Review

Ion channels and epilepsy

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Introduction

In order for cells to retain their integrity to water and yet permeate charged ions, the phospholipid cell membrane contains transmembrane proteins that allow the passage of specific ions from the interior of the cell to outside, and vice versa. There is a huge diversity of these ion channels. Some are tissue-specific; others are widely distributed throughout the body. They contribute to the maintenance of the negative resting membrane potential inside cells. Unsurprisingly, these membrane channels are integral to the processes of electrical signalling and excitation that are central to the functioning of the nervous system. Figure 1a shows a generic ion channel.

The past 15 years have seen rapid expansion in the discovery of disease-causing mutations in genes encoding ion channel proteins. These manifest as neurological, cardiac, renal and respiratory disorders, the most common being cystic fibrosis. This disparate collection of syndromes is now referred to as the channelopathies, a descriptive term referring to the common underlying pathophysiology of ion channel dysfunction. Ion channels themselves are divided into two broad categories, depending on their mode of activation. Voltage-gated channels are controlled by changes in membrane potential, ligand-gated channels by ligand binding (Table 1).

Epilepsy affects up to 1% of the population and causes significant morbidity. Most cases are idiopathic, with suspected polygenic inheritance of susceptibility loci that may be influenced by environmental and developmental factors. So far, investigation of complex risk factors has provided little to illuminate disease pathogenicity. The study of the rarer monogenic epilepsy syndromes is technically easier, and may help to identify susceptibility loci for the more common polygenic forms of disease. Over the past decade, the molecular defect has been elucidated in several monogenic epilepsy syndromes, the vast majority being mutations in cellular membrane ion channel genes. Table 1 summarizes these findings.

Functional assays

The effect of mutated ion channel subunits on channel function can be studied in vitro in Xenopus laevis oocytes (frog eggs). These cells are large, allowing the physiological characterization of mutant channels. Mutant transcripts are injected directly into the cell, which translates them into a mutant protein that is packaged into the cell membrane. Voltage-clamping techniques can then be used to assess the current amplitude, activation and inactivation kinetics (methods reviewed in reference 1). As these are autosomal dominant diseases, co-injection of mutant and wild-type subunits can delineate the effect, if any, of mutant on normal subunits, mimicking the situation in patients. These studies can also be performed in mammalian cell lines.
Idiopathic generalized epilepsy (IGE) is characterized by recurrent generalized seizures in the absence of detectable brain lesions or metabolic abnormalities. There are electroencephalogram (EEG) changes of generalized, symmetrical, bilateral synchronous discharges. IGE comprises various syndromes that differ in age of onset, including childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME) and epilepsy with grand mal seizures on awakening (EGMA).² Benign neonatal familial convulsions (BNFC) was also included in this classification, but is now recognized as a partial epilepsy syndrome. Generalized epilepsy with febrile seizures plus syndrome (GEFS+) is also difficult to classify, as patients may experience partial seizures in addition to generalized attacks. All known Mendelian forms

**Mendelian epilepsy syndromes**

Idiopathic generalized epilepsy (IGE) is characterized by recurrent generalized seizures in the absence of detectable brain lesions or metabolic abnormalities. There are electroencephalogram (EEG) changes of generalized, symmetrical, bilateral synchronous discharges. IGE comprises various syndromes that differ in age of onset, including childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME) and epilepsy with grand mal seizures on awakening (EGMA).² Benign neonatal familial convulsions (BNFC) was also included in this classification, but is now recognized as a partial epilepsy syndrome. Generalized epilepsy with febrile seizures plus syndrome (GEFS+) is also difficult to classify, as patients may experience partial seizures in addition to generalized attacks. All known Mendelian forms
Focal epilepsy

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)

Features of this unique syndrome include focal-onset frontal-lobe seizures, almost exclusively occurring during drowsiness or sleep, and variable severity of symptoms in family members. Milder cases are often undiagnosed, or misdiagnosed as nightmares, sleep or functional disorders. Neuroimaging is normal, and treatment with carbamazepine is dramatically effective. Although recognition of this syndrome is important for appropriate therapy and genetic counselling, underestimation of cases is likely. The clinical delineation of this as a separate epilepsy syndrome has allowed genetic analysis. Neuronal nicotinic acetylcholine receptors (nAChR) are heteropentamers consisting of varying combinations of subunits (\( \alpha_2-8, \beta_2-4 \)) arranged in a ring around a central pore. These are differentially expressed throughout the brain, forming receptors with distinct physiological properties. The most common human adult brain receptor comprises two \( \alpha_4 \) and three \( \beta_2 \) subunits. A large Australian kindred showed linkage to a region of chromosome 20, where the nAChR \( \alpha_4 \) subunit is located (CHRNA4). Mutation analysis revealed the substitution of a serine residue, which is highly conserved in most AChR \( \alpha \) subunits across many diverse species. The nAChR is widely expressed throughout the brain, and is thought to regulate neurotransmitter release. To explain frontal lobe epileptiform activity
in ADNFLE would therefore imply the formation of different nAChRs using alternative subunits in distinct brain regions, or an increased vulnerability of the frontal lobes to cholinergic effects during sleep.4

The importance of nAChR in epilepsy has been confirmed by the discovery of a mutation in the nAChR β2 subunit, CHRNΒ2, in a family with eight affected members. This affects a conserved residue, again in the second transmembrane domain analogous to the α4 subunit mutations. Functional expression showed prolonged inward current after the application of a cholinergic stimulus, suggesting gain of function. It was hypothesized that prolonged activation of presynaptic nAChRs leads to hyper-excitability and increased neuronal firing, leading to seizures.5 In total, four CHRNA46–10 and three CHRNΒ2 mutations5,11,12 have been identified. Hence, mutations in both the α4 and β2 subunits lead to clinically indistinguishable phenotypes. However, approximately 91% of ADNFLE families do not have mutations in either of these genes.13

Mixed focal and generalized epilepsies

Benign familial neonatal convulsions (BFNC)

Benign familial neonatal convulsions is a syndrome characterized by brief unprovoked partial or generalized seizures occurring a few days after birth that cease spontaneously weeks to months after birth. The EEG shows initial suppression of activity followed by generalized discharges of spikes and slow waves. There are no abnormal neurological signs and patients have normal development with no associated neuropsychological morbidity. However, there is an increased seizure risk in later life, estimated to be 16%, as opposed to the cumulative population risk of 2%.14 Knowledge of this disorder can prevent needless and potentially harmful anticonvulsant therapy.

