The genetic landscape of the epileptic encephalopathies of infancy and childhood

Amy McTague*, Katherine B Howell*, J Helen Cross, Manju A Kurian, Ingrid E Scheffer

Epileptic encephalopathies of infancy and childhood comprise a large, heterogeneous group of severe epilepsies characterised by several seizure types, frequent epileptiform activity on EEG, and developmental slowing or regression. The encephalopathies include many age-related electroclinical syndromes with specific seizure types and EEG features. With the molecular revolution, the number of known monogenic determinants underlying the epileptic encephalopathies has grown rapidly. De-novo dominant mutations are frequently identified; somatic mosaicism and recessive disorders are also seen. Several genes can cause one electroclinical syndrome, and, conversely, one gene might be associated with phenotypic pleiotropy. Diverse genetic causes and molecular pathways have been implicated, involving ion channels, and proteins needed for synaptic, regulatory, and developmental functions. Gene discovery provides the basis for neurobiological insights, often showing convergence of mechanistic pathways. These findings underpin the development of targeted therapies, which are essential to improve the outcome of these devastating disorders.

Introduction

Severe epilepsies of infancy and childhood are a group of devastating disorders characterised by frequent epileptic seizures associated with developmental delay or regression. These conditions encompass a large group of disorders known as epileptic encephalopathies, in which the infant or child typically has several types of seizures and abundant epileptiform activity on EEG, associated with developmental slowing or regression that might follow seizure onset or exacerbation. The onset of epileptic encephalopathies might occur against a background of normal or delayed development. Comorbidities are common, including autism spectrum disorder, and behavioural and movement disorders; the outcome is often poor.

Epileptic encephalopathies comprise many age-related epilepsy syndromes characterised by specific seizure types, and EEG and neurological features (table). Evolution from one age-related epilepsy syndrome to another might occur. For example, Ohtahara syndrome begins in the first 2 months of life, often evolving to West syndrome, and later, to Lennox-Gastaut syndrome. Not all patients can be classified as having a known epilepsy syndrome, but with rapidly evolving scientific discoveries, new disorders are emerging. As more genes causing epileptic encephalopathies are identified, specific genetic encephalopathies are being delineated with distinctive electroclinical features and comorbidities, enabling classification of disorders in patients for whom this was not previously possible. Epilepsy is most common in childhood, with an incidence of 70-1 per 100 000 children aged younger than 2 years. A prospective population-based study identified an epileptic encephalopathy in 22 (39%) of 57 infants, but the overall incidence of epileptic encephalopathies was probably underestimated because many disorders in children were not classifiable despite severe neurodevelopmental sequelae. The most common epileptic encephalopathies of infancy are West syndrome with an incidence of 25–42 per 100 000 per year, and Dravet syndrome, with an incidence of one per 22 000.

In this Review, we explore the genetic landscape of the epileptic encephalopathies by focusing on how growth in gene discovery has radically changed our understanding of this severe group of diseases. Major insights have been made into mechanisms of inheritance and biological pathways involved. We aim to untangle the relation between genotype and phenotype, and describe present and emerging genetic technologies responsible for this new era of gene discovery. We show how, for the first time in epileptic encephalopathies, the new genetic era is informing understanding of pathogenesis, which is being translated to tailored precision management to improve patient outcomes. Finally, we address remaining research questions and future directions.

The concept of an epileptic encephalopathy

The concept underpinning an epileptic encephalopathy is that the epileptic activity itself contributes to the severe cognitive and behavioural impairment, above that expected from the underlying pathology alone. West syndrome, in which infantile spasms are associated with hypsarrhythmia and developmental regression, is an archetypal epileptic encephalopathy related to continuous epileptiform activity. In epilepsy with myoclonic–atonic seizures, periods of cognitive regression might be associated with potentially treatable episodes of myoclonic status epilepticus, or in Lennox-Gastaut syndrome, with non-convulsive status epilepticus.

