Ion channels and neurological disease: DNA based diagnosis is now possible, and ion channels may be important in common paroxysmal disorders

Ion channels are large transmembrane proteins which are essential for the normal function of all eukaryotic cells. They are especially important in excitable cells because they determine the membrane potential, both at rest and during firing, and also play a critical part in neurotransmitter release. Ion channels may be broadly classified into voltage gated and ligand gated, although many voltage gated channels are also affected by intracellular messengers. It is well recognised that autoimmune attack of the nicotinic acetylcholine receptor underlies acquired myasthenia gravis. Research over the past few years has, however, established that genetic defects in both ligand and voltage gated ion channels also cause some inherited neurological disorders. Collectively, these immunological and genetic conditions have become known as the neurological channelopathies. The genetic channelopathies are listed in tables 1 and 2.

The genetic advances have increased our understanding of the molecular pathogenesis of several relatively rare muscle and CNS diseases. The immediate benefits of this are an improved classification of these disorders and the availability of DNA based diagnosis. However, an important principle has also emerged: permanent ion channel dysfunction can cause a paroxysmal neurological disturbance. By extrapolation, ion channel defects are strong candidates for other paroxysmal disorders. Some of the recently established genetic channelopathies represent rare forms of more common disorders such as migraine and epilepsy. This has led to the suggestion that the genetic susceptibility known to exist in these common disorders

### Table 1 Genetic voltage gated channelopathies

<table>
<thead>
<tr>
<th>Ion channel</th>
<th>Disorder</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle disorders:</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle sodium channel</td>
<td>HyperPP</td>
<td>Dominant</td>
<td>SCN4A</td>
<td>17q23-25</td>
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<tr>
<td></td>
<td>PMC</td>
<td>Dominant</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>PAM</td>
<td>Dominant</td>
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</tr>
<tr>
<td></td>
<td>NormoPP</td>
<td>Dominant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle DHP sensitive calcium channel</td>
<td>HypoPP</td>
<td>Dominant</td>
<td>CACLN1A3</td>
<td>1q31-32</td>
</tr>
<tr>
<td></td>
<td>MH</td>
<td>Dominant</td>
<td></td>
<td></td>
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<tr>
<td>Skeletal muscle ryanodine calcium channel</td>
<td>MH</td>
<td>Dominant</td>
<td>RYR1</td>
<td>19q13.1</td>
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<td></td>
<td>Central core disease</td>
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<td>Myotonia congenita</td>
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<td>Thomsen's disease</td>
<td>Dominant</td>
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<tr>
<td></td>
<td>Becker's myotonia</td>
<td>Dominant</td>
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<tr>
<td>Skeletal muscle chloride channel</td>
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<tr>
<td>CNS disorders:</td>
<td></td>
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<tr>
<td>Brain and nerve potassium channel</td>
<td>Episodic ataxia type I with myokymia</td>
<td>Dominant</td>
<td>KCNA1</td>
<td>12p</td>
</tr>
<tr>
<td>Brain (P/Q-type) calcium channel</td>
<td>Episodic ataxia type II</td>
<td>Dominant</td>
<td>CACNL1A4</td>
<td>19p13</td>
</tr>
</tbody>
</table>

Standard gene nomenclature is used for abbreviations. HyperPP=Hyperkalaemic periodic paralysis; HypoPP=hypokalaemic periodic paralysis; NormoPP=normokalaemic periodic paralysis; PMC=paramyotonia congenita; PAM=potassium aggravated myotonia; MH=malignant hyperthermia; FHM=familial hemiplegic migraine; SCA6=spinocerebellar ataxia type 6.

### Table 2 Genetic ligand gated channelopathies

<table>
<thead>
<tr>
<th>Ion channel</th>
<th>Disorder</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuromuscular disorder:</td>
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<td>Nicotinic acetylcholine receptor</td>
<td>Congenital myasthenia</td>
<td>Dominant and recessive</td>
<td>CHRNA</td>
<td>2q</td>
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<td>CNS disorder:</td>
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<td></td>
<td></td>
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<tr>
<td>Glycine receptor</td>
<td>Hyperekplexia</td>
<td>Dominant</td>
<td>GLRA-1</td>
<td>5q</td>
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<td>Neuronal nicotinic receptor</td>
<td>ADNFLE</td>
<td>Dominant</td>
<td>CHRNA4</td>
<td>20q13.2-13.3</td>
</tr>
</tbody>
</table>

Standard gene nomenclature is used for abbreviations. ADNFLE=Autosomal dominant nocturnal frontal lobe epilepsy.
Skeletal muscle channelopathies

Conventionally, the periodic paralyses would be classified separately from the myotonias. An important result of the genetic progress in this field has been to show that different mutations of the same channel can cause diseases that blur this distinction.

