genetic and functional variation in influencing susceptibility to common forms of migraine and epilepsy are also discussed.

VOLTAGE-GATED ION CHANNELS

Action-potential generation and synaptic transmission in the central and peripheral nervous systems depend on the coordinated activity of voltage-gated ion channels, and most of the genetic neurological channelopathies that have been defined at the molecular genetic level are caused by mutations in this type of channel.[1]

Given their vital function, it is not surprising that the structures of voltage-gated ion channels have been highly conserved through evolution (see Figure 1 for a schematic representation of a 'typical' voltage-gated ion channel). The neuronal voltage-gated sodium channel exhibits the typical features of such a channel. The α or pore-forming subunit of this channel is a large transmembrane protein of approximately 2,000 amino acid residues. In common with most voltage-gated ion channels, the sodium channel is composed of four homologous domains that contain well-characterized voltage-sensing and pore regions. Each domain comprises six membrane-spanning segments, each of which has an α-helical structure.[3] The four domains form a sodium-permeable pore within the membrane that is remarkably selective for the individual ion that it conducts. Depolarizing activation of the sodium channel results in passage of sodium into the muscle fiber or neuron, thereby forming the depolarizing upstroke of the action potential.

Each sodium channel α subunit associates with one or more different β subunits. This association with such auxiliary subunits has an important influence on the voltage dependence, kinetics and cell-surface expression of most voltage-gated ion channels. Although most

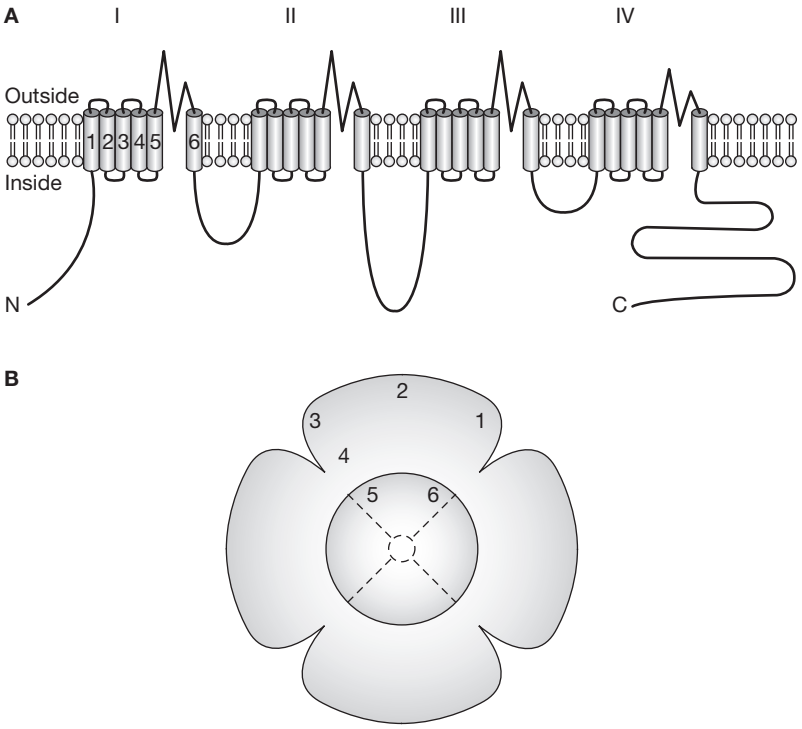

Figure 1 Schematic representation of a voltage-gated ion channel in a membrane. (**A**) There are four repeated domains (I–IV), each containing six membrane-spanning segments (1–6). The voltage sensor is segment 4. (**B**) Axial view illustrating the topology of the assembled channel. The interlinkers between segments 5 and 6 line the central pore of the channel (Figure 1B courtesy of Dr TD Graves).
C, carboxy terminus; N, amino terminus.

neurological channelopathies associated with dysfunction of voltage-gated ion channels are caused by mutations in the gene encoding the pore-forming subunit, mutations in auxiliary subunits have been shown to be responsible in some cases.[4–6]

THE GENETIC SKELETAL MUSCLE CHANNELOPATHIES

Periodic paralysis and inherited myotonia were the first human neurological disorders to be attributed to dysfunction of ion-channel genes.[4,7–10] These disorders are characterized by disturbances in the excitability of skeletal muscle-fiber membranes.

The periodic paralyses are autosomal dominant disorders in which patients experience focal or generalized episodes of muscle weakness of variable duration. These disorders have been characterized on the basis of a change in serum potassium levels during an attack. In

Table 1 Skeletal muscle channelopathies—a genetic classification.