In other cases, whether the epileptiform activity per se accounts for developmental slowing is unclear. A good example is Dravet syndrome, in which seizures begin at 6 months classically with recurrent febrile status epilepticus. Development is normal until aged 1–2 years, despite frequent, prolonged seizures. To add to this complexity, the EEG is often normal until age 2 years, despite development plateauing before frequent epileptiform activity begins. More than 80% of patients...
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<td>Early infantile epileptic encephalopathy (Ohtahara syndrome)</td>
<td>STXBP1 in ~30%, KCNQ2 in ~20% (emerging syndrome with discrete features); SCN2A in ~10%</td>
<td>Equal; exception: ARX X-linked and mainly affects boys</td>
<td>0-3 months</td>
<td>Tonic seizures; might have seizure types including focal seizures and infantile spams; myoclonus rare</td>
<td>Intercital: burst suppression on pattern; ictal diffuse attenuation or low-voltage fast activity; focal rhythmic spiking seen with focal seizures; often evolves to hypsarrhythmia</td>
<td>Limited response to treatments; ketogenic diet</td>
<td>75% evolve to West syndrome; ongoing seizures in most</td>
<td>Early myoclonic encephalopathy</td>
<td>Severe-to-profound delay</td>
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<td>Early myoclonic encephalopathy</td>
<td>ERBB4, PIGA, SETBP1, SIK1, SLC5A22</td>
<td>Equal; rare</td>
<td>0-3 months</td>
<td>Fragmentary myoclonus; might have seizure types including tonic and focal seizures</td>
<td>Intercital: burst suppression, which is worse in sleep and persists beyond infancy; ictal myoclonias do not show EEG correlation</td>
<td>Resistant to several antiepileptic drugs</td>
<td>No evolution in most, but persistent myoclonic and focal seizures</td>
<td>Early infantile epileptic encephalopathy</td>
<td>Severe-to-profound delay</td>
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<td>Epilepsy of infancy with migrating focal seizures</td>
<td>KCNQ1 in ~50%, SCN2A in ~50%, PIGA, LGP2, SNN1A, SLC25A22, SLC35A2, SLC1A5</td>
<td>Equal;</td>
<td>0-6 months (median 7 weeks)</td>
<td>Focal seizures that migrate from one hemisphere to the other</td>
<td>Intercital: can be normal initially, becomes slow with multifocal epileptiform abnormalities; ictal: migrating ictal focus between hemispheres</td>
<td>Resistant to several antiepileptic drugs (most patients); beneficial (some patients): phenytoin, ketogenic diet, levetiracetam, rufinamide, corticosteroids, bromides</td>
<td>Infantile spasms develop later in ~7%; ongoing seizures in some; others have infrequent seizures after first year of life</td>
<td>Early-onset epileptic encephalopathy (starting before 3 months)</td>
<td>Severe-to-profound delay</td>
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<td>West syndrome</td>
<td>CDKL5 in ~10% (emerging syndrome with discrete features); STXBP1 in ~2%; ARX, ALC1, DOCK7, DM1, FXYD1 (duplications), GABRB1, GABRB2, GABRA1, GRIN1, GRIN2A, GRIN2B, MAG2, MIF2C, NEDD4, NDRG1, NPY1, PIGA, PIGB, PDEN, PDEF1, PIGC, PIGD, PIGF, PIGK, PIGK2, PIKA, PIGL, PIKN, PIGQ, SLCO2A1, SLCO2B1, SLC1A5</td>
<td>Equal; exceptions: ARX affects boys, CDKL5 affects girls more than boys, 12 000 000-6000</td>
<td>2-12 months (peak 6 months)</td>
<td>Infantile spasms</td>
<td>Intercital: hypsarrhythmia; ictal: electroencephalogram response or high-amplitude midline-slow wave with admixed fast activity</td>
<td>First-line treatment: corticosteroids (vigabatrin first-line for tuberous sclerosis); other beneficial treatments: vigabatrin, ketogenic diet</td>
<td>Might evolve to Lennox-Gastaut syndrome or develop other seizure types</td>
<td>Pre-seizures, some have a period of normal development; regression or plateau occurs in many after seizure onset; outcome: severe-to-profound delay in most</td>
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<td>Dravet syndrome</td>
<td>SCN1A in 90% (mutations in &gt;85%, copy number variants in &lt;5%–&gt;90% occur de novo, 5–10% inherited; mostly missense mutations); PCDH19, * GABRG1, GABRG2, HCN1, * STXBP1</td>
<td>Equal; exception: PCDH19 mainly affects girls (&lt;95%); 12 0000-40000</td>
<td>5-16 months (peak 5-8 months)</td>
<td>Febrile or afebrile hemidetonic or generalised tonic-clonic seizures, usually as status epilepticus</td>
<td>Normal for first 1–2 years; generalised and multifocal epileptiform abnormalities; photosensitivity common</td>
<td>Resistant to several antiepileptic drugs; beneficial: topiramate, stiripentol in combination with sodium valproate and clobazam, levetiracetam, ketogenic diet; exacerbatation: carbamazepine, lamotrigine</td>
<td>Ongoing seizures; from 1 to 5 years: focal, myoclonic, or absence seizures, with or without non-convulsive status epilepticus; from second decade: brief nocturnal convulsive seizures with or without focal dyscognitive seizures, subtle myoclonus</td>
<td>Epilepsy in females with mental retardation; epilepsy with myoclonic-atonic seizures; Lennox-Gastaut syndrome</td>
<td>Normal development in first year of life; slows between age 1 year and 2 years; mean age of walking 17 months, regression might occur with episodes of status epilepticus; outcome: mild-to-moderate delay (rare cases of normal development reported)</td>
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*Review*
Epilepsy (Continued from previous page)