Two groups (via different strategies) simultaneously discovered the potassium channel mutations responsible for BFNC. One identified a novel potassium channel gene, KCNQ2, which is expressed throughout the brain, using positional cloning in a large Australian BFNC pedigree. A 5-bp insertion mutation into a highly conserved region caused translational frameshift and predicted premature termination of synthesis of the KCNQ2 protein. Functional expression of this mutant KCNQ2 in Xenopus laevis oocytes showed complete loss of potassium currents. Cells co-injected with both mutant and wild-type constructs showed reduced potassium currents, suggesting that haplo-insufficiency may be the pathogenic mechanism in vivo.15 The other group identified a small deletion of 20q13.3 in a BFNC pedigree, which co-segregated with the phenotype. Five mutations in BFNC pedigrees were then identified, all of which affect functionally important regions in the KCNQ2 channel.16 Many further mutations have since been identified.

Using a positional cloning strategy, KCNQ3, a homologue to KCNQ2, was located on chromosome 8. A missense mutation leading to the loss of a charged amino acid in the highly conserved pore region17 was found in a previously described large BFNC pedigree.18 However, most families with BFNC have KCNQ2 mutations; mutations in KCNQ3 are a much rarer cause of this disease.19

KCNQ2 and KCNQ3 are heteromeric ion channels with high sequence homology, which co-assemble to produce the inhibitory M current.20,21 The physiological role of voltage-gated potassium channels is to repolarize neuronal membranes that have been depolarized by sodium and calcium influx. Potassium channels are also thought to repolarize neuronal membranes after activation of excitatory neurotransmitter ligand-gated ion-channel receptors, e.g. glutamate and acetylcholine. Thus, these highly penetrant mutated potassium channels (KCNQ2/3) are postulated to show reduced function in vivo, taking longer to repolarize excitatory neurones and hence permitting the increased excitatory stimulus, which leads to seizures.22 To date, 48 mutations in KCNQ2 have been identified,13 suggesting that there are no common recurrent mutations, and genetic testing will need to be sequence-based. The association of potassium channel mutations and a circumscribed seizure syndrome (BFNC), which disappears early in post-natal life, suggests developmental changes in the expression levels of these proteins, perhaps with adult brain relying on a different configuration of potassium channel subunits.24 Dynamic changes in the expression of ion channel subunit genes may explain the resolution of other seizure disorders restricted to childhood. Remission in adult-onset seizure disorders with time could also represent the substitution of alternative subunits, not usually expressed in adulthood.

BFNC/myokymia syndrome

In this rare subtype, patients with BFNC are affected by myokymia in later life. Myokymia is the clinical manifestation of persistent peripheral nerve stimulation, and is seen as continuous muscle twitching,
especially in the fingers and around the eyes. It is an integral part of episodic ataxia type 1 (EA1), an autosomal dominant potassium channelopathy,\textsuperscript{25} (see below) and Isaac’s syndrome, its autoimmune correlate,\textsuperscript{26} and can be detected by electromyography. A German family was investigated, one of whom had exercise-induced myalgia and generalized myokymia, worsened by fever, cold, alcohol and pregnancy, and improved by carbamazepine. Her twin daughters had neonatal convulsions and (rarely) generalized tonic-clonic seizures (GTCS), sometimes associated with febrile illness. All were found to have a mutation in the KCNQ2 gene, which removed a positive charge from the voltage sensor region of the potassium channel. Functional expression assays revealed a shift in the voltage dependence to more positive voltages, leading to a severe slowing of activation and loss of potassium current. There was also a dominant negative effect on wild-type channels,\textsuperscript{27} implying that the mutant subunit interacts adversely with wild-type subunits, leading to the disease phenotype. BFNC/myokymia syndrome suggests that the KCNQ2 channel is expressed at the peripheral nerve in addition to the brain. Supporting this hypothesis, KCNQ2 was localized to the nodes of Ranvier and initial axon segments of peripheral nerves.\textsuperscript{28}

**Generalized epilepsy with febrile seizures plus syndrome (GEFS+)**

Generalized epilepsy with febrile seizures plus syndrome (GEFS+) is a pleomorphic familial epilepsy syndrome including febrile, afebrile generalized (tonic-clonic, absence, myoclonic, atonic) and partial seizures. Febrile seizures (FS) occur during episodes of fever and are usually restricted to early childhood, but in GEFS+, they may persist beyond the age of 6 years.\textsuperscript{29} There is genetic heterogeneity, with mutations found in the voltage-gated sodium channel \( \beta_1 \) subunit gene (\textit{SCN1B}),\textsuperscript{30} the voltage-gated sodium channel \( \alpha_1 \) subunit gene (\textit{SCN1A})\textsuperscript{31} and the GABA receptor \( \gamma_2 \) subunit gene (\textit{GABRG2}).\textsuperscript{32,33} There is also phenotypic heterogeneity, as within the same family patients may have different seizure semiology, response to treatment and prognosis. Voltage-gated sodium channels are composed of an \( \alpha \) subunit with one or more accessory \( \beta \) subunits. There are at least four known brain \( \alpha \) subunits, each possessing four homologous repeats with six transmembrane domains, the fourth containing the voltage sensor. The linker between segments 5 and 6 is thought to line the ion channel pore (Figure 1). The voltage-gated sodium channel \( \beta_1 \) subunit consists of a single transmembrane domain with a prominent extracellular N-terminal domain, which is thought to modulate channel gating.

**GEFS+ type 1**

An extensive pedigree from Tasmania with 42 affected individuals was studied. Linkage to 19q13.1 led to consideration of \textit{SCN1B} (which encodes the voltage-gated sodium channel \( \beta_1 \) subunit) as a candidate gene. A mutation in a highly conserved cysteine residue of \textit{SCN1B} was identified. This is predicted to disrupt the formation of a disulphide bridge in the extracellular domain. Functional expression showed slowed inactivation of sodium channels, suggesting loss of function and reduced ability to modulate channel gating.\textsuperscript{30} Analysis of further pedigrees has shown this to be a rare form of GEFS+, with analysis of 40 GEFS+ families yielding only one mutation in a large pedigree.\textsuperscript{34}

**GEFS+ type 2**

Two French families had linkage to 2q24-33, where the sodium channel \( \alpha \) subunit cluster (\textit{SCN1A}, \textit{SCN1B} and \textit{SCN1C}) is located. After finding no mutations in \textit{SCN1B}, the human \textit{SCN1A} sequence was deduced from a rat orthologue, to which it has 98% amino acid identity. Two mutations were identified in the \( \alpha_4 \) voltage-sensor of domains II and IV, both at conserved residues. One of these changed a positively charged residue to an uncharged polar residue.\textsuperscript{31} Functional expression analysis showed mild loss of function and persistent inward sodium current, leading to hyperexcitability.\textsuperscript{35} Functional studies of all types of \textit{SCN1A} mutations have not provided an inclusive hypothesis for epileptogenesis. This is in part due to the sensitivity of the sodium channel to its cellular environment, particularly the cell type used for expression. Also, these \textit{in vitro} systems do not detect abnormal membrane assembly or trafficking. However, using a computational method, a theoretical increase in the rate of action potential firing could be produced in all mutations studied, leading to the electrical excitability required for seizure propagation, despite the results of previous functional analysis.\textsuperscript{36} Since identification of the genetic basis of this disease, many further mutations have been reported (reviewed in reference 37).

A missense mutation in the sodium channel type \( II \) \( \alpha_2 \) subunit gene (\textit{SCN2A}) was found in a Japanese patient with FS associated with epilepsy. This affects a residue that is highly conserved across all sodium channels. Functional analysis showed slowed channel inactivation, which may allow persistent repetitive firing during depolarization, a gain of channel
function leading to seizure activity. However, no further mutations have been identified in this gene in other GEFS+-like pedigrees, suggesting that this is either a rare cause, or is population-specific.

Sodium channels govern the production of action potentials critical for neuronal excitability, and a variety of anticonvulsant medications, including carbamazepine and phenytoin act on sodium channels. Thus, sodium channels have an important role in human epilepsy (see also below).

**GEFS+ type 3**

There is a cluster of GABA<sub>Α</sub> receptor subunit genes on 5q34. GABA receptors are heteropentameric proteins with an integral chloride channel, which mediate rapid synaptic inhibition and contain binding sites for GABA, benzodiazepines, barbiturates and steroids. The main channel expressed in adult brain is composed of α<sub>1</sub>, β<sub>2</sub> and γ<sub>2</sub> subunits. Using the GABA<sub>Α</sub> receptor γ<sub>2</sub> subunit gene (GABRG2) as a candidate in a large French family, a missense mutation (K289M) was found. This leads to the substitution of a charged amino acid for a neutral residue in the highly conserved extracellular loop linking the second and third transmembrane segments. Functional expression in *Xenopus laevis* oocytes showed that mutant cells produced only 10% of the current amplitude of wild-type cells, and GABA-induced currents were also inhibited. This is significant, as reduced GABA-mediated synaptic inhibition is potentially epileptogenic. Interestingly, the equivalent mutation in the glycine receptor (K276E) is implicated in hereditary hyperekplexia; another ion channel mediated paroxysmal disorder. This suggests that this is a very important residue for ligand-gated ion channel function.

Recently, in small GEFS+-pedigrees, two putative missense mutations have been identified in the GABRD gene, encoding the GABA<sub>Α</sub> receptor δ subunit. There are some inconsistencies in the genetic data but this is strengthened by the functional studies, which showed reduced maximal current in the G177A mutant. The R220C mutation showed no functional difference to wild-type channels. The authors suggest this is a susceptibility factor for the development of GEFS+, rather than a monogenic trait.

**Severe myoclonic epilepsy of infancy (SMEI)**

This is a rare disorder, which until recently was not considered to be genetic. It is characterized by generalized tonic, clonic, and tonic-clonic seizures that are initially induced by fever, developing during the first year of life. Later, other seizure types develop, including absence, myoclonus, and simple and complex partial seizures, leading to a malignant epileptic syndrome. Psychomotor development becomes abnormal during the second year of life. After the identification of *SCN1A* mutations in GEFS+, and given that both GEFS+ and SMEI involve fever-associated seizures, seven patients with SMEI were screened for mutations in *SCN1A*. Seven mutations were identified, all de novo. SMEI is now considered to be the most severe phenotype within the spectrum of GEFS+. Confirmation of the inherited basis of this syndrome came with a larger study, in which probands were initially thought to be sporadic. However, 32% had a positive family history of FS, and 12% of family members had epilepsy. This emphasizes the need for obtaining a thorough family history in patients with epilepsy, and may reflect reduced penetrance or variable expressivity in the parents of severely affected offspring. Despite this, *SCN1A* mutations were only identified in 35% of patients, suggesting genetic heterogeneity. Many further nonsense and missense mutations have since been identified, most in sporadic cases, suggesting a high new mutation rate. Missense mutations seem to cluster in the pore region (S5–S6) of the channel (52%, compared with GEFS+ mutations at 29%), perhaps explaining the more severe phenotype. Further expansion of the phenotypic spectrum occurred with the discovery of a *SCN1A* mutation in a patient with infantile spasms, part of the triad of West's syndrome.

Further evidence for SMEI as a subtype of GEFS+ was provided with the identification of a GABRG2 mutation in a family with GEFS+, including an individual with SMEI. This mutation lies in the intracellular loop between the third and fourth transmembrane domains, and introduces a premature stop codon. GABA sensitivity in *Xenopus laevis* oocytes expressing the mutant subunit was completely abolished, and fluorescent microscopy showed that receptors containing GFP (green fluorescent protein)-labelled mutant subunits were retained in the lumen of the endoplasmic reticulum. This suggests that loss of function is due to the inability to form functional channels at the cell membrane, due to difficulties in packaging or trafficking of subunits.

**Intractable childhood epilepsy with generalized tonic-clonic seizures (ICGTCS)**

These patients resemble SMEI, and differ only by the absence of myoclonus and the reduced severity of psychomotor impairment. Missense mutations in
SCN1A were identified in 8/10 patients with ICEGTCS. Functional analysis in HEK (human embryonic kidney) cells showed a wide range of defects, including two mutants that were non-functional. These predict both loss and gain of function in vivo, but all showed reduced use-dependent inhibition, suggesting that this may be the common pathogenesis. Two of the probands had parents with a GEFS+ phenotype; interestingly they were both male and inherited the mutation from their mothers, suggesting gender or hormonal influences on channel function. This further illustrates the difficulties in genotype-phenotype correlation in SCN1A mutations.

Benign familial neonatal-infantile seizures (BFNIS)

This newly described benign epilepsy syndrome has age of onset intermediate to BFNC and benign familial infantile seizures. Two families had seizures beginning at a mean of 1.9 months and ceasing by a mean of 3.8 months. Mixed seizure types were noted, including focal and generalized seizures, but only one patient had FS. All patients had normal development and intellect. Two mutations were found in the sodium channel α2 subunit gene SCN2A, previously mutated in a patient with FS and epilepsy. This further increases the difficulty of providing genotype-phenotype correlations in epilepsy due to ion channel mutations. Recently the phenotype has been more extensively classified, and six mutations discovered in a further eight families. All show autosomal dominant inheritance with high penetrance. The median age of seizure onset was 13 weeks, with the predominant seizure type being secondarily generalized seizures. Seizure activity had ceased in all cases by 12 months. Any EEG abnormalities present in the first year of life had disappeared by later studies, and neuroimaging was normal in all tested. This is an important condition to recognize, as the prognosis is excellent, obviating the need for extensive investigations and treatment. Despite the identification of these eight mutations, the electrophysiological effect on the SCN2A channel remains untested.

Generalized epilepsies

Childhood absence epilepsy (CAE)

Childhood absence epilepsy accounts for 5–15% of childhood epilepsies. Symptoms occur at around 6–7 years of age, in contrast to juvenile absence epilepsy, which begins around puberty. Absence seizures are characterized by a paroxysmal loss of consciousness with sudden onset and cessation, associated with bilateral synchronous bursts of spike and wave discharges on the EEG. GTCS may develop during adolescence. Absence seizures occur many times per day, and may either remit or persist into adulthood. About 10–15% of patients have a history of FS. A large Australian pedigree with CAE and FS was studied. A genome-wide screen revealed a linked marker on chromosome 5, near the cluster of GABAA receptor subunits. By sequencing the GABRG2 gene, a missense mutation (R43Q) was identified at an arginine residue, which is not only highly conserved in all known human and animal GABAA receptor subunits, but also in other neurotransmitter receptors such as the glycine receptor and the acetylcholine receptor CHRNA4. It has recently been shown that the pathogenic mechanism of the R43Q mutation is reduced cell surface expression, due to the retention of mutant receptors in the endoplasmic reticulum. There is also reduced inter-subunit contact, leading to poorly assembled receptors and hence reduced receptor function. Although this syndrome is classified as CAE, some individuals fulfill the criteria for GEFS+, so this may represent a further extension of the GEFS+ phenotype. A splice site mutation was identified in an unrelated pedigree with a similar CAE phenotype. However, mutations in GABA receptors are not a common cause of CAE, as a Japanese study of 52 patients failed to identify mutations in GABRA1, GABRB2 or GABRG2. Therefore mutations in GABRG2 are associated with three different epilepsy phenotypes (SMEI, CAE, GEFS+), which occur in different age groups, and hence GABRG2 is postulated to have age-dependent effects on neuronal networks.

An ion channel gene recently implicated in IGE is the brain T-type calcium channel α subunit CACNA1H. Calcium channel structure is similar to that of sodium channels (Figure 1a), except that in addition, calcium channels possess α2δ and γ accessory subunits (Figure 1b). T-type calcium channels are low-threshold voltage-gated channels, opened by a small depolarization from the resting membrane potential. It is thought that they are involved in thalamocortical circuitry and facilitate the burst firing mode, which usually occurs during non-REM sleep. Rhythmic activation of the cortex in this manner while awake results in the characteristic EEG and clinical findings of absences. Ethosuximide, a specific anti-absence drug, is thought to act by blocking T-type calcium channels, offering supportive evidence for this hypothesis.
between domains I and II. Six were in conserved residues, and a further three in functionally important regions of the channel protein.\textsuperscript{56} Functional analysis has shown that two of these mutations allow increased calcium influx during physiological activation, while another results in channel opening at more hyperpolarized potentials, which may underlie the propensity for seizures.\textsuperscript{57,58} A recent study has failed to replicate these findings in Caucasian mixed IGE pedigrees, finding only four sequence variants that did not segregate with disease status or phenotype.\textsuperscript{57,58} This suggests that this gene may be more important in the polygenic inheritance of epilepsy. All of the original variants have now been studied functionally. Computer simulations suggested that seven of these would lead to increased neuronal firing.\textsuperscript{60} One variant is recurrent (G773D) and commonly co-inherited with another SNP (single nucleotide polymorphism), which is found in normal controls (R788C). The G773D-R788C combination was found in three individuals on the same allele, and had a greater functional effect than either variant alone.\textsuperscript{60} Sequence variation in \textit{CACNA1H} as a risk factor for the polygenic inheritance of epilepsy may be population-specific, as \textit{R788C} has a high frequency in the Japanese (13.7\%) but is less common in Caucasian populations (5\%).\textsuperscript{60}

**Juvenile absence epilepsy (JAE)**

Despite the misleading name of this syndrome, patients may experience several seizure types, including absence seizures, GTCS, GTCS on awakening and myoclonic seizures. These begin at around the time of puberty. In a family with JAE, a missense mutation in the chloride channel gene \textit{CLCN2} was identified.\textsuperscript{61} CIC-2 (the protein product of \textit{CLCN2}) is widely expressed in the brain, particularly in neurons that receive inhibitory input via GABAergic neurons and is thought to maintain the low intracellular chloride concentration required to facilitate an inhibitory GABA response. Functional studies of the mutant channel in mammalian cells showed increased outward current of chloride during depolarization. This gain of function mutation is hypothesized to lead to membrane hyperexcitability and seizures.\textsuperscript{61}

**Juvenile myoclonic epilepsy (JME)**

Juvenile myoclonic epilepsy is a common epilepsy syndrome affecting up to 26\% of all patients with IGE. Affected individuals have seizures and myoclonic jerks that begin in adolescence. Isolated myoclonic jerks usually occur in the morning, and do not necessarily lead to GTCS. A family history of epilepsy is common. A French-Canadian family with eight affected members was studied, of whom half had associated absence seizures. A genome-wide screen revealed linkage to chromosome 5q34, a region that includes the GABA\textsubscript{A} receptor subunit cluster. A mutation in \textit{GABRA1}, the gene encoding the GABA\textsubscript{A} receptor \textit{\alpha}1 subunit, was identified. This is located in the third transmembrane domain and affects a residue conserved in \textit{\alpha}1 subunits across several species and in all human \textit{\alpha} subunits. Functional expression showed lower-amplitude GABA-activated currents than wild-type channels, suggesting a loss of inhibitory input, leading to seizures.\textsuperscript{62}

Another family with four members affected by autosomal dominant JME and one with epilepsy with grand mal seizures on awakening was studied. All experienced frequent GTCS and myoclonic jerks. Seizure-free remission was only achieved when treated with combination anticonvulsants. A 1-bp insertion in the \textit{CLCN2} gene was identified that predicts a premature stop codon and a truncated protein lacking the ionic pore. Functional studies showed that mutant channels had no detectable chloride currents, and had a dominant negative effect on wild-type channels. Mutant subunits were visible in the cell membrane, suggesting that impaired channel function mediates these effects, rather than a failure of channel assembly.\textsuperscript{61} Native CIC-2 channels control chloride efflux, maintaining the transmembrane chloride gradient required for inhibitory GABA responses. This suggests that in cells expressing mutant channels, the resultant intracellular chloride accumulation would reduce GABA-mediated inhibition and result in neuronal hyperexcitability and seizures, analogous to the JAE mutation.\textsuperscript{61} However, a more recent study has shown that the mutant subunits do not reach the plasma membrane, suggesting the pathogenic mechanism is haploinsufficiency.\textsuperscript{63} Another group sequenced \textit{CLCN2} in 112 probands with IGE, and identified a splice site variant in two siblings with JME. However, their father also carried the mutation, with no epilepsy phenotype. There was no change in mRNA splicing in blood, although this does not exclude an effect in brain.\textsuperscript{64} Whether this is a true mutation with reduced penetrance, or a rare polymorphism, remains unclear.

A frequently occurring SNP, R220H in \textit{GABRD}, which encodes the GABA\textsubscript{A} receptor \delta subunit, has been suggested as a possible susceptibility factor for the development of JME, due to the presence of a homozygote with JME. However, the phenotype was not compared with that of a heterozygous affected
offspring, and this SNP has a similar frequency in both patients and controls. Functional studies showed a reduced peak current amplitude, with a greater reduction in homozygous mutant channels. These data show that SNPs in GABRD have functional effect and may contribute to an epilepsy phenotype, but further study is required.

**Epilepsy with grand mal seizures on awakening (EGMA)**

Epilepsy with grand mal seizures on awakening is characterized by GTCS occurring predominantly on awakening (independent of the time of day) or during evening leisure time. GTCS can be isolated or associated with the other sub-syndromes of IGE in childhood or adolescence. The common precipitant is lack of sleep, and sleep itself is often unstable or disturbed. In a pedigree with several members affected with EGMA, an 11-bp deletion in intron 2 of the CLCN2 gene, close to the splice acceptor site was identified. This predicts an in-frame deletion of 44 amino acids and the deletion of helix B of the channel protein. Expression studies showed a non-functional chloride channel that exerted a dominant negative effect on wild-type subunits. Recent studies have shown that mutant subunits do not assemble at the plasma membrane. Using a minigene approach, there was no difference between exon-skipped and wild-type mRNA, although mRNA levels in brain would need to be tested to provide conclusive proof.

Hence, three mutations in the same gene (CLCN2) are associated with different IGE subtypes (IAE, JME and EGMA). All three mutations predict hyperexcitability of the post-synaptic membrane of the GABAergic synapse, which taken together with mutations in GABAA subunits found in GEFS+, CAE and JME provide further evidence for the role of dysfunction of GABAergic inhibition in epileptogenesis.