Gene	Channel	Disease	Inheritance	Function
CACNA1S[a]	Calcium channel L-type calcium α subunit	HypoPP1	AD	Uncertain
		Malignant hyperthermia	AD	Uncertain
SCN4A[a]	Sodium channel Nav1.4 α subunit	HyperPP	AD	Gain
		PMC	AD	Gain
		PAM	AD	Gain
		HypoPP2	AD	Loss
KCNJ2[a]	Potassium channel Kir2.1	ATS	AD	Loss
KCNQ2	Potassium channel	Neuromyotonia and BNFC	AD	Loss
CLCN1[a]	Chloride channel CIC1	Myotonia congenita	AD/AR	Loss
		DM1[b]	AD	Loss
		DM2[b]	AD	Loss
RYR1	Ryanodine receptor	Malignant hyperthermia	AD	Gain
	Calcium-release channel	Central core disease	AD	Gain
			AR	Gain

[a]Indicates that DNA-based diagnosis is available in the UK. [b]Indicates that altered splicing of *CLCN1* has been shown in both forms of myotonic dystrophy as the basis of the myotonia.
AD, autosomal dominant; AR, autosomal recessive; ATS, Andersen–Tawil syndrome; BFNC, benign familial neonatal convulsions; DM1, Myotonic dystrophy type 1; DM2, Myotonic dystrophy type 2; HyperPP, hyperkalemic periodic paralysis; HypoPP1, hypokalemic periodic paralysis type 1; PAM, potassium aggravated myotonia; PMC, paramyotonia congenita.

hyperkalemic periodic paralysis (HyperPP), potassium triggers an attack that can be ameliorated by glucose ingestion. By contrast, patients with hypokalemic paralysis (HypoPP) will notice improvement with potassium ingestion, but worsening with glucose. This clinically useful potassium-based classification has now been supplemented by a genetic classification (Table 1).[1,2] Mutations in any of three skeletal muscle ion-channel genes—the voltage-gated sodium channel gene *SCN4A*, the voltage-dependent calcium channel gene *CACNA1S*, and the voltage-independent potassium channel gene *KCNJ2*—can be associated with human periodic paralysis.[7,8,11] In all forms of periodic paralysis, the muscle-fiber membrane becomes electrically inexcitable during an attack, but the mechanisms leading to this state vary, as described below.

Myotonia is a clinical disorder in which patients experience muscle stiffness because of a failure of normal electrical inactivation of activated muscle. Myotonia can result from mutations in either the *CLCN1* gene, which is located on chromosome 7q35 and encodes the muscle voltage-gated chloride channel (dominant or recessive myotonia congenita), or the voltage-gated sodium channel gene *SCN4A* (dominant paramyotonia congenita [PMC]).[12–14]

Periodic paralysis and myotonias caused by sodium channel dysfunction

HyperPP is characterized by attacks of weakness starting in the first decade of life. Precipitators include rest following exercise, cold, potassium ingestion and stress. Attacks, which usually last less than 2 hours, vary in severity from mild weakness to total paralysis. Typically, the attack frequency declines with age, but patients often develop a fixed myopathy of variable severity. Death from HyperPP is extremely rare in humans. Cardiac arrhythmias are also uncommon, except in one type of periodic paralysis known as Andersen–Tawil (or Anderson's) syndrome (ATS; see below).

HyperPP is caused by gain-of-function point mutations in the *SCN4A* gene, which encodes the α subunit of a muscle sodium channel. These mutations lead to defective fast inactivation of the channel.[8,15,16] HyperPP-causing point mutations are generally located in the inner regions of the transmembrane segments or in the intracellular interlinking loops. The resulting persisting inward sodium current impairs repolarization and increases membrane excitability.[17] Depending on the degree of membrane excitability, a patient might experience myotonia or paralysis. Some genotype/phenotype correlations can be made; for example, the most frequent point mutation, T704M, which causes

60% of cases of HyperPP, frequently causes permanent late-onset muscle weakness. Another frequent mutation, I1592M, is often associated with myotonia as well as paralysis.

Many attacks of HyperPP are brief and do not require treatment. If necessary, acute attacks can be terminated by ingestion of carbohydrate or inhaled salbutamol.[18] Preventative treatment with acetazolamide or a thiazide diuretic might be required.[19] Whether reducing attack frequency with such agents reduces the likelihood of the subsequent development of myopathy is not known.

PMC is a form of myotonia that appears during exercise and worsens with continued activity. Electromyography at rest frequently shows some myotonia. Low temperature often precipitates symptoms, and cooling produces repetitive spontaneous motor-unit discharges with a decrement in the muscle-action-potential amplitude. Like HyperPP, PMC is caused by mutations in *SCN4A*,[4] and it is a highly penetrant autosomal dominant trait. Mutations have been found throughout the gene, although exon 24 appears to be a hot spot.[20] The PMC-associated point mutations are gain-of-function, but the resulting impairment of fast inactivation is less marked than that associated with HyperPP. Mexiletine hydrochloride is an effective symptomatic treatment for PMC.[21] Milder forms of myotonia without cold sensitivity have also been described in association with different sodium channel point mutations, and are known collectively as the potassium-aggravated myotonias.[22]