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<td>Epilepsy with myoclonic–atonic seizures</td>
<td>SLC2A1 in 5%, SLC6A1 in 4%, GABRA1, GABRG2, SCN1A, SCN1B, KCNQ2</td>
<td>2:1 (boys:girls) when onset at age &gt;1 year, equal when onset at age &lt;1 year; 1:10 000</td>
<td>7 months–6 years (peak 3–4 years)</td>
<td>Several seizure types: myoclonic–tonic with or without myoclonic, absences, or tonic-clonic seizures, and episodes of non-convulsive status epilepticus</td>
<td>Intercital: hypersynchronous theta or delta slowing; generalised spike-wave or generalised polyspike-wave activity, increasing in sleep, photosensitivity in some</td>
<td>Most patients resistant to several antiepileptic drugs; beneficial: ketogenic diet (&gt;50% improve), corticosteroids</td>
<td>Remission in most within 3–5 years of onset; persistent seizures in severe cases, usually nocturnal tonic or tonic–clonic seizures</td>
<td>Benign myoclonic epilepsy of infancy, Dravet syndrome, Lennox–Gastaut syndrome, atypical benign rolandic epilepsy, late-onset epileptic spasms, other myoclonic epilepsies</td>
<td>Early development normal in most; regression often occurs with epilepsy onset; outcomes vary from normal intellect (26–62%) to severe intellectual disability</td>
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| Lennox–Gastaut syndrome | ALG13, CACN1A1, CORK, CNT2, DNM1, FLNA, GABBR1, GRIN1, HNRNAP, HNRNPH1, IQSEC1, IQSEC2, KCNQ3, MTOR, SCN1A, SCN2A, SCNBA, STXB1 | Equal; 1:200 000 | 1–8 years (peak 3–5 years), rare adult-onset cases | Several seizure types: tonic seizures with or without atypical absence, tonic, myoclonic, or generalised tonic-clonic seizures, spasms, focal seizures, episodes of tonic or non-convulsive status epilepticus | Intercital: slow background, slow (<2.5 Hz) spike-wave, generalised paroxysmal fast activity in sleep, ictal: electroencephalogram or low-voltage fast activity (tonic seizures), slow spike-wave (atypical absences), generalised spike-wave or polyspike-wave activity (myoclonic seizures) | Resistant to several antiepileptic drugs; if focal lesion, surgical resection might be curative | Seizures persist into adulthood in ~80% | Epilepsy with myoclonic–tonic seizures, Dravet syndrome, epilepsy-aphasia spectrum | Developmental delay precedes epilepsy onset in 20–60%; cognitive impairment in 90% by 5 years after seizure onset, learning difficulties in remainder |