THE PERIODIC PARALYSES AND PARAMYOTONIA CONGENTITA

The periodic paralyses are disorders of muscle fibre membrane excitability, which are classified on the basis of the serum potassium at or close to the onset of the attack into hyperkalaemic, normokalaemic, or hypokalaemic. During an attack, often precipitated by exercise followed by rest, the muscle fibre membrane enters a partially depolarised inexcitable state. 6–10 Before molecular genetic advances, electrophysiological work had demonstrated that muscle fibres from patients with hyperkalaemic periodic paralysis (HyperPP) show defective inactivation of sodium channels. 10 When the subunit of the voltage gated sodium channel gene in skeletal muscle was cloned, it was the potassium aggravated myotonias. 9 (Patients with paramyotonia congenita alter the rate and voltage dependence of the associated hyperkalaemia. Mutations seen in paramyotonia congenita, and which includes acetazolamide responsive myotonia, are also due to mutations in this gene (SCN4A) and are the cause of most cases of HyperPP. 11–13 Different mutations in the same channel are also associated with paramyotonia congenita (either alone or in combination with HyperPP), and with a group of disorders which have become known as the potassium aggravated myotonias. 14 (Patients with paramyotonia, as opposed to myotonia, experience a worsening of stiffness with exercise.) Expression studies have confirmed that all sodium channel mutations investigated result in impaired fast inactivation of the channel. This is an abnormal gain of function, which is consistent with the dominant mode of inheritance seen in these disorders. HyperPP mutations give rise to a small residual current that fails to inactivate at positive membrane potentials. 8 Impaired membrane repolarisation causes extracellular potassium accumulation, especially in the T tubules, which further depolarises the muscle fibre membrane. This instability may explain both the episodic nature of HyperPP and the associated hyperkalaemia. Mutations seen in paramyotonia congenita alter the rate and voltage dependence of inactivation, resulting in the characteristic repetitive discharges. Available evidence suggests that normokalaemic periodic paralysis is also a sodium channel disorder. 10

Although muscle fibre membrane inexcitability also characterises attacks of hypokalaemic periodic paralysis (HypoPP), there was no consensus on a candidate channel before genetic studies. 14 A genomewide linkage search led to the discovery that three mutations in a muscle voltage gated calcium channel (CACNL1A3) are responsible for most cases of HypoPP. 14 15 This is perhaps surprising, as this calcium channel is not known to have a role in determining muscle fibre membrane excitability. Expression studies have shown that the three known CACNL1A3 mutations alter channel function, but the pathogenesis of the attacks remains unclear. 16 Both of the common forms of periodic paralysis may be associated with a disabling vacuolar myopathy which seems to be mutation specific. 10 16

DNA based diagnosis of periodic paralyses is now obviating the need for time consuming and potentially hazardous provocative testing. 16 In addition to avoiding precipitating factors, drugs which can be effective in both types of periodic paralysis include acetazolamide and thiazide diuretics. 17 A recent placebo controlled trial suggests that dichlophenamide (a more potent carbonic anhydrase inhibitor than acetazolamide) is specifically effective in HypoPP. 17 Salbutamol can be useful in HyperPP. 15 Some patients are, however, resistant to all treatments.