In summary, sodium channel diseases are caused by slow and incomplete sodium channel inactivation, which results in altered membrane excitability. Mutations associated with severe sodium channel inactivation defects create a condition in which the membrane depolarizes to an inexcitable state typical of periodic paralysis. By contrast, milder defects in sodium channel inactivation create a state of repetitive firing, as in paramyotonia and pure myotonia. Elegant modeling studies by Cannon and colleagues have provided support for the relationship between the biophysical defect and the electroclinical phenotype.[23]

Hypokalemic periodic paralysis

HypoPP is an autosomal dominant condition, with *de novo* dominant mutations accounting for one-third of cases.[2] Attacks are precipitated by rest after a period of exercise or by carbohydrate loading. Attacks typically develop in the early hours of the morning, and can last for hours or even days. Serum potassium levels are typically low at the onset, but can normalize quickly. Attack frequency tends to decline with age, but a fixed myopathy sometimes develops. Myotonia does not occur in HypoPP.[2] As with HyperPP, very few humans die from this condition.

Point mutations in two separate muscle channel genes can cause HypoPP. Most cases harbor one of three point mutations in the L-type calcium channel gene *CACNA1S*; this form of the disease is known as HypoPP type 1.[7,10] Far less frequently, mutations have been described in the muscle sodium channel gene *SCN4A*; this form is known as HypoPP type 2.[24–27] All *CACNA1S* mutations lead to arginine substitutions in the voltage sensor (S4) of the channel protein. It remains unclear how defects in CACNA1S, which does not have a major role in determining muscle membrane excitability, result in attacks of paralysis. The normal channel has two roles: as a slow voltage-activated calcium channel, and in excitation–contraction coupling with the ryanodine receptor. Molecular expression studies of mutated channels have shown enhanced inactivation of the channel.[7] Loss of normal channel function would therefore seem responsible for the attacks of paralysis.

HypoPP associated with *CACNA1S* mutations exhibits almost complete penetrance in males, but only 50% penetrance in females.[28] About half of the women who have an R528H mutation, and one-third of those with an R1239H mutation, are asymptomatic. By contrast, more than 90% of males with a disease-causing mutation are symptomatic. Specific mutations appear to have discrete clinical features; for example, the common R528H mutation is associated with late disease onset and myalgias.

HypoPP can also be caused by missense mutations that affect the voltage sensor of domain 2 of SCN4A.[24–26] Expression studies indicate that the *SCN4A* mutations associated with HypoPP cause loss of function of the channel. There is some evidence that such HypoPP cases might experience worsening of attacks, with prominent myalgia, when exposed to acetazolamide. In this setting, an alternative carbonic anhydrase inhibitor dichlorphenamide seems to be effective;[26] however, the availability of this drug varies in different countries. My colleagues and

GLOSSARY

INWARDLY RECTIFYING POTASSIUM CHANNEL
These channels are called inward rectifiers because current flows through them more easily into than out of the cell

DOMINANT-NEGATIVE
Describes a mutant molecule that forms a complex with, and disrupts the function of, its wild-type counterpart

I found *SCN4A* mutations to be an uncommon cause of HypoPP in the UK.[25]

Normokalemic periodic paralysis

Patients with periodic paralysis in whom no change in serum potassium can be documented at the time of the attacks have been reported. My research group undertook DNA analysis on the first family that was reported to have normokalemic periodic paralysis. This analysis confirmed that the family harbored a sodium channel mutation, indicating that this is a sodium channel disease. Other workers have also identified sodium channel diseases in similar patients, and the term potassium-sensitive normokalemic periodic paralysis was introduced to describe this disorder.[29,30]

Periodic paralysis and cardiac arrhythmias

Most cases of periodic paralysis are not associated with cardiac arrhythmias, as the responsible gene (*CACNA1S* or *SCN4A*) is not expressed in cardiac muscle. ATS, however, is a form of dyskalemic periodic paralysis in which cardiac involvement is frequent. In addition to periodic paralysis, patients with this syndrome might have atrial arrhythmias, ventricular arrhythmias, or both, and might also have characteristic facial and skeletal features.[31,32] This disorder should be considered in any case of periodic paralysis with arrhythmia. It is not uncommon for the resting electrocardiogram to show bigeminy (double heartbeat) in this condition.

ATS is caused by mutations in the INWARDLY RECTIFYING POTASSIUM CHANNEL Kir2.1, which is encoded by the *KCNJ2* gene on chromosome 17q23.[11] The functional channel is a homotetramer that is important for cardiac and skeletal muscle membrane hyperpolarization, and also has a role in skeletal bone precursor cell migration and fusion during development. Functional expression studies have shown loss of function resulting from a DOMINANT-NEGATIVE effect on wild-type channel subunits, thereby producing a reduced inwardly rectifying potassium current.[32,33] My research group studied 12 families in the UK, and identified six new mutations. Expression studies confirmed a dominant-negative effect (Figure 2).[32]

The severity of ATS varies within families, and partial manifestation of the phenotype is common. Serum potassium levels are usually low during an attack, but have been observed to be normal or even high in some cases. In patients with hypokalemia, oral potassium supplements might improve the weakness. In some families, increasing the plasma potassium concentration with acetazolamide improves arrhythmias but exacerbates weakness.[34] Once the diagnosis is made, detailed cardiac assessment is needed. The optimum management to prevent malignant arrhythmias, however, has not yet been established.