| Epilepsy-aphasia spectrum (including Landau–Kleffner syndrome, epileptic encephalopathy with continuous spike–wave discharges in slow wave sleep, and atypical benign rolandic epilepsy) | GRIN2A in 10–20% | Unknown for whole epilepsy-aphasia spectrum; 3:2 (boys:girls) for benign epilepsy with centrotemporal spikes | 3–7 years | Landau–Kleffner syndrome: rolandic seizures in 70%, epileptic encephalopathy with continuous spike-wave discharges in slow wave sleep: rolandic seizures, atypical benign rolandic epilepsy: rolandic seizures, negative myoclonus, atonic seizures | Atypical benign rolandic epilepsy: centrotemporal spikes, often bilateral, becoming synchronised and increasing in sleep; Landau–Kleffner syndrome and CSWS: electrical status in sleep (>85% non-REM sleep); | Resistant to several anti-epileptic drugs; beneficial: steroids, benzodiazepines such as valproate, ethosuximide, levetiracetam; exacerbating: carbamazepine | Epilepsy 6 age limited, resolving by mid-teens in almost all patients | Lennox–Gastaut syndrome | Pre-seizure development normal in most; regression occurs with seizure onset in many (language, global, or motor), outcome varies from normal to severe delay |

REM=rapid eye movement. All genes are described in further detail in the appendix. *Most cases have a syndrome that can be readily distinguished from Dravet syndrome. † Lamotrigine and carbamazepine are exacerbating in the context of SCN1A-mutation-positive Dravet syndrome.

Table: Epileptic encephalopathies—electroclinical syndromes and known genetic determinants
with Dravet syndrome have mutations of the sodium channel α-1 subunit gene, SCN1A, which encodes the voltage-gated channel Na\(_{1\cdot1}\). Voltage-gated ion channel subunits such as Na\(_{1\cdot1}\) are named by the following convention: principal ion (Na); main physiological regulator (v for voltage); number of subfamily (1); and number of isoform (0-1), usually named in the order of discovery.\(^{15}\) Mutant Na\(_{1\cdot1}\) channels affect development, as shown by patients with autism spectrum disorder with SCN1A mutations.\(^{10}\) Animal models of Dravet syndrome provide initial evidence that cognitive dysfunction might be mediated by loss of Na\(_{1\cdot1}\) function in neuronal networks, independent of seizures.\(^{16,17}\) Evidence exists of seizure-independent cerebellar dysfunction manifesting as ataxia and a cognitive profile reminiscent of cerebellar cognitive affective syndrome, presumed to be due to loss of Na\(_{1\cdot1}\) function in Purkinje neurons, as observed in animal models.\(^{16,17}\) Contrary to the view that epileptic encephalopathy causes developmental slowing and regression, one study\(^ {18}\) has postulated that gradual decline relative to age-matched abilities occurs in Dravet syndrome, which later stabilises independent of seizures and EEG manifestations. Therefore, in many epileptic encephalopathies, the underlying cause often results in a mixed developmental and epileptic encephalopathy, and unravelling the contribution of the gene to each specific component is not possible at present.

**Aetiology**

Until 2001, the cause of epileptic encephalopathies was unknown, and they were thought to probably be due to a so-called symptomatic cause such as an acquired insult. A minority of cases undoubtedly have symptomatic causes in which a child has a structural aetiology such as a stroke or hypoxic-ischaemic encephalopathy underlying their epileptic encephalopathy. An exception is West syndrome, in which almost 30% of patients have an acquired aetiology.\(^ {19}\) The structural abnormality is associated with an epileptiform focus, leading to epilepsy and developmental regression. Similarly, malformations of cortical development can be associated with an epileptic encephalopathy, as exemplified by tuberous sclerosis complex. In these cases, the underlying cause of the malformation should still be sought and is often genetic,\(^ {20,21}\) although environmental causes are well recognised.\(^ {22}\)

A genetic cause has been identified in many different epileptic encephalopathies, with many previously unknown genes emerging.\(^ {23,24}\) The genetic causes of epileptic encephalopathies are heterogeneous; de-novo mutations in the affected individual are most commonly reported; rare cases are due to chromosomal anomalies (eg, trisomy 21) and inborn errors of metabolism (eg, phenylketonuria). Therefore, epileptic encephalopathies can be due to structural, metabolic, and chromosomal defects. The genetic basis of these disorders, with the exception of glucose transporter 1 deficiency syndrome is beyond the scope of this Review.