**Generalized epilepsy with paroxysmal dyskinesias (GEPD)**

Recently, an autosomal dominant pedigree was described as having a combination of generalized epilepsy and paroxysmal non-kinesigenic dyskinesia (PNKD), which the authors termed generalized epilepsy with paroxysmal dyskinesia (GEPD). PNKD is characterized by spontaneous hyperkinetic attacks that are precipitated by alcohol, coffee, stress and fatigue. Sixteen affected members were reported: of these, four had epilepsy alone, seven developed PNKD and five had both. They harbour a mutation which segregates with disease status in the ω subunit of the BK (maxi-K) potassium channel KCNMA1, on chromosome 10q22. The 1301 A→G mutation leads to the substitution of a negatively charged for a neutral amino acid (D434G), at a highly conserved residue in the RCK domain of the protein. BK channels are activated by increased intracellular calcium concentration or membrane depolarization. They are large conductance channels that are important in the control of neuronal excitability, by tempering calcium influx via voltage-gated calcium channels. Structurally, there are seven transmembrane domains and a large C-terminus, which includes the RCK domain where regulatory ligands (e.g. calcium and magnesium) bind. Expression studies indicated that the mutant BK channel exhibited increased sensitivity to ambient calcium ion concentration, resulting in an increase in BK channel open probability: a gain of channel function. It is proposed that enhanced BK channel activity in vivo may lead to increased neuronal excitability by inducing rapid repolarization, thereby facilitating fast neuronal firing, which may underlie the seizures and PNKD. Interestingly, ethanol can activate BK channels in C. elegans, hence providing a neat explanation of the propensity for dyskinesic attacks to be induced by alcohol in patients with PNKD.

**Other autosomal dominant channelopathies associated with epilepsy**

**Episodic ataxia type 1 (EA1)**

EA1 begins in childhood and consists of brief attacks (less than 10 min) of ataxia, dizziness without vertigo and visual blurring with no associated nystagmus. Exacerbating factors include abrupt change in posture, emotion, startle or vestibular stimulation. Neuromyotonia (continuous spontaneous muscle fibre activity) or myokymia may occur during and between episodes of ataxia. EA1 is due to mutations in the Kv1.1 potassium channel encoded by KCNA1. A mutation leading to the replacement of a highly conserved residue in the second transmembrane segment was identified in a family with five affected members, two of whom also had complex partial seizures. This abolished potassium currents and had a dominant negative effect on wild-type channels, predicting a reduced potassium efflux and delayed repolarization, leading to seizures. The investigators reviewed all published cases of EA1 and found an increased risk of epilepsy.
Episodic ataxia type 2 (EA2)

EA2 is a similar condition, but begins earlier in childhood and leads to more prolonged attacks (30 min to many hours) of ataxia, dysarthria and nystagmus. There may be associated vertigo, nausea, vomiting and headache. Weakness may occur during attacks, and can precede the onset of ataxia. Attacks are precipitated by physical or emotional stress. EA2 is due to mutations in the P/Q-type voltage-gated calcium channel $\alpha_2$ subunit CACNA1A. This is a high voltage-activated channel expressed throughout the brain and is a multimeric complex of $\alpha_1$, $\beta$, $\gamma$ and $\alpha_2\delta$ subunits (Figure 1b). Functionally, the $\alpha_1$ subunit is most important, acting as both a voltage sensor and ion-conducting pore. Mutations in this gene are also associated with epilepsy. A truncation mutation has been found in a patient with absence seizures, mental retardation and cerebellar ataxia. Recently, a large family with an EA2 absence epilepsy phenotype was identified. These patients harboured a novel CACNA1A mutation, which leads to impaired calcium channel function due to reduced membrane trafficking. As other groups have also described patients with EA2 and seizures, it seems that this is a genuine, if under-recognized, association.

Mutations in the P/Q-type calcium channel $\beta_4$ subunit gene CACNB4 have been found in two pedigrees with idiopathic generalized epilepsy (IGE) and one with a late-onset EA2 phenotype. Eight of the nine affected family members were tested; all carried the mutation. One family had typical JME, while the other had a mixed IGE syndrome, including EGMA, absences and praxis-induced seizures. Hence mutations in two of the subunits that constitute the P/Q-type calcium channel are both associated with an epilepsy phenotype.

Familial hemiplegic migraine (FHM)

Familial hemiplegic migraine is a rare, severe form of migraine associated with hemiparesis. FHM1 is due mainly to missense mutations in CACNA1A, and is therefore allelic to EA2. A study of CACNA1A-negative FHM pedigrees revealed linkage to 1q23, where the ATP1A2 gene is located. This encodes the $\alpha_2$ subunit of the Na$^+/K^+$-ATPase pump and loss of function mutations were identified. After publication of this mutation, a family with co-segregation of FHM2 and benign familial infantile convulsions was studied. A glutamate to arginine substitution in an intracellular linker region of ATP1A2 was found. This residue is highly conserved in the $\alpha$ subunits of many ATPases from several species. The Na$^+/K^+$-ATPase pump comprises $\alpha$, $\beta$ and $\gamma$ subunits, and exchanges intracellular Na$^+$ for extracellular K$^+$, hence loss of function may lead to depolarization of the cell membrane and excitability. In mice, the $\alpha_2$ subunit is only expressed in neurons in the early neonatal stage; this also seems to be the case in humans, where adult expression is confined to astrocytes. This provides a neat explanation of the infantile seizure phenotype seen in this family.

In a patient who displayed episodic ataxia, hemiplegic migraine, coma and seizures, no mutations were found in either the calcium channel CACNA1A or the Na$^+/K^+$-ATPase pump ATP1A2. Given the hypothesized role of glutamate, an excitatory neurotransmitter in the genesis of these symptoms, the gene encoding the excitatory amino acid transporter type 1 (EAAT1) was sequenced and a mutation, C1047G, was identified. This leads to the substitution of arginine at a highly conserved proline residue in the fifth transmembrane domain. Further, functional analysis using a glutamate uptake assay showed reduced uptake via mutant transporters and a dominant negative effect on cell surface expression. The mutation therefore causes disease by both reduced level of protein function and reduced expression. Calcium influx triggers the release of neurotransmitter vesicles at the presynaptic nerve terminal, and the Na$^+/K^+$-ATPase pump maintains the steep sodium gradient required for glutamate uptake by EAAT1. Hence, increased synaptic glutamate, via gain of function CACNA1A mutations in FHM1, loss of function ATP1A2 mutations in FHM2 and loss of function EAAT1 mutations, can all lead to paroxysmal neurological symptoms, including epilepsy.

Association studies and IGE

Ion channels are attractive candidates to account for the polygenic inheritance of seizure susceptibility. Ion channels are vital to neuronal functioning by maintaining cellular ionic and electrical homeostasis. They have an appropriately narrow tissue distribution, in that subunits are expressed exclusively in brain, and dysfunction could therefore lead to an isolated epilepsy phenotype. As outlined above, there are many examples of pathogenic ion-channel gene mutations leading to changes in ion flux. It is possible that coding or non-coding polymorphisms in ion channel genes may also predispose to seizures. However, as in other complex trait diseases, there is conflicting evidence
as to the association of genetic variation in ion channel genes and the more common idiopathic epilepsy phenotypes. The study of ion channel SNPs as epilepsy risk factors is proving more difficult than the investigation of single-gene disorders. Genetic association studies in epilepsy have generally been performed in small numbers of patients, and therefore lack statistical power. Those that have shown a positive association have either not been independently replicated, or have produced conflicting results in different studies. Others have found no association, failed to reach statistical significance, or have not systematically examined the entire gene. A detailed examination of this field is beyond the scope of this review, but interested readers are directed to the excellent review by Tan et al. The results from genetic association studies of ion channel genes are summarized in Table 2. There is clearly more work to do in this field, given our current (lack of) understanding and the conflicting results so far. Also, many ion channels proven to be pathogenic in single-gene disorders have yet to be investigated in non-hereditary epilepsy populations.

Modern techniques involve examining as much genetic diversity in the region of the gene of interest as physically (and economically) possible. This usually involves genotyping many SNPs. However, some previous epilepsy association studies have just typed one SNP, mutation or triplet repeat. These results therefore do not indicate the association, or lack thereof, of that gene with epilepsy, but rather whether the SNP (or whatever was examined) is/is not associated with epilepsy. The advent of high-throughput genotyping techniques, genetic databases and statistical methods for study design and choice of SNPs has revolutionized this area, and should provide statistically significant results in large numbers (i.e. >500, but ideally many thousands) of patients. Only when all genetic association studies enter this era may the true nature of common genetic variation in epilepsy be elucidated.

**Drug responsiveness**

Recently a functional variant in SCN1A has been associated with drug responsiveness in epilepsy. Both carbamazepine and phenytoin act by blocking sodium channels. An intronic polymorphism was highly associated with the maximum dose of both of these drugs. The polymorphism destroys the consensus sequence of the S’ splice donor site of a fetal alternatively spliced exon (exon 5N). In epileptic brain, the proportion of 5N transcripts was increased in temporal lobe, relative to hippocampus, only in those with homozygous wild-type genotype. This suggests that seizures may upregulate inclusion of a fetal exon in adults, but further work is required to confirm these findings. This further highlights the important role of SCN1A in human epilepsy.

**Anticonvulsant medications**

All commonly used anticonvulsants exert their anti-seizure action via ion channels. Therefore, seizures may be caused by increased excitation via mutations in ion channel genes and can be treated with medications which reduce excitation by acting on ion channels, further emphasizing the importance of ion channels in epilepsy. Voltage-gated ion channels are required for action potential generation, firing and neurotransmitter release. Hence, modulation of these channels can reduce the propensity to seizures. Ligand-gated ion channels mediate synaptic inhibition and excitation, therefore reduced excitation or increased inhibition via modification of these channels can produce anticonvulsant effects, including burst suppression and limitation of seizure spread. The mode of action of all available anticonvulsants is summarized in Table 3. A detailed discussion of the mode of action of all anticonvulsants is beyond the scope of this paper; readers are directed to the review by Rogawski & L"oscher. The most established and well-understood anticonvulsants act on sodium channels. Blockage of voltage-gated sodium channels reduces high-frequency repetitive firing and thereby reduces seizure spread. This is the mechanism of action of many anticonvulsants, the archetypal drugs being phenytoin and carbamazepine. These produce increased block on hyperpolarized (more negative) channels and those which are firing repetitively. Drugs that block these channels are thus more effective on active channels than on those which are firing normally. Sodium channel blockers promote fast inactivation of channels, rendering them unavailable for further action potential firing, and hence reduce seizures. These drugs have a slow onset and offset of action, as long depolarizations are required to block the channel, therefore they are only effective against pathological sustained depolarizations with high frequency discharges and not on normal action potentials.

The detailed knowledge of ion channel gene mutations on cellular function in Mendelian epilepsy disorders should aid drug design in providing appropriate channel blockers and openers, as has already been achieved with the discovery of retigabine, a KCNQ2/KNCQ3 channel activator. Given the limited number of ion channel types (sodium, potassium, etc.) in the CNS, it is likely that at least some of these drug discoveries will be
### Table 2 Genetic association studies of ion channel genes