Myotonia congenita

Dominant and recessive forms of myotonia congenita (Thomsen's disease and Becker's disease, respectively) are recognized. Patients experience differing degrees of myotonia and muscle hypertrophy. Myotonia arises because of impaired voluntary muscle relaxation. Typically, muscle stiffness is most marked at the onset of physical activity, but declines with repeated activity—the so-called 'warm-up phenomenon'. There is usually normal power at rest, although a minority of individuals have proximal weakness. Muscle hypertrophy and myalgia can occur in both forms, but are more prominent in the commoner recessive cases. Electrophysiologically, the myotonia (and paramyotonia) is characterized by uncontrolled repetitive action potentials at the sarcolemma that are initiated by a voluntary activation. This persistent involuntary activation prevents the patient from relaxing the muscle, resulting in muscle stiffness and limitation in free-flowing movements.[2]

Both forms of myotonia congenita are caused by mutations in the muscle voltage-gated chloride channel gene *CLCN1*.[12] The homodimeric CLCN1 protein has a different membrane topology to that described previously (Figure 1), and there is evidence that, unlike other voltage-gated ion channels, this channel has two separate ion pores through which chloride ion passage might occur.[35] The stability of the resting membrane potential in skeletal muscle depends largely on the chloride channel conductance. Mutations in channel subunits associated with the dominant disease interfere with dimer formation by exerting a dominant-negative effect on wild-type subunits.[35] During electrical activity in skeletal muscle, potassium accumulates in the T-tubules and causes an afterdepolarization following action potentials. This afterdepolarization is prevented in normal muscle by the high chloride conductance. In the absence of functional

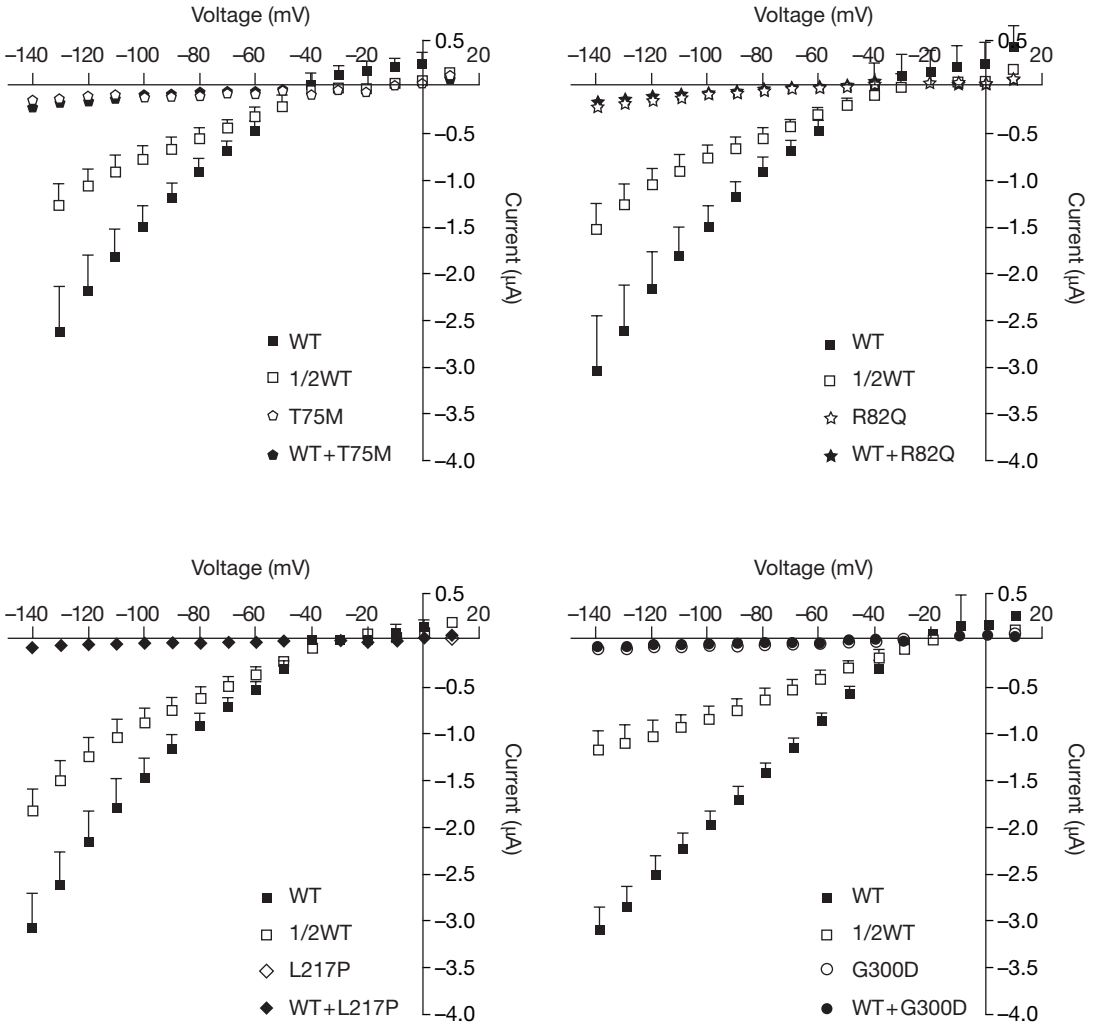

Figure 2 Functional effects of T75M, R82Q, L217P and G300D Kir2.1 mutations. Instantaneous current–voltage relationships for oocytes injected with wild-type, half wild-type, mutant, and coinjected wild-type and mutant complementary RNA (cRNA). Currents were elicited by step depolarizations from +10 to −140 mV, from a holding potential of −10 mV. Oocytes were injected with 9.2 ng total cRNA, with the exception of half wild-type, which was injected with 4.6 ng wild-type cRNA. Currents induced by injection of 4.6 ng wild-type were approximately half the magnitude of those induced by 9.2 ng wild-type Kir2.1. Data are means ± SEM; $n = 8$–10 oocytes for each group. These data confirm that the mutations identified exert a dominant-negative effect on wild-type channels. This action predicts impaired cardiac and skeletal muscle repolarization, which is seen in the patients. Figure reproduced with permission from reference 32 © (2005) AAN Enterprises, Inc.
1/2WT, half wild-type; WT, wild-type.

chloride channels, such afterdepolarizations cause repetitive action potentials and, therefore, clinical myotonia.[14]

By contrast, recessive mutations are simple loss-of-function, and the mutated subunits do not interact with wild-type monomers. Two mutant monomers are required to reduce chloride conductance sufficiently to induce myotonia.

Myotonic dystrophy types 1 and 2

Myotonic dystrophy (MD) is characterized by myopathy, myotonia, subcapsular cataracts, cardiac conduction defects and endocrinopathy. Two types of MD, dystrophia myotonica 1 (DM1) and dystrophia myotonica 2 (DM2; also known as proximal myotonic myopathy or PROMM), are clinically recognized. Both types exhibit the characteristic clinical features

Table 2 Neuronal channelopathies—a genetic classification.

Gene	Channel	Disease	Inheritance
GLRA1	Glycine receptor α_1 subunit	Hyperekplexia	AD/AR
CACNA1A	Calcium channel Cav2.1 α_1 subunit	EA2 FHM SCA6	AD AD AD
KCNA1	Potassium channel Kv1.1	EA1	AD
KCNQ2/KCNQ3	Potassium channel	BFNC	AD
CHRNA4/CHRNB2	Nicotinic acetylcholine receptor α4 and β2 subunits	ADNFLE	AD
SCN1A	Sodium channel Nav1.1	GEFS+ SMEI FHM	AD Sporadic AD
SCN2A	Sodium-channel Nav1.2	GEFS+	AD
SCN1B	Sodium channel β_1 subunit	GEFS+	AD
CLCN2	Chloride channel	IGE	AD

AD, autosomal dominant; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; AR, autosomal recessive; BFNC, benign familial neonatal convulsions; EA1, episodic ataxia type 1; EA2, episodic ataxia type 2; FHM, familial hemiplegic migraine; GEFS+, generalized epilepsy with febrile seizures plus; IGE, idiopathic generalized epilepsy; SCA6, spinocerebellar ataxia type 6; SMEI, severe myoclonic epilepsy of infancy.

of MD, and clinically useful distinguishing features of DM2 include proximal distribution of the muscle weakness and prominence of pain, also usually in a proximal distribution. DM1 is caused by an unstable CTG repeat in the 3' untranslated region of the MD protein kinase gene (*DMPK*) on chromosome 19, whereas DM2 is associated with a CCTG repeat in the zinc finger protein gene *ZNF9* on chromosome 3q. Recently, it has been demonstrated that reduced expression of the chloride channel CLC1 at the muscle-fiber membrane is the basis of the myotonia in both DM1 and DM2. The reduced expression seems to be at the level of processing of the primary RNA transcript of the chloride channel gene.[36]

Diagnosis of skeletal muscle channelopathies

Increasingly, diagnosis of the muscle channelopathies described above is accomplished by DNA testing. In view of the increasing number and the large size of the genes involved, however, appropriate gene selection is important. Recently, Fournier and colleagues have provided evidence that careful clinical electrophysiological evaluation can help significantly in gene selection.[37]

THE GENETIC NEURONAL CHANNELOPATHIES

Ion channels and neuronal function

Ion channels are as important for neuronal signaling as they are for muscle contraction. Although sodium channels have a very similar role in both cell types, the resting membrane potential of neurons is determined mainly by potassium channels. Membrane repolarization in neurons is mediated mainly by an outward potassium flux supported by voltage-sensitive potassium channels. There is additional complexity in the CNS with regard to the distribution of ion-channel expression, and also the variety of types of voltage-gated and ligand-gated ion channel. Distinct ion channels are expressed in different compartments of the neuron, which include the soma, the action-potential initiation segment of the axon, nodal and paranodal segments of myelinated axons, presynaptic varicosities and terminals, and synaptic and extrasynaptic areas of dendrites and dendritic spines. The genetic neuronal channelopathies are summarized in Table 2.

Epilepsy

The discovery that single-gene mendelian forms of epilepsy can be caused by mutations in various neuronal ion-channel genes has sparked significant interest in the role of ion channels

in common forms of human epilepsy.[38] Many studies have indicated a strong genetic contribution to the risk of developing common forms of epilepsy, such as idiopathic generalized epilepsy and febrile seizures.[38] To date, single-gene epilepsy disorders have been associated with mutations in several neuronal ion-channel genes, including the sodium channel α and β subunit genes *SCN1A*, *SCN2A* and *SCN1B*,[39–42] the voltage-gated potassium channel genes *KCNQ2* and *KCNQ3*,[43–45] the nicotinic acetylcholine receptor subunit genes *CHRNA4* and *CHRNB2*,[46,47] and the chloride channel gene *CLCN2*.[48] Furthermore, on the basis of animal models (but with little supporting evidence to date in humans), mutations in the voltage-dependent calcium channel gene *CACNA1A* and the voltage-gated ion potassium channel gene *KCNA1* seem to have a role in epilepsy.[49,50] The neuronal sodium channels that have been implicated in human epilepsy have been extensively studied, and will be considered in detail here.

Generalized epilepsy with febrile seizure plus (GEFS+) is a recently recognized autosomal dominant epilepsy syndrome. Affected individuals have febrile seizures in childhood, febrile seizures persisting beyond childhood (or both), or afebrile seizures.[48] Missense mutations in the neuronal sodium channel genes *SCN1A, SCN1B* and *SCN2A* have all been linked with this familial syndrome.[40–42] Interestingly, the same sodium channel mutation can produce a wide range of epilepsy severity in different individuals of the same family.

The first gene that was associated with GEFS+, *SCN1B*, encodes the β_1 sodium channel subunit,[42] an auxiliary subunit of the α pore-forming subunit encoded by *SCN1A*. Coexpression studies have shown that this subunit facilitates fast inactivation of sodium channels. There is evidence that this β_1 subunit might co-assemble with either of two α pore-forming subunits—Nav1.1 and Nav1.2—which are encoded by *SCN1A* and *SCN2A* respectively.

The GEFS+-associated mutation in *SCN1B* (C121W) is predicted to disrupt a cysteine bond in the β_1 subunit, and has been shown to impair fast inactivation of the sodium channel; that is, the mutation causes sodium channels to close more slowly than normal.[42] The net effect of this mechanism is a gain of function with a persistent inward neuronal sodium current, leading to neuronal hyperexcitability. This effect is very similar to the molecular pathophysiology observed in the muscle *SCN4A* mutations that cause HyperPP and PMC, as described earlier. GEFS+-associated missense mutations in *SCN1A* have also been shown to impair fast inactivation of sodium channels.[41,42,51,52]

Our understanding of the role of sodium channels in human epilepsy was further advanced by the discovery that certain mutations cause a severe epilepsy syndrome known as severe myoclonic epilepsy of infancy (SMEI).[40] SMEI is a pediatric epilepsy, in which patients experience episodes of status epilepticus, and develop cognitive and motor regression. Death in childhood from complications of this type of epilepsy is common. Most cases of SME1 arise *de novo* without a family history.

Several studies have shown that *de novo* heterozygous mutations of *SCN1A*—mainly premature stop codons and splice-site mutations predicted to give rise to nonfunctional proteins—occur in a large proportion of children with SMEI.[40,52] In fact, in excess of 150 mutations in *SCN1A* have now been identified in association with SMEI. From a genetic viewpoint, these observations indicate that SMEI is likely to be caused by a loss of sodium channel function. Haploinsufficiency of *SCN1A*—a quantitative reduction in gene expression to 50% of normal levels—is likely to underlie the SMEI phenotype. In support of this idea, heterozygous *SCNA1* knockout mice exhibit a severe epilepsy phenotype.[53] The mechanisms by which *SCNA1* haploinsufficiency causes epilepsy are currently unknown.

Taken together, the observations made to date in relation to sodium channels and epilepsy raise several intriguing questions. It seems that both gains and losses of sodium channel function can associate with an epilepsy phenotype, the phenotype associated with gain-of-function mutations (GEFS+) generally being milder than that associated with loss-of-function mutations. The observation that certain gain-of-function mutations might also exhibit loss-of-function properties, such as current reduction, adds to the complexity of understanding these disorders. How loss of sodium channel function should generate an epilepsy phenotype is not entirely clear—indeed, medications that are effective for treating many types of epilepsy operate by blocking sodium channel function. It seems clear that these common drugs should be avoided in patients with SMEI. It is also interesting to note the variability of disease expression, even within the same family. Other

GLOSSARY

LINKAGE DISEQUILIBRIUM
The tendency of specific combinations of alleles at linked loci to segregate together on the same chromosome more frequently than would be expected by chance

DYSARTHRIA
Slurred or impaired speech caused by damage to the brain regions involved in the control of muscles responsible for word formation

modifying genes, environmental factors or even accumulation of somatic mutations might be relevant, but further study of these possibilities is required. Another important question is whether genetic variation in the sodium channel genes might affect susceptibility to common epilepsy phenotypes. My group and others are studying this possibility using population genetic techniques, including LINKAGE DISEQUILIBRIUM mapping.

Episodic ataxias

The episodic ataxias are well-characterized neuronal channelopathies that principally affect cerebellar function. The study of these disorders has provided some important insights into their molecular pathophysiology. Two main forms of episodic ataxia are recognized, both of which are inherited in an autosomal dominant manner.

Patients with episodic ataxia type 1 (EA1) experience brief attacks (seconds to minutes) of incoordination, dominated by gait ataxia and often triggered by sudden movements and stress. Between attacks, patients often exhibit neuromyotonia or myokymia, manifesting as muscle stiffness, twitching, and small-amplitude involuntary movements of the fingers. In some patients, neuromyotonia is detectable only by electromyographic recordings.[2] Epilepsy is more common among patients with EA1 than in the general population.[49] The severities of the ataxia and neuromyotonia vary among kindreds, as does the response to treatment with carbamazepine or acetazolamide.

EA1 is associated with mutations in the voltage-gated ion potassium channel gene *KCNA1*.[54] This gene encodes the pore-forming subunit Kv1.1, which assembles in a tetramer to form a delayed rectifier-type channel. The channel opens relatively slowly following membrane depolarization, allowing potassium ions to flow out of the neuron and thereby contributing to repolarization. Kv1.1 co-assembles either as a homotetramer, or with other members of the Kv1 family to form heterotetramers, as well as with cytoplasmic auxiliary subunits. Kv1.1 is expressed widely in the CNS, as well as in motor axons, where it contributes to repolarization of the membrane after action potentials.

EA1-associated mutations are loss-of-function, and have variable deleterious effects on channel assembly, trafficking and kinetics.[55,56] This mechanism of action gives rise to the prediction that, following membrane depolarization, neurons that normally express Kv1.1 should show impaired repolarization. The spontaneous motor axon activity that is presumed to result from this impairment is analogous to the repetitive muscle-fiber action potentials in myotonia congenita. The phenotype of a mutant mouse expressing a human EA1-associated mutation provides support for the hypothesis that ataxia arises from excessive release of γ-aminobutyric acid (GABA) from inhibitory synaptic terminals in the cerebellum.[57] My colleagues and I observed that, when comparing different mutations, there is an approximate correlation between the severity of the clinical syndrome and the degree to which potassium channel function is reduced, with the most 'severe' mutations associated with a dominant-negative effect on coexpressed wild-type channel subunits.[58] As the channel dysfunction is persistently present, it is unclear why the ataxia in EA1 patients should be paroxysmal.

The other main category of episodic ataxia is episodic ataxia type 2 (EA2). EA2 is more common than EA1, and patients experience paroxysms of cerebellar dysfunction lasting hours to days. The attacks are dominated by DYSARTHRIA, diplopia (double vision) and gait ataxia. Patients often have nausea and dysphoric symptoms during attacks, which are sometimes diagnosed as basilar migraine. Over half of patients with EA2 experience severe migraine headache during an attack. Patients generally also have evidence of a mild but slowly progressive cerebellar disorder, with prominent interictal nystagmus. Attacks are frequently precipitated by stress, emotion or intercurrent illness, but not by sudden movement (in contrast to EA1). The attacks can usually be prevented, or at least ameliorated, by acetazolamide.[2]

EA2 is caused by mutations in the calcium channel gene *CACNA1A*, which encodes the α_1 pore-forming subunit of Cav2.1, also known as the P/Q-type channel.[59] This channel is highly expressed in cerebellar granule and Purkinje's cells. The channel is also present presynaptically at most synapses in the brain, and at the neuromuscular junction, where it mediates the calcium influx that is responsible for neurotransmitter release.

EA2 is associated with loss-of-function mutations in *CACNA1A*.[50,59,60] The degree

of reduction of calcium channel flux can be partial, as shown for some missense mutations, or complete, as expected to occur for premature stop codons or splice-site mutations. It has not been established why a loss of or a reduction in calcium channel function should cause a paroxysmal and progressive cerebellar disorder. There are some spontaneously generated inbred mouse strains that have mutations in *Cacna1a*, and these mice exhibit various combinations of cerebellar degeneration and movement disorders, and also epilepsy. Indeed, these mouse strains are generally regarded as good models of primary generalized epilepsy. The role of *CACNA1A* in human epilepsy is unclear, although my research group has identified families with *CACNA1A* mutations that segregate with an epilepsy phenotype.[50,60]

Familial hemiplegic migraine

Familial hemiplegic migraine (FHM) is an autosomal dominant severe form of migraine with aura, in which the aura can include reversible hemiparesis.[61] Mutations in three genes—*CACNA1A*, *ATP1A2* and, most recently, *SCN1A*—have been found to cause FHM.[59,62,63]

All of the FHM-associated mutations in *CACNA1A* are of the missense type.[59] There is some evidence for genotype–phenotype correlation; for example, the T666M mutation associates with progressive cerebellar ataxia in addition to FHM.[61] Other variable features include reversible coma and a myasthenic syndrome.[61]

The issue of whether FHM-associated *CACNA1A* mutations are loss-of-function or gain-of-function has been debated. Although most of the mutations reduce the maximal calcium current mediated by Cav2.1 relative to wild-type when expressed *in vitro*, some work has indicated that the current passing through a single channel might be increased.[64] Alterations of expression of other calcium channels might also occur, however, which might have different consequences in cerebellar neurons and transmitter-release sites.[65] A mutant mouse strain expressing a human FHM mutation shows a lowered threshold for cortical spreading depression,[66] which is presumed to be the substrate of the aura.

The second gene linked to FHM, *ATP1A2*, encodes a sodium–potassium pump[62] that has a crucial role in maintaining the transmembrane ion gradients that underlie resting potentials and action potentials. Therefore, although *ATP1A2* does not code for an ion channel, normal function of ion channels depends on the membrane gradients that this pump generates. The mutations of this gene are loss-of-function, so they can be expected to lead to depolarization and impaired ion homeostasis in the brain. These changes might explain the lowered threshold for cortical spreading depression and aura.[62]

The third gene that is associated with FHM is the neuronal sodium channel gene *SCN1A*.[63] Recently, a heterozygous missense mutation (Q1489K) in *SCN1A* was identified in four families with FHM. Expression studies using the homologous *SCN5A* sodium channel gene indicated that mutation in this gene induced a twofold to fourfold accelerated recovery from fast inactivation. These findings strengthen the proposed molecular relationship between migraine and epilepsy, but it remains to be seen how the observed biophysical defect relates to the clinical phenotype in these FHM patients.

CONCLUSIONS

Many single-gene mendelian disorders have been established as neurological channelopathies. Certain muscle channelopathies caused by dysfunction of muscle sodium and chloride channels have been characterized in detail, and the molecular pathophyisology elucidated. For other muscle disorders associated with dysfunction of the calcium channel, precise disease mechanisms have not been determined. For all muscle channelopathies, DNA-based diagnosis is becoming increasingly available and, in combination with specialized electrophysiology, facilitates rapid diagnosis, genetic counseling and treatment choice.

Study of the single-gene neuronal channelopathies has provided new information about disease mechanisms in epilepsy, cerebellar ataxia and migraine. DNA diagnosis is not yet readily available for this group, partly because of the large size of the genes and a lack of common mutations. Genetic susceptibility to common forms of migraine and epilepsy might be imparted by variation in the sequences of genes that encode ion channels, or pumps that control the ionic milieu. Large-scale population genetic studies are required to establish the role of such genes in these common paroxysmal neurological diseases. If the molecular mechanisms can be elucidated, there should be considerable potential for tailoring drug treatments to specific types of ion-channel dysfunction.

KEY POINTS

- The normal function of neurological tissues, such as brain, peripheral nerves and skeletal muscle, relies on the complex interplay between key ion channels, which determine membrane excitability
- It is becoming clear that genetically induced dysfunction of ion channels is the underlying cause of many single-gene neurological diseases, including forms of periodic paralysis, myotonia, episodic ataxias, migraine and epilepsy
- Mutations in the voltage-gated sodium and calcium channel genes *SCN4A* and *CACNA1S*, and the voltage-independent potassium channel gene *KCNJ2*, are associated with human periodic paralysis
- Myotonia can result from mutations in either the muscle voltage-gated chloride channel gene *CLCN1* or the voltage-gated sodium channel gene *SCN4A*
- Single-gene epilepsy disorders have been associated with mutations in genes that encode sodium channel subunits, potassium channels, nicotinic acetylcholine receptor subunits and chloride channels
- Episodic ataxia type 1 (EA1) is associated with mutations in the voltage-gated potassium channel gene *KCNA1*, whereas EA2 is caused by mutations in the calcium channel gene *CACNA1A*
- Mutations in three genes—*CACNA1A*, *ATP1A2* and *SCN1A*—have been found to cause familial hemiplegic migraine

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