**Gene discovery and mechanisms of inheritance**

A genetic cause for an epileptic encephalopathy was first recognised in 2001, with the finding that all seven children in a study of Dravet syndrome had a de-novo SCN1A mutation.\(^ {10}\) With the advent of molecular techniques, such as chromosomal microarray and next-generation parallel sequencing of multiple genes, a rapid growth in gene discovery for epileptic encephalopathies has occurred.\(^ {23,24,27,29}\)

Copy number variation is an important molecular cause of epileptic encephalopathy, with up to 8% of cases showing a causative or potentially contributing copy number variant (CNV).\(^ {30}\) CNVs in the form of microdeletions and microduplications exist in every human molecular karyotype, and many represent normal human variation.\(^ {31}\) When a CNV is identified, its pathogenicity needs to be ascertained according to several factors: presence in the general population; whether it contains many genes or a gene with a crucial function; size (a deletion of more than 1 Mb is more likely to be pathogenic); and segregation (a CNV in an unaffected parent is less likely to be causative). Chromosomal microarray studies looking for pathogenic CNVs are now a standard early investigation for all patients with epileptic encephalopathy. Chromosomal microarrays are also important for novel gene discovery; a deletion or duplication in one patient might implicate a candidate gene, which can then be sequenced in patients with similar phenotypes, as shown by the discovery of STXBP1, among many others.\(^ {32}\)

Next-generation sequencing involves shearing of genomic DNA, hybridisation to a library of probes designed to capture either all coding (exonic) regions or a limited panel of genes, massively parallel sequencing of patient DNA fragments, and alignment to the reference genome. Increased efficiency and reduced cost of this technology have enabled different experimental designs to discover new epileptic encephalopathy genes. For example, whole-exome sequencing (WES) refers to sequencing of all protein-coding exons, which comprise 1–0–1–5% of the human genome. By applying WES with a patient–parent trio design, a mean of 1–68 de-novo mutations is reported in the patient, often enabling rapid identification of the patient’s causative gene.\(^ {33}\) WES is not without limitations; coverage of coding regions is usually not 100%, and detection of insertions, deletions, and duplications is suboptimum, albeit improving. An alternative approach is use of panels in which a targeted set of known or candidate genes enables denser coverage than is possible with WES.\(^ {33}\) This approach enables identification of variants of interest, and segregation can be studied in the parents of affected individuals. In a study of 400 patients with early-onset epilepsy tested with a gene panel, 71 (18%) of 400 had a causative mutation identified with a diagnostic rate of 39% in those with seizure onset within the first 2 months (Scott R, personal communication).
Therefore, a genetic cause can be identified in a substantial proportion of patients with epileptic encephalopathy. Most have de-novo dominant mutations,\textsuperscript{25,36,37,35,36} which probably occur in the gamete or early in embryogenesis.\textsuperscript{8} Most mutations are exonic, but interest is increasing in interrogating the regulatory and non-coding regions of DNA, including microRNAs, for mutations.\textsuperscript{8,37}

X-linked inheritance might occur with recessive (eg, ARX), dominant (eg, DCX),\textsuperscript{22} and male-sparing patterns (eg, PCDH19).\textsuperscript{8} Autosomal recessive inheritance deserves special consideration, with homozygous mutations more likely in consanguineous unions. Compound heterozygote mutations are more frequent in outbred couples, in which the child inherits different mutations of the same gene from each parent. Mitochondrial disorders can follow maternal or autosomal recessive inheritance.\textsuperscript{8}

So far, the monogenic causes, in which a gene of major effect is mutated, have provided easy targets for gene discovery. Some patients are likely to have epileptic encephalopathy with a polygenic basis, owing to interaction of several genetic variants of mild-to-moderate, or even severe, risk.\textsuperscript{8,40} Insights into these disorders are scarce at present, but an example of a disorder with polygenic inheritance is Dravet syndrome, in which 10% of patients have an inherited SCN1A mutation.\textsuperscript{41} Relatives of these patients who have the same SCN1A mutation have milder forms of epilepsy consistent with the phenotypic spectrum of genetic epilepsy with febrile seizures plus or might even be unaffected, suggesting that additional genes might contribute to the severe phenotype of Dravet syndrome in the proband.\textsuperscript{41}

A different, but fascinating, molecular clue to the disparate seizure phenotypes within families is explained by mosaicism, which is emerging as crucial in the genetics of epilepsies and other disorders.\textsuperscript{8} Mosaicism refers to two populations of cells in a human being, one with the gene mutation and the other with the normal allele.\textsuperscript{8} A parent might have gonadal mosaicism, in which a mutation is confined to the egg or sperm. Gonadal mosaicism becomes evident when parents produce two affected offspring with the same mutation, despite the mutation being undