

Molecular genetics of the epilepsies

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Introduction

A genetic contribution to aetiology has been estimated to be present in about 40% of patients with epilepsy.

It is useful to categorise genetic epilepsies according to the mechanisms of inheritance involved. This identifies three major groups:

Mendelian disorders, in which a single major locus can account for segregation of the disease trait

Non-mendelian or 'complex' diseases, in which the pattern of familial clustering can be accounted for by the interaction of several loci together with environmental factors, or by the maternal inheritance pattern of mitochondrial DNA

Chromosomal disorders, in which a gross cytogenetic abnormality is present.

A second useful distinction is between the 'symptomatic' epilepsies, in which recurrent seizures are one component of a multifaceted neurological phenotype, and the 'idiopathic' epilepsies, in which recurrent seizures occur in individuals who are otherwise neurologically and cognitively intact and who have no detectable anatomical or metabolic abnormality of the brain.

There are over 200 mendelian diseases which include epilepsy as part of the phenotype. Many of these are associated with obvious structural lesions: e.g. tuberous sclerosis, neurofibromatosis type 1, malformations of cortical development and the progressive myoclonic epilepsies.

A small number of mendelian epilepsies are 'pure' idiopathic epilepsy syndromes, and these may be either generalised epilepsies (e.g. benign familial neonatal convulsions) or partial epilepsies (e.g. autosomal dominant nocturnal frontal lobe epilepsy, familial temporal lobe epilepsy). The mendelian idiopathic epilepsies are individually rare, and probably account for no more than 1% of patients, but their study has been critical in advancing our understanding of the pathogenetic basis of idiopathic epilepsy.

The 'common' non-familial idiopathic epilepsies tend to display 'complex' inheritance. They include well-characterised entities including the various forms of idiopathic generalised epilepsy (IGE) such as juvenile myoclonic epilepsy (JME) and childhood absence epilepsy (CAE), as well as the idiopathic partial epilepsies.

Several gross chromosomal aberrations are associated with epilepsy, including Down syndrome, trisomy 12p and ring chromosome 20. Recent progress has also seen the first report of chromosomal micro-deletions associated with epilepsy (e.g. Helbig et al¹) extending the observation that variation in gene copy number (CNVs) plays an important role in susceptibility to epilepsy.

‘Primary’ idiopathic mendelian epilepsies

A few primary epilepsies are inherited in a mendelian fashion. They are rare and together account for only a small fraction of all epilepsy. However, they form an important group because recognition of the characteristic features and presence of a family history will enable the correct diagnosis to be made. Identification of genes responsible for some of these disorders has provided valuable insights into the molecular mechanisms underlying epilepsy. Table I lists the known genes implicated in idiopathic mendelian epilepsy. By definition, each gene was identified using linkage analysis in an extended pedigree. Meticulous clinical characterisation of epilepsy within each pedigree was instrumental in the discovery of each gene, and the identification of each has been a significant advance in our understanding of epilepsy. Some of these are discussed in greater detail below.

Benign familial neonatal convulsions (BFNC): mutations in KCNQ2 and KCNQ3

Benign familial neonatal convulsions (BFNC) is a rare autosomal dominant idiopathic epilepsy, first described in 1963, and was the first epileptic syndrome to be localised by linkage². Seizures occur in otherwise well neonates from the second or third day of life and remit by week 2–3. The characteristic seizures comprise tonic posturing with ocular and autonomic features, followed by a clonic phase with motor automatisms. The prognosis for neurological and intellectual development is favourable, although seizures recur later in life in about 10% of individuals.

BFNC is a good illustration of genetic heterogeneity, which can be explained by the underlying molecular genetics. The first locus (*EBN1*) identified in 1989 was localised to chromosome 20q by linkage analysis in a four-generation family with 19 affected individuals³. Six French pedigrees confirmed this linkage⁴. However, in a study of two North American families, one family which showed linkage to *EBN1* included family members with seizures persisting up to two years of age, and in one individual into adolescence⁵. The other family, none of whose members had seizures after two months of age, could be excluded from linkage to *EBN1*, and was subsequently linked to a second locus (*EBN2*) on chromosome 8q⁶.

The gene for *EBN1*, subsequently named *KCNQ2*, was identified by characterisation of a sub-microscopic deletion on chromosome 20q13.3 in affected individuals and was found to show significant homology with a voltage-dependent delayed rectifying potassium channel gene, *KCNQ1*⁷. Members of the *KCNQ* potassium channel family comprise six transmembrane-spanning segments (S1–S6), a pore forming loop linking S5 and S6, and intracellular N and C termini. These channels open on membrane depolarisation and are involved in the repolarisation of the action potential and thus in the electrical excitability of nerve and muscle. Mutations in *KCNQ1* can cause the paroxysmal cardiac dysrhythmias long QT syndrome and Jervell-Lange-Nielson cardioauditory syndrome^{8,9}. Six allelic variants of *KCNQ2* have now been identified to segregate with the disease in families with BFNC, including one family whose affected members subsequently developed myokymia (spontaneous contractions of skeletal muscle fibres)¹⁰. All mutations involve regions of the gene important for ion conduction, including the S6 domain, the channel pore and the C terminus.