MYOTONIA CONGENTITA AND THE POTASSIUM AGGRAVATED MYOTONIAS

Myotonia congenita is characterised by generalised symptomatic myotonia from a young age. This is generally milder in the rare dominant form (Thomsen’s disease) than in the much more common recessive form (Becker’s generalised myotonia). 18 Electrophysiological experiments on skeletal muscle preparations from patients (and on the myotonic goat model of the disease) established that the resting chloride conductance of the muscle fibre membranes is reduced. 10 11 As the chloride conductance plays a major part in determining the resting membrane potential, a decrease in this conductance lowers the threshold for depolarisation and therefore predisposes to myotonia. When the human skeletal muscle chloride channel CLCN1 was cloned, both forms of myotonia congenita were found to be due to different mutations in this gene. 20 Expression studies have shown that both dominant and recessive mutations result in decreased chloride currents. By contrast with sodium and calcium channels, the functional chloride channel is a homodimer, possibly explaining why the disease can be either dominant or recessive. The recessive mutations result in loss of function. The dominant mutations, on the other hand, may result in haploid insufficiency (50% of the normal gene product is not enough for normal chloride conductance), or have a dominant negative effect, in which mutant and wild-type subunits dimerise to result in abnormal or non-functional channels. 10 The diagnosis is usually straightforward on clinical grounds. Although DNA based diagnosis is available, it is very time consuming as over 30 point mutations have been described. 15 Treatment with antimuscarinic drugs is often needed, of which mexilitene is the drug of choice.

Cloning of the muscle sodium channel has also led to a better understanding of another previously poorly defined group of myotonic disorders. It is now clear that diseases in this group, which has been named “atypical” myotonia congenita, and which includes acetazolamide responsive myotonia, are also due to mutations in the sodium channel SCN4A. A prominent feature of the myotonia in this group is its marked exacerbation after potassium ingestion, which is not a feature of the chloride channel disorders. They are now known as the “potassium aggravated myotonias.”

MALENGH HYPERTHERMIA AND CENTRAL CORE DISEASE

Malignant hyperthermia is a rare but serious disorder, generally coming to light when otherwise healthy people undergo general anaesthesia. 22 Exposure to triggering agents, including halogenated volatile anaesthetics and depolarising muscle relaxants, results in hyperthermia, muscle rigidity, and rhabdomyolysis. Without rapid intervention with supportive measures and dantrolene this is often fatal. 23 Malignant hyperthermia is now known to be a disorder of regulation of skeletal muscle calcium. The triggering substances lead to an increased myoplasmic calcium concentration because of excessive release from the sarcoplasmic reticulum. Extrapolation from genetic studies on the porcine model of malignant hyperthermia
(porcine stress syndrome) led to the discovery that mutations in the human ryanodine receptor gene cause malignant hyperthermia in some families. The ryanodine receptor is a voltage-gated ion channel located in the membrane of the sarcoplasmic reticulum, and which releases calcium ions, thereby contributing to excitation-contraction coupling. Depolarisation normally leads to brief activation of the ryanodine receptor via an interaction with the skeletal muscle calcium channel (CACNL1A3). Ryanodine receptor gene mutation analysis can now be used to identify those at risk of malignant hyperthermia in families with a known mutation in this gene. This should reduce the need for the invasive in vitro contracture test.

In some patients susceptible to malignant hyperthermia central cores are seen in muscle biopsies, and conversely, patients with central core disease, a congenital myopathy, are at risk for developing malignant hyperthermia. These findings have recently been explained by the discovery that central core disease and malignant hyperthermia may both be caused by mutations in the ryanodine receptor gene. Malignant hyperthermia is, however, genetically heterogeneous. Interestingly, a family has been identified, in which a point mutation in the calcium channel CACNL1A3 segregates with the disease. This is the channel that triggers the ryanodine receptor to open, implying that defective interactions between the two proteins can cause the malignant hyperthermia phenotype. HypoPP and some cases of malignant hyperthermia may thus be regarded as allelic disorders.

CNS channelopathies

CALSUM CHANNELOPATHIES: EPISODIC ATAXIA TYPE 2,
FAMILIAL HEMIPLEGIC MIGRAINE, AND SPINOCEREBELLAR
ATAXIA TYPE 6

Perhaps the most remarkable recent discovery in the field of channelopathies is that three different autosomal dominant disorders are associated with mutations in the same gene, CACNL1A4, coding for the α1A subunit of the brain P/Q-type voltage gated calcium channel.

Episodic ataxia type 2 (EA2), familial hemiplegic migraine in some families, and SCA6 are allelic disorders caused by different mutations in this gene. The channel is widely expressed in the brain, with particularly high levels in cerebellar Purkinje cells.

Spinocerebellar ataxia type 6 is the commonest genetic adult onset pure dominant cerebellar ataxia. Onset is usually in the fifth decade and the syndrome progresses slowly, with some patients becoming wheelchair bound after 20 years. The pathological features include marked cerebellar atrophy with loss of Purkinje and granule cells as well as cells in the dentate nucleus and inferior olive. It is another example of a genetic disease in which the mutation is an abnormally expanded trinucleotide repeat sequence (CAG).