<table>
<thead>
<tr>
<th>Ion channel gene</th>
<th>No. of patients</th>
<th>Epilepsy syndrome</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ligand-gated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GABBR1 GABA&lt;sub&gt;B&lt;/sub&gt; receptor</td>
<td>141</td>
<td>TLE</td>
<td>Positive association with a coding non-synonymous SNP</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>118</td>
<td>IGE</td>
<td>No association with two non-synonymous SNPs and a silent SNP</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>CAE</td>
<td>No association with 3 intronic SNPs and one silent coding SNP</td>
<td>85</td>
</tr>
<tr>
<td><strong>GABRG2 GABA&lt;sub&gt;A&lt;/sub&gt; receptor γ subunit</strong></td>
<td>104</td>
<td>FS</td>
<td>Silent SNP associated</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>FS</td>
<td>Attempted replication by another group failed to replicate initial findings in an unrelated population</td>
<td>87</td>
</tr>
<tr>
<td><strong>CHRNA4 Acetylcholine receptor α&lt;sub&gt;4&lt;/sub&gt; subunit</strong></td>
<td>103</td>
<td>IGE</td>
<td>Silent SNP associated, but with borderline significance</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>182</td>
<td>IGE</td>
<td>Attempted replication by another group failed to replicate initial findings in an unrelated population</td>
<td>89</td>
</tr>
<tr>
<td><strong>GLRA3 Glycine receptor α&lt;sub&gt;3&lt;/sub&gt; subunit</strong></td>
<td>104</td>
<td>IGE</td>
<td>No association with four silent SNPs</td>
<td>92</td>
</tr>
<tr>
<td><strong>Voltage-gated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNJ3 M-channel</td>
<td>71</td>
<td>IGE</td>
<td>No association with intragenic repeats</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>119</td>
<td>JME</td>
<td>Association with intragenic repeats</td>
<td>94</td>
</tr>
<tr>
<td>KCNJ3 M-channel</td>
<td>115</td>
<td>IGE</td>
<td>No association with coding SNP</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>FS</td>
<td>No association with two silent SNPs</td>
<td>96</td>
</tr>
<tr>
<td><strong>KCNJ3 Inwardly-rectifying potassium channel</strong></td>
<td>187</td>
<td>IGE</td>
<td>Silent SNP associated with borderline statistical significance</td>
<td>97</td>
</tr>
<tr>
<td><strong>hKCa3 Calcium-activated potassium channel</strong></td>
<td>126</td>
<td>IGE</td>
<td>No association with trinucleotide repeat length</td>
<td>98</td>
</tr>
<tr>
<td><strong>TASK-3 Tandem-pore domain potassium channel</strong></td>
<td>65</td>
<td>JAE/CAE</td>
<td>No association with silent SNP</td>
<td>99</td>
</tr>
<tr>
<td><strong>CACNA1A P/Q-type calcium channel α subunit</strong></td>
<td>188</td>
<td>IGE</td>
<td>Silent SNP associated</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>354</td>
<td>IGE</td>
<td>Attempted replication by another group failed to replicate initial findings in an unrelated population</td>
<td>101</td>
</tr>
<tr>
<td><strong>CACNA1H T-type calcium channel α subunit</strong></td>
<td>118</td>
<td>CAE</td>
<td>Coding non-synonymous SNPs associated with IGE in Chinese Han population (see CAE section above)</td>
<td>56</td>
</tr>
<tr>
<td><strong>CACNA1G T-type calcium channel α-subunit</strong></td>
<td>192</td>
<td>CAE</td>
<td>No association of two silent SNPs</td>
<td>102</td>
</tr>
<tr>
<td><strong>SCN2A Sodium channel type II α&lt;sub&gt;1&lt;/sub&gt; subunit</strong></td>
<td>46</td>
<td>IGE</td>
<td>No association of two silent SNPs</td>
<td>103</td>
</tr>
<tr>
<td><strong>SCN2B Sodium channel type II β&lt;sub&gt;1&lt;/sub&gt; subunit</strong></td>
<td>92</td>
<td>IGE</td>
<td>No association of silent SNP</td>
<td>104</td>
</tr>
<tr>
<td><strong>Transporters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP1A2 Sodium/potassium co-transporter</td>
<td>56</td>
<td>TLE</td>
<td>4 bp insertion not associated</td>
<td>105</td>
</tr>
<tr>
<td>EAAT2 Glutamate transporter</td>
<td>133</td>
<td>IGE</td>
<td>No association of silent SNP</td>
<td>106</td>
</tr>
</tbody>
</table>

TLE temporal lobe epilepsy. Adapted from reference 76.
effective even in epilepsy syndromes not directly related to specific ion channel mutations. With in vitro cellular models available to directly test efficacy, the drug discovery process should gather momentum. Given the tissue-specific distribution of these ion channels, such new treatments should theoretically have a lower side-effect profile.

Conclusions

During the past 10 years, there has been an upsurge in the discovery of epilepsy-causing mutations in genes encoding ion channel proteins. Good genotype–phenotype correlation is possible in BFNC, BFNIS and ADNFLE, where little intrafamilial variability is seen. However, in GEFS+ and epilepsy caused by CLCN2 mutations, the clinical phenotype can be highly variable. This has led to the hypothesis of channel mutations as risk factors only when expressed on the correct inherited genetic background, in the presence of polymorphisms or a mutation in another gene. However, digenic inheritance is very rare. Little is known about the control of gene expression of ion channels. There are conceivably many modifying genes that are completely unknown at present. In the future, when such mechanisms have been elucidated, we will have a greater understanding of the role of ion channel gene mutations and epileptogenesis. Perhaps the variable penetrance and clinical phenotype depends on the availability of alternative ion channel subunits to replace those which are dysfunctional. The differing severities of these syndromes may represent the non-redundancy of multiple ion-channel subunits in distinct channel types, so that (for example) an α1 subunit could be replaced by an α2 subunit. Even in syndromes with little phenotypic variability, there remains genetic heterogeneity (e.g. ADNFLE), with no obvious clinical correlates to indicate which gene is affected, making molecular diagnosis difficult. This suggests that dysfunction of a single ion channel type per se does not lead to the seizure phenotype; rather, it is due to a more general effect on neuronal excitability. Our understanding of epileptogenesis in ion channel diseases may lie in the elucidation of the final common pathway in neuronal signalling, downstream to the individual neurone.

In all Mendelian epilepsy syndromes described above, with the exception of CHRNA4, there are no common recurrent mutations. Ion channel genes are large, encoding complicated proteins. Therefore, DNA sequencing as a screening tool is time-consuming and impractical, with the result that molecular diagnosis currently remains within the domain of research and not routine clinical diagnosis. The introduction of automated sequencing techniques and other technological advances may make this more feasible in the future.

In the last few years, the number of discrete autosomal dominant epilepsy syndromes due to ion channel mutations has expanded rapidly (Table 1), such that some are not included in the ILAE classification. Perhaps future classification systems will include the molecular genetic basis of disease, such as those used in hereditary motor and sensory neuropathy and spinocerebellar ataxia, in addition to clinical syndromes.

These discoveries provide a starting point in the elucidation of epileptogenesis in inherited IGE...
syndromes, and perhaps in the more common types of epilepsy, and may in the future provide specific targets for new anticonvulsant therapies.

Acknowledgements

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