Table I. Idiopathic mendelian epilepsy genes identified by linkage analysis in extended pedigrees (from M.R. Johnson. The genetic contribution to epilepsy: the known and missing heritability. In: The Causes of Epilepsy, eds S.D. Shorvon et al, pp 63–67. Cambridge University Press, Cambridge, 2011).

Class	Gene	Gene product	Epilepsy syndrome	Reference
Voltage-gated ion channel genes				
	<i>SCN1A</i>	Sodium channel α 1 subunit	GEFS+, Dravet	Escayg et al, 2000
	<i>SCN1B</i>	Sodium channel β 1 subunit	GEFS+	Wallace et al, 1998
	<i>SCN2A</i>	Sodium channel α 2 subunit	BFNIS	Heron et al, 2002
	<i>KCNQ2</i>	Potassium channel subunit	BFNC	Singh et al, 1998
	<i>KCNQ3</i>	Potassium channel subunit	BFNC	Charlier et al, 1998
	<i>KCNA1</i>	Potassium channel subunit	EA1 and epilepsy	Zuberi et al, 1999
Ligand-gated ion channel genes				
	<i>CHRNA4</i>	Acetylcholine receptor α 4 subunit	ADNFLE	Steinlein et al, 1995
	<i>CHRNA2</i>	Acetylcholine receptor α 2 subunit	ADNFLE	Aridon et al, 2006
	<i>CHRNA2</i>	Acetylcholine receptor β 2 subunit	ADNFLE	De Fusco et al, 2000
	<i>GABRA1</i>	GABA _A receptor α 1 subunit	AD JME, CAE	Cossette et al, 2002
	<i>GABRG2</i>	GABA _A receptor γ 2 subunit	GEFS+, CAE	Wallace et al, 2001; Baulac et al, 2001
Others				
	<i>LGII</i>	Leucine-rich glioma inactivated	ADLTE	Kalachikov et al, 2002
	<i>EFHC1</i>	Protein with EF-hand motif	IGE, particularly JME	Suzuki et al, 2004
	<i>PCDH19</i>	Protocadherin 19	EFMR	Dibbens et al, 2008
	<i>ATP1A2</i>	Na/K ATPase pump	FHM and epilepsy (including BFNIC)	Vanmolkot et al, 2003
	<i>POLG1</i>	Mitochondrial DNA polymerase	Mixed epilepsy phenotypes	Engelsen et al, 2008

AD = autosomal dominant, ADNFLE = autosomal dominant frontal lobe epilepsy, ADLTE = autosomal dominant lateral temporal lobe epilepsy, AE = absence epilepsy, BFNC = benign familial neonatal convulsions, BFNIS = benign familial neonatal-infantile seizures, CAE = childhood absence epilepsy, EA1 = episodic ataxia type 1, EFMR = epilepsy and mental retardation limited to females, FHM = familial hemiplegic migraine, GEFS+ = generalised epilepsy with febrile seizures plus, GLUT-DS = glucose transporter type 1 deficiency syndrome, JME = juvenile myoclonic epilepsy, IGE = idiopathic generalised epilepsy, PED = paroxysmal exercise-induced dyskinesia

Following identification of *KCNQ2*, a BLAST search was made of the human expressed sequence tag (EST) database, to find cDNA sequences showing significant homology to *KCNQ2*. Rather fortuitously, a novel gene, *KCNQ3*, was identified with 69% similarity to *KCNQ2*, which mapped to the *EBN2* critical region on chromosome 8q24, and was found to be mutated in affected members of the BFNC/*EBN2* family¹¹. The missense mutation identified altered a conserved amino acid in the critical pore-forming region (the same amino acid found to be mutated in *KCNQ1* in a patient with long QT syndrome⁷).

KCNQ2 and *KCNQ3* are co-expressed in most areas of the brain, especially the hippocampus, neocortex and cerebellum. They have been shown to coassemble and form a heteromeric channel with essentially identical biophysical properties and pharmacologic sensitivities to the native neuronal M-channel¹². The M-channel is a slowly activating and deactivating potassium conductance that plays a critical role in determining the subthreshold electroexcitability of neurons. It can thus be seen how mutations in either *KCNQ2* or *KCNQ3* disrupt the native M-current and result in an identical disease phenotype

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE): mutations in CHRNA4 and CHRNB2

Six families from Australia, UK and Canada with an autosomal dominantly inherited form of partial epilepsy were described^{13,14}. Seizures occurred during sleep and had frequently been misdiagnosed as nightmares, night terrors, hysteria, sleep paralysis and paroxysmal nocturnal dystonia. The familial nature of the condition had therefore previously gone unrecognised. Seizures begin predominantly in childhood and persist into adulthood. They occur in clusters of between four and eleven episodes a night, usually soon after falling asleep or before waking. Several individuals report an aura which may be sensory, somatosensory or psychic. The majority of subjects are aware throughout the seizure, although many develop secondarily generalised seizures at some time. Affected individuals are neurologically and intellectually normal. The inter-ictal EEG is usually normal although the ictal EEG may show sharp and slow-wave activity in the anterior quadrants bilaterally.

Segregation analysis performed in the five families described supported autosomal dominant inheritance with 69% penetrance and variable expression. Linkage studies performed in a single large Australian pedigree assigned the gene to chromosome 20q13.2, the same region to which *EBN1* maps¹⁵. This region of chromosome 20q contains a candidate gene, *CHRNA4*, which encodes the $\alpha 4$ subunit of the nicotinic acetylcholine receptor. A missense mutation that replaces a serine with phenylalanine at codon 280 (codon 248 based on numbering in the original report) has been demonstrated in *CHRNA4* in the chromosome 20-linked family¹⁶. This highly conserved amino acid lies in the second transmembrane domain and it is likely that mutation at this site would cause disease. Two further mutations have been described in the M2 domain: c873-874insGCT associated with Leu301-302¹⁷ and c851C>T associated with Ser284Leu (Ser252Leu in old numbering) in a Japanese family¹⁸.

A second ADNFLE locus was mapped to chromosome 15q24¹⁹. This region is close to the *CHRNA3/CHRNA5/CHRNB4* cluster, although no mutations were found on screening the exons that encode the pore-forming region of *CHRNA3*, *CHRNA5*, and *CHRNB4*. Two mutations have been identified at the *CHRNB2* locus on 1q. These are both missense mutations, changing the valine residue at 287 to leucine²⁰ or to methionine²¹. The ADNFLE loci at the three chromosomal regions are designated epilepsy, nocturnal frontal lobe (ENFL) type 1: 20q13.2, type 2: 15q24 and type 3: 1q.

In the mammalian brain most neuronal nicotinic acetylcholine receptors are heteromultimers consisting more commonly of three $\alpha 4$ and two $\beta 2$ subunits, the very two

subunits encoded by the genes mutated in ADNFLE. It is not clear how mutations in genes encoding these subunits cause an age-dependant, nocturnal frontal lobe epilepsy²². The functional effects of these naturally occurring mutations have been examined *in vitro* by expression in *Xenopus* oocytes and other cells. Analysis of four mutations demonstrated an increase in acetylcholine sensitivity indicating a gain of function²³.

Generalised epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy of infancy (SMEI): mutations in SCN1A, SCN2A, SCN1B and GABRG2

This syndrome was first described in 1997 in an Australian family originating from Yorkshire in the UK²⁴. Genealogical information was obtained on 2000 family members dating back to the mid-1700s, and clinical information obtained on 289 individuals, of which 28 had seizures. The commonest phenotype, denoted as 'febrile seizures plus' (FS+) comprised a childhood onset of multiple febrile seizures persisting beyond the age of six, as well as a spectrum of afebrile seizures including absences, myoclonic seizures, atonic seizures and rarely myoclonic-astatic epilepsy. The pattern of inheritance was autosomal dominant.

A second Australian family with generalised epilepsy and febrile seizures plus was linked to chromosome 19q13.1, and a point mutation identified in *SCN1B*, which encodes the $\alpha 1$ subunit of the voltage gated sodium channel²⁵.

Neuronal voltage-gated sodium channels are essential for the generation and propagation of the action potential. They contain a large α subunit associated with two smaller β subunits. The pore-forming α subunit contains four homologous domains each containing six membrane-spanning units. The β subunits contain a single transmembrane region and modulate the gating properties of the channel and are required for normal inactivation kinetics. Mutations in α subunit genes cause several paroxysmal disorders of muscle, including hyperkalaemic periodic paralysis, paramyotonia congenita (*SCN4A*) and long QT syndrome (*SCN5A*)²⁶. Sodium channels are also modulated by antiepileptic drugs such as phenytoin and carbamazepine²⁷. They are thus good candidate genes for epilepsy.

The *SCN1B* mutation segregated with disease status in the 19q13.1-linked GEFS+ family. It changes a conserved cysteine residue that disrupts a disulfide bridge normally maintaining an extracellular immunoglobulin-like fold in the β subunit. This alters the secondary structure of the extracellular domain, which modulates channel gating. The predicted effects of the disruption are to reduce sodium channel expression, slow inactivation, and slow recovery from inactivation. Coexpression of α and mutant $\beta 1$ subunits exerts significant modulatory effects on the channel gating kinetics and expression levels. The net effect is to cause persistent inward neuronal sodium currents, increased membrane depolarisation, and neuronal hyperexcitability. This may also exaggerate the normal effects of temperature on both conductance and gating of neuronal sodium channels, explaining the apparent temperature dependence of the GEFS+ phenotype.

Two further families with GEFS+ showed linkage to chromosome 2q24, and mutations were identified in *SCN1A*, the gene encoding the sodium channel $\alpha 1$ subunit²⁸. *De novo* mutations in this gene have also been identified in patients with severe myoclonic epilepsy of infancy (SMEI), which also involves fever-associated seizures²⁹. In fact, many patients with SMEI have a family history of seizures consistent with the spectrum of seizure phenotypes seen in GEFS+, suggesting that SMEI is the most severe phenotype in the GEFS+ spectrum³⁰. A mutation in the gene encoding the $\alpha 2$ sodium channel subunit, *SCN2A*, has now been identified in a patient with febrile seizures associated with afebrile seizures, consistent with GEFS+. This mutation also slows channel inactivation, suggesting involvement in the epilepsy phenotype³¹. *SCN2A* mutations have also been found in families with benign familial neonatal-infantile seizures (BFNIS), an epilepsy similar to

benign familial neonatal convulsions but in which seizures begin after one month of age³². The functional effects of three *SCN1A* mutations have been investigated by heterologous expression with $\beta 1$ and $\beta 2$ subunits in cultured mammalian cells³³. All three mutations alter channel inactivation, resulting in persistent inward sodium current. Thus neuronal hyperexcitability is again likely to result from increased membrane depolarisation, as with the *SCN1B* mutation. Most of the *SCN1A* mutations causing SMEI introduce a stop codon with truncation of the protein and predicted loss of function. Thus severe *de novo* truncation mutations of *SCN1A* underlie many cases of SMEI. Cases occurring within GEFS+ families suggest that *SCN1A* mutations interact with modifier genes to determine the phenotype.

The GEFS+ phenotype is not only caused by mutations in voltage-gated sodium channels. In two GEFS+ families, mutations have been identified in the GABA_A receptor γ subunit gene, *GABRG2*^{34,35}. GABA_A receptors are anion-selective ligand-gated channels which mediate fast synaptic inhibition. Binding of GABA opens an integral chloride channel, producing an increase in membrane conductance that results in inhibition of neuronal activity. The first GEFS+ mutation substitutes a serine for a methionine in the extracellular loop between transmembrane segments M2 and M3, which was shown to decrease the amplitude of GABA-activated currents when expressed in *Xenopus* oocytes^{34,36}. The second mutation introduces a premature stop codon in the mature protein³⁵. This completely abolished GABA sensitivity in *Xenopus laevis* oocytes expressing the mutant $\gamma 2$ -subunit. Receptors containing the mutant subunit failed to reach the cell surface when expressed in HEK293 cells. Mutations in *GABRG2* also cause a phenotype of childhood absence epilepsy and febrile seizures³⁷.

Autosomal dominant juvenile myoclonic epilepsy (ADJME): mutation in GABRA1

A mutation of *GABRA1* has been described in a large French Canadian family with autosomal dominant segregation of a phenotype consistent with juvenile myoclonic epilepsy (JME)³⁸. A genome scan provided evidence of linkage to chromosome 5q34 encompassing a cluster of GABA_A receptor subunit genes: *GABRB2*, *GABRA1* and *GABRG2*. A mutation, Ala322Asp, in *GABRA1* was found to segregate in a heterozygous state with affected individuals. GABA_A receptors are the major sites of fast synaptic inhibition in the brain and dysfunction of this receptor has long been suspected in the development of epilepsy. This mutation was not found in 83 patients with sporadic idiopathic generalised epilepsy (IGE) (including JME and CAE), some of whom had a positive family history for various epilepsy syndromes. However this is the first example of a mutation segregating with a mendelian phenotype corresponding to a common IGE syndrome.

Autosomal dominant partial epilepsy with auditory features (ADPEAF): mutations in LGII

Autosomal dominant partial epilepsy with auditory features (ADPEAF) (also called lateral temporal lobe epilepsy) was first described in a three-generation family with 11 members diagnosed with an idiopathic/cryptogenic epilepsy³⁹. The epilepsy was clearly localised in all but one of these cases, with both simple and partial complex seizures progressing to secondarily generalised tonic-clonic seizures. Six of those with idiopathic epilepsy reported auditory disturbances as a simple partial component of their seizures. The seizures were infrequent with an age of onset between eight and 19 years.