The CAG repeat is translated into a polyglutamine tract located in the C terminal region of the calcium channel α1A subunit. This repeat is much smaller and seems to be more stable than the other disease causing CAG repeats described to date. It is not known if the disease mechanism relates to altered calcium channel function, or involves abnormal protein deposition as has been suggested in other CAG repeat diseases.

Episodic ataxia type 2 is characterised by episodes of midline cerebellar disturbance with nystagmus, vertigo, ataxia, and dysarthria. Headache with nausea, leading to a diagnosis of basilar migraine, occurs in 50% of families. Attacks may last for hours to days and are typically precipitated by stress, exercise, and fatigue. Between attacks there may be progressive cerebellar dysfunction with nystagmus, and imaging often shows cerebellar atrophy. This disorder is usually very responsive to acetazolamide, although the precise therapeutic mechanism remains uncertain. An MRS study has shown a disturbance of cerebellar pH in patients with EA2 off treatment. Initially, splice site and frame shift mutations were described in the CACNL1A4 gene in patients with EA2, implying that truncation of the protein may be important in pathogenesis. However, recently the SCA6 CAG repeat has also been described in patients with EA2. One family has, moreover, been reported in which a missense mutation in CACNL1A4 is associated with a severe progressive cerebellar ataxia in one member, whereas other members had episodic ataxia and vertigo, more suggestive of EA2. The genetic distinction between SCA6 and EA2 is thus far from clearcut.

In families with familial hemiplegic migraine patients experience headache preceded by an aura, which includes a hemiparesis lasting hours to days. The onset is usually in childhood, and interictal nystagmus, ataxia, and dysarthria may occur. Four missense mutations in CACNL1A4 have been identified. Up to 50% of families with familial hemiplegic migraine linked to CACNL1A4 exhibit mild cerebellar ataxia and have cerebellar atrophy on imaging. A recent genotype-phenotype correlation study indicates that the presence of cerebellar atrophy may be mutation specific. The same study also identified a CACNL1A4 mutation in a case of "non-hemiplegic" migraine indicating that such mutations may associate with commoner forms of migraine.

Interestingly, a missense mutation in the orthologous mouse gene produces the tottering (tg) phenotype which exhibits absence seizures, motor seizures and ataxia. The frame shift and splice site mutations in EA2 are similar to the gene defects in another mouse model, the leaner mouse (tg) which is allelic to the C57BL mouse but more severely affected, exhibiting absence seizures, severe ataxia and premature death. Abnormalities on EEG are reported in patients with familial hemiplegic migraine and EA2 which, in the light of the findings in the mouse models, raises the question whether CACNL1A4 is relevant to epilepsy in humans.

Recently families with familial hemiplegic migraine not linked to CACNL1A4 have been shown to be linked to 1q31 at or near a different (R type) voltage gated channel α1R subunit gene. The molecular pathogenesis of disease associated with frame shift and splice site mutations of CACNL1A4, and the CAG repeat, has yet to be studied. The four known missense mutations associated with familial hemiplegic migraine have, however, been expressed. Three of the mutations were associated with altered inactivation gating, suggesting that neuronal instability plays a part in migraine attacks.

EPISODIC ATAXIA TYPE 1

The most diverse group of human voltage and ligand gated ion channels are the potassium channels, reflecting their early evolutionary origin. Point mutations in the human potassium channel gene KCNA1 cause the dominant disorder episodic ataxia type I (EA1). This channel is expressed widely, but especially in the cerebellum and in peripheral nerve. Patients experience brief attacks of cerebellar ataxia lasting up to minutes, which are not usually accompanied by abnormal eye signs as in EA2. There is no interictal cerebellar dysfunction, and brain imaging is normal. Stress, emotion, or sudden movement may precipitate attacks. Interictally there are continuous spontaneous repetitive discharges on EMG sometimes termed neuro-myotonia, although this is not always clinically obvious. The therapeutic response in EA1 is less consistent than in EA2. Many families respond to carbamazepine, and others have a favourable response to acetazolamide, although...
some cases are resistant to both treatments. Several point mutations are now described, and expression studies indicate that they impair channel function both by reducing the amplitude of the potassium current and by altering its voltage dependent kinetics. These changes would be predicted to increase neuronal excitability, which is the basis of the neuromyotonia and presumably the attacks of ataxia. Homozygous deletion of the orthologous gene in the mouse has recently been described to cause severe epilepsy, raising the question whether KCNA1 mutations are involved in human epilepsy. In support of this, there is evidence for an overrepresentation of epilepsy in cases of EA1. Recently, mutations in two novel potassium channel genes have been shown to associate with benign familial neonatal convulsions, providing more direct evidence that potassium channel dysfunction may cause epilepsy in humans.

Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFLE)

This dominant disorder is characterised by clusters of nocturnal frontal lobe seizures, each typically lasting less than 1 minute. They usually occur while falling asleep or just before waking. Most patients respond well to carbamazepine. Linkage to chromosome 20q13.2 was established in 1995, followed soon after by the discovery of a point mutation in the α4 subunit of the neuronal nicotinic acetylcholine receptor. A second mutation has recently been described. The neuronal acetylcholine receptor is a pentameric protein composed of varying combinations of subunits. It is mainly located presynaptically, and influences the release of other neurotransmitters. Expression studies of the two known mutations show a loss of channel function, which is thought to be the basis of the epilepsy in these cases. It remains to be seen whether other neuronal nicotinic receptor subunits are important in commoner forms of epilepsy.

Conclusions

It is now established that genetic defects of both ligand and voltage-gated ion channels can cause diverse neurological disease. To date, these are all relatively rare disorders. The detailed clinical, genetic, and biophysical analysis of neurological channelopathies over the past few years allows common themes to be identified. From a genetic viewpoint it is clear that both dominant and recessive modes of inheritance occur, and in some instances different mutations in the same ion channel gene may exhibit different inheritance. The mutations in CACLN1A4 indicate that different mutations in the same ion channel gene may result in quite different phenotypes, and also suggest that the relation between genotype and phenotype is not always simple. Another interesting finding is that either genetic or immunological insults to the same or closely related ion channels may produce neurological disease. For example, potassium channel antibodies may produce neuromyotonia, which also occurs in association with mutations in the KCN1A gene. Some congenital syndromes that mimic acquired myasthenia gravis are also caused by mutations of the peripheral nicotinic receptor. This raises the possibility that immunological counterparts may be found for other genetic channelopathies.

Ion channel dysfunction is often susceptible to external factors such as stress and changes in pH, ion concentration, and temperature, and many respond to acetazolamide. The natural history of these disorders is variable. Mutations may produce paroxysmal disorders with normal interictal tissue function (for example, EA1), paroxysmal disorders with progressive tissue dysfunction (for example, EA2), or progressive tissue dysfunction alone, without clear episodic symptoms (for example, SCA6).

It is likely that other neurological channelopathies will be identified. Strong CNS candidate diseases include paroxysmal movement disorders such as paroxysmal dystonic choreoathetosis and familial generalisepasm. Of the skeletal muscle diseases, the myotonic Schwartz-Jampel syndrome is another candidate. It is perhaps surprising that genetic channelopathies affecting peripheral nerve have not been described (other than EA1, which includes neuromyotonia in the phenotype).

Most intriguing is the possibility that ion channels may be important in common neurological diseases such as migraine and epilepsy. The clinical overlap between some of the calcium channel phenotypes and migraine is striking. One study suggests the possibility that CACLN1A4 may be important in commoner forms of migraine, although this requires confirmation. The mouse models mentioned above also make CACLN1A4 a good candidate for the genetic susceptibility known to exist in absence epilepsy. Ligand gated channels are also strong candidates for commoner forms of primary epilepsy. These include postsynaptic receptors mediating fast excitatory or inhibitory transmission, as well as presynaptic receptors such as nicotinic and kainate receptors, which modulate the release of GABA. An allelic association has indeed been reported between a kainate receptor subunit and juvenile absence epilepsy.

It remains to be seen if ion channel dysfunction is important in these common disorders. If it is, a new chapter in pharmacogenetic research with the aim of tailoring therapies to specific genotypes and modes of ion channel dysfunction is likely to follow.

Further information about neurological channelopathy DNA based diagnosis is available from the DNA-laboratory, National Hospital, Queen Square, London, UK.

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