A genome screen identified linkage over a 10cM region on chromosome 10q23.3-24³⁹. This region was narrowed to 3cM by a genome screen in a large Basque family segregating lateral temporal lobe epilepsy with auditory and visual features⁴⁰. Further families confirmed the linkage but failed to narrow the region. Construction of a physical map identified 28 putative genes of which 21 were sequenced in an affected individual from three families, and mutations subsequently checked in a further two families⁴¹. Mutations were identified in the leucine rich, glioma-inactivated 1 gene, *LGII*, in all affected individuals and obligate carriers, as well as six unaffected members, consistent with a 71%

disease penetrance. The five mutations identified were not present in 123 unrelated controls.

LGII is a member of the leucine-rich repeat (LRR) superfamily, in particular the adhesive proteins and receptors. It was first described following the observation that it was disrupted by translocation in a glioblastoma multiforme cell line and in over one quarter of primary tumours. The *LGII* protein consists of an extracellular domain with LRR repeat motifs, a transmembrane segment and an intracellular segment of unknown function⁴². The extracellular portion aligns most closely with a group of proteins, including slit, toll and tartan, involved in CNS development and in which the LRRs bind nerve growth factor and other neurotrophins. Interestingly, a C-terminal repeat motif, now referred to as the EAR (epilepsy-associated repeat) domain, has been identified in both *LGII* and the *MASSI* gene, which is mutated in the Frings mouse model of audiogenic epilepsy and in a family with febrile and afebrile seizures^{43,44}. This EAR domain is likely to play a role in the pathogenesis of epilepsy. *LGII* is expressed predominantly in brain (cerebellum, cortex, medulla, occipital pole, frontal lobe, temporal lobe and putamen), muscle and spinal cord. Of the five mutations identified in ADPEAF, three were missense mutations with predicted premature truncation of the *LGII* protein, one was a non-synonymous point mutation in the highly conserved extracellular and C-terminal region, and one was an intronic mutation predicted to alter a splice site. *LGII* and *MASSI* are the first non-ion channel gene identified as causing an idiopathic epilepsy in humans.

X-linked infantile spasms (ISSX) and X-linked myoclonic epilepsy with generalised spasticity and intellectual disability (XMESID): mutations in ARX

Infantile spasms are divided into those that are symptomatic and those that are cryptogenic or idiopathic. The majority (70–80%) are symptomatic and may be attributed to a prenatal, perinatal or postnatal cause, of which prenatal aetiologies are the most common (50%). Many of these are genetically determined, including disorders of brain development, neurocutaneous syndromes, metabolic disorders and chromosomal abnormalities.

Most cases of idiopathic infantile spasms are sporadic, and the recurrence risk is less than 1%⁴⁷. However several familial cases have been identified consistent with X-linked inheritance. Feinberg and Leahy first reported five affected males in four sibships of a three-generation family⁴⁸. Subsequently, five further families have been identified, some of which also include individuals with X-linked mental retardation without infantile spasms⁴⁹⁻⁵³. Linkage analysis in these families mapped the disease gene to chromosome Xp21.3-Xp22.1⁵⁰⁻⁵². The aristaless-related, homeobox gene, *ARX*, was considered a candidate on the basis of its expression pattern in fetal, infant and adult brain. Screening of this gene identified mutations in four of the five families with infantile spasms⁵³. Mutations were also identified in five families with mental retardation together with myoclonic seizures or dystonia, but no infantile spasms. Two recurrent mutations identified in seven of the nine families result in expansion of polyalanine tracts of the *ARX* protein. These are likely to cause protein aggregation, as has been demonstrated in other human diseases caused by alanine expansions⁵⁴.

XMESID, a rare X-linked recessive myoclonic epilepsy with spasticity and intellectual disability in boys, has now also been associated with a missense mutation in *ARX*⁵⁵. Hyperreflexia was found in carrier women. Homeobox-containing genes are known to be important in the regulation of key stages of development. *ARX* encodes one of a class of proteins incorporating a C-terminal aristaless domain thought to be particularly important in the differentiation and maintenance of specific neuronal subtypes in the cerebral cortex⁵⁶.

Mendelian disorders in which epilepsy forms part of the phenotype

Progressive myoclonic epilepsies (PMEs)

The PME are a clinically and aetiologically heterogeneous group of rare inherited disorders characterised by the association of epilepsy, myoclonus and progressive neurological deterioration, in particular ataxia and dementia. The most common forms of PME are: Unverricht-Lundborg disease, Lafora disease, the neuronal ceroid lipofuscinoses, myoclonus epilepsy with ragged-red fibres (MERRF), and sialidosis.

Unverricht-Lundborg disease – Baltic myoclonus

Progressive myoclonic epilepsy of the Unverricht-Lundborg type (ULD, locus symbol *EPM1*) is an autosomal recessive disorder which is enriched in the Finnish population with an incidence of one in 20,000 births. Stimulus sensitive myoclonus begins between the age of about six and 15 years and mild mental retardation, dysarthria and ataxia develop with time. Non-specific histological changes are found in the brain; Lafora bodies or autofluorescent lipopigment are not found. Evidence suggested that ULD, Baltic myoclonus⁵⁷, and so-called Mediterranean myoclonus⁵⁸ are genetically homogenous. The combination of a high degree of consanguinity and a risk rate for siblings of one in four demonstrate that inheritance is autosomal recessive⁵⁹.

ULD was mapped to the long arm of chromosome 21 in a group of 11 nuclear pedigrees from Finland. A genome search was undertaken and linkage found after testing 64 marker loci⁶⁰. A maximum multipoint lod score of 10.08 was obtained with three loci in 21q22.3. The localisation has been further refined⁶¹, most recently using the technique of linkage disequilibrium mapping to a region of about 0.3 cM. Linkage studies in non-Finnish families have demonstrated genetic (locus) homogeneity within this phenotype.

The *EPM1* gene was isolated by positional cloning and shown to be the gene encoding cystatin B⁶². Cystatin B, a small protein that is a member of a superfamily of cysteine protease inhibitors, is thought to inactivate proteases that leak out of the lysosome. Of great interest is the observation that the majority of disease causing mutations are due to expansion of an unstable repeat in the 5' flanking region⁶³⁻⁶⁵.

Cystatin B is ubiquitously expressed. Its role in the development of Unverricht-Lundborg disease is not known. Mice lacking cystatin B develop myoclonic seizures and ataxia, associated with cerebellar granule cell loss, and cellular changes characteristic of apoptotic cell death⁶⁶. Cystatin B, therefore, may protect against cerebellar apoptosis. Caspases are cysteine proteases involved in the initiation of apoptosis. Cystatin B may block apoptosis by direct inhibition of caspase activity, or via inhibition of cathepsins, which activate caspases. Cystatin B may prevent apoptosis more indirectly via control of proteolysis.

Lafora disease

Progressive myoclonus epilepsy with polyglucosan intracellular inclusion bodies was first described in 1911 by Lafora and has become known as Lafora disease^{67,68}. It is an autosomal recessive disease characterised by the presence of periodic acid-Schiff-positive cytoplasmic inclusion bodies, known as Lafora bodies, in neurons, heart, liver and muscle. During adolescence affected individuals develop a seizure disorder which may include generalised tonic-clonic seizures, absences, drop attacks or focal occipital seizures. Soon after presentation, subjects develop asymmetric myoclonic jerks. Dementia rapidly follows accompanied by ataxia and visual loss. The EEG shows high-voltage bilateral synchronous, spike-wave and polyspike-wave complexes. Diagnosis is based on the presence of Lafora bodies in the eccrine sweat duct cells, most readily detected on axillary skin biopsy.

Linkage analysis performed in nine families with Lafora disease produced a maximum two-point lod score of 10.54 at the marker *D6S311*, localising the gene to 6q23-25⁶⁹. Homozygosity mapping in four consanguineous families revealed a region of homozygosity extending over a 17cM interval from *D6S292* and *D6S420*. Using a positional cloning approach, the gene mutated in Lafora disease, *EPM2A* was cloned⁷⁰. *EPM2A* is predicted to encode an intracellular protein tyrosine phosphatase (PTP), laforin. *EPM2A* is expressed in many tissues, including brain. PTPs act to oppose the action of tyrosine kinases in cell signalling pathways and regulate levels of phosphotyrosine in cells.

Mutation analysis of 30 families with Lafora disease detected mutations in ten families, which segregate with disease status. These are predicted to cause deleterious effects in laforin.

The neuronal ceroid lipofuscinoses

The neuronal ceroid lipofuscinoses (NCL) are a group of neurodegenerative encephalopathies characterised by psychomotor deterioration, visual failure, seizures and the accumulation of autofluorescent lipopigment in neurons and other cell types. There are five types that present as a progressive myoclonus epilepsy: classical late infantile or *CLN2* (Jansky-Bielschowsky's disease), juvenile or *CLN3* (Spielmeyer-Vogt-Sjögren's or Batten's disease), adult or *CLN4* (Kufs' or Parry disease), late infantile Finnish variant or *CLN5*, and late infantile variant or *CLN6*. Inheritance is autosomal recessive except for the adult form, which may present autosomal dominant inheritance. The phenotypic subtypes are classified on the basis of age of onset, clinical features and ultrastructural content of the storage material. The main clinical features include failure of psychomotor development, impaired vision and epilepsy. The major component of storage bodies in both late-infantile and juvenile (but not infantile) NCL is the protein subunit c of the mitochondrial ATP synthase complex.

Advances in human molecular genetic techniques have allowed positional cloning strategies to be applied to identification of the defective genes and their protein products. So far, six disease gene loci have been mapped and all of these genes have been isolated⁷¹.

Non-mendelian epilepsies

The considerable progress in identifying genes for mendelian idiopathic epilepsy has not been matched by similar progress in identifying genetic susceptibility to more common sporadic forms of the disease. Two (non-competing) hypotheses have been proposed to account for the genetic susceptibility to common genetic disorders such as epilepsy. The 'common variant common disease' hypothesis proposes that genetic variants that confer low risk for disease (Odds Ratio 1.1–1.2) are present at high frequency in a population (>1%) and thus account for a large proportion of cases. In contrast, the 'rare variant common diseases' hypothesis proposes that mutations that confer risk for disease may rarely arise in a large number of genes and so confer a high mutation to the disease class.

At the time of writing, there is no validated common variant associated with epilepsy. That is not to say that such variants are unlikely to exist, but rather that the systematic large-scale collaborative efforts required to identify common variants for epilepsy have yet to be undertaken.

In contrast, considerable incremental progress has been made in identifying rare variants for sporadic idiopathic epilepsy. Here, astute clinical connections made between rare mendelian forms of epilepsy with known genes and sporadic forms of epilepsy allowed the spectrum of candidate genes that might harbour rare variants to be narrowed to a degree that permitted the detection of rare variants. There is now good evidence for a causal

relationship between rare variants in 16 genes and sporadic epilepsy (Table II). As with the identification of genes for mendelian epilepsy, the identification of each of these is a tribute to the clinical method in epilepsy genetics.

Although the genes listed in Table II are likely to be only a fraction of the many hundreds of rare variants required to explain heritable epilepsy, they are already sufficient in number to suggest that epilepsy may emerge as a 'rare variant common disease' condition. Exciting developments in very high throughput DNA sequencing technology will soon offer the potential for whole-genome re-sequencing that will ultimately define all the rare (and common) variant contributions to epilepsy. The challenge will then be how to translate these understandings to better therapies and improved patient care.

A further recent advance along the rare variant hypothesis for epilepsy has come in the identification of rare, recurrent microdeletions or duplications in a range of epilepsies. Copy number variation is a type of genetic variability that arises when short segments of DNA are duplicated or deleted as a result of mis-alignment of homologous chromosomes at meiosis and subsequent non-allelic homologous recombination. Copy number variants (CNVs) may be small or large (the cut-off is arbitrary but large CNVs are generally considered to be 1 megabase or more in length and may result in the deletion or duplication of several contiguous genes). When a particular CNV arises repeatedly in a population it is termed 'recurrent'. For technical reasons, the focus of attention so far has been on large, rare CNVs and several important studies have demonstrated an association between this class of CNV and epilepsy, autism, schizophrenia, MR/ID and attention deficit hyperactivity disorder (ADHD)⁷²⁻⁷⁹. These findings add epilepsy to the list of neuropsychiatric disorders with susceptibility conferred by CNV. Of particular relevance is the observation that an individual recurrent CNV can contribute risk to more than one type of neuropsychiatric disorder. In Table III, for the three rare, large, recurrent CNVs robustly associated with epilepsy, are listed the other neuropsychiatric phenotypes that are also associated with each particular CNV. The fact that an individual CNV can contribute risk for several different neuropsychiatric conditions means that although it accounts for only a small proportion of the total genetic basis for each trait, it is still able to confer significant burden of neurodevelopmental disease⁸⁰. The pleiotropic relationship between a particular CNV and its neuropsychiatric phenotype has led to the suggestion that, at least for schizophrenia, autism and mental retardation, these conditions may not be individual diseases but rather overlapping phenotypes from shared neural development⁸¹.

Attempts to inform the causal mechanisms of CNVs associated with neuropsychiatric disease have focused on the search for underlying biological themes among the set of genes impacted by pathogenic copy number variation. However, as yet there is no clear understanding of precisely which CNV genes are causally involved in epilepsy. Although each CNV results in the deletion of several contiguous genes, large CNV deletions may, because of their functional interconnections, also disrupt the expression of adjacent or distant chromosomal regions and so may have remote effects on gene expression beyond simple haploinsufficiency. It has been postulated that microdeletion of the region containing the ion-channel gene *CHRNA7* may be responsible for the epilepsy phenotype associated with 15q13.3 deletion⁸², but it is unknown whether haploinsufficiency alone is enough to cause epilepsy and sequence analysis of *CHRNA7* has so far yielded no data to support the unmasking of recessive variants in this gene⁸³. Similarly, sequencing of 10 individuals with epilepsy and deletions at 16p13.11 provided no evidence for the unmasking of functional, recessive variants by this CNV⁷⁹.

Mitochondrial disorders

The mitochondrial genome (mtDNA) is a circular DNA molecule, 16,569 bp long, present in up to ten copies per mitochondrion and, therefore, in up to several hundred copies per

Table II. *De novo* arising rare variants associated with sporadic epilepsy.

Gene	Gene product	Epilepsy syndrome	Reference
SCN1A	Sodium channel α 1 subunit	Dravet Syndrome	Claes et al, 2001
<i>LGII</i>	Leucine-rich glioma inactivated	ADLTE	Bisulli et al, 2004
<i>GABRA1</i>	GABA _A receptor α 1 subunit	IGE	Maljevic et al, 2006
<i>CACNA1H</i>	T type calcium channel subunit	IGE	Heron et al, 2007
<i>CLCN2</i>	Chloride channel subunit	IGE	Kleefuss-Lie et al, 2009; Saint-Martin et al, 2009
GABRD	GABAA receptor γ 2 subunit	GEFS+	Dibbens et al, 2004
<i>KCND2</i>	Voltage-gated potassium channel	TLE	Singh B. et al, 2006
<i>SCL2A1</i>	Glucose transporter type 1 (GLUT1)	Early onset AE	Suls et al, 2009
<i>KCNJ11</i>	Pore-forming subunit of K(ATP) channel	DEND Syndrome	Gloyn et al, 2006
<i>ABCC8</i>	ATP binding cassette transporter protein	DEND Syndrome	Proks et al, 2006
<i>EFHC1</i>	Protein with EF-hand motif	IGE	Stogmann et al, 2006; Medina et al, 2008
15q13.3 microdeletion (copy number variation)		IGE	Helbig et al, 2009; Dibbens et al, 2009
<i>POLG1</i>	Mitochondrial DNA polymerase	Mixed epilepsy phenotypes	Uusimaa et al, 2008; Engelsens et al, 2008
<i>STXBP1</i>	Syntaxin binding protein	Ohtahara syndrome	Saitsu et al, 2008
<i>CDKL5</i>	Cyclin-dependent kinase-like 5	IS and Rett-like syndrome	Bahi-Buisson et al, 2008
<i>ARX</i>	Paired type homeobox	West, Ohtahara syndrome	Kato et al, 2003; Kato et al, 2007

DEND = developmental delay, epilepsy and neonatal diabetes, IS = infantile spasms

Table III. Large, rare CNVs associated with epilepsy.

Cytonband	Maximum coordinates	Epilepsy phenotypes	Other phenotypes	References
16p13.11	14.7–18.26	PE and GE	MR, ASD, ADHD	Heinzen et al, 2010; Mefford et al, 2010; de Kovel et al, 2010; Williams et al, 2010
15q11.2	19.7–21.01	Mostly GE, Some PE	SZ, MR, ASD	de Kovel et al, 2010; Heinzen et al, 2010
15q13.3	26.7–30.7	GE	SZ, MR, ASD	Dibben et al, 2009; Helbig et al, 2010; de Kovel et al, 2010

GE = generalised epilepsy, PE = partial epilepsy, MR = mental retardation, ASD = autism spectrum disorder, SZ = schizophrenia, ADHD = attention deficit hyperactivity disorder

cell. Mitochondrial DNA encodes two ribosomal RNAs, 22 transfer RNAs and 13 messenger RNAs encoding components of the inner mitochondrial membrane respiratory chain. The entire mitochondrial genotype of an individual is inherited from the mother.

Human diseases due to mutations of mtDNA include myopathies, encephalopathies, cardiomyopathies and various multi-system disorders. Two diseases with CNS involvement manifested in part as epilepsy have been described which are caused by point mutations in mitochondrial transfer RNA genes. These are so-called myoclonic epilepsy with ragged-red fibres (MERRF) and mitochondrial encephalomyopathy, lactic-acidosis and stroke-like episodes (MELAS).

MERRF is characterised by epilepsy, intention myoclonus, muscle weakness, progressive ataxia and deafness. An A to G transition mutation at nucleotide pair 8344 in the pseudouridyl loop of the tRNA lys gene was first described in three unrelated MERRF families¹⁰⁶. This mutation has now been described in most MERRF families. The patients are heteroplasmic. Both normal and mutated mtDNA populations are found. Variability of the clinical phenotype appears to depend on the amount and tissue distribution of mutant mtDNA in each individual.

An A to G transition at nucleotide 3243 was reported in 26 out of 31 unrelated Japanese patients with MELAS. This mutation affects a nucleotide position in the dihydrouridine loop of the transfer RNA for leucine. Again, heteroplasmy was present with 50.92% of mutant mtDNA present¹⁰⁷. Maternal transmission was documented in one family.

These observations confirm that seizures can be caused by deficiencies in mitochondrial energy production and raise the interesting question of whether mutations in mtDNA could contribute to the unexplained but well documented maternal influence on the transmission of epilepsy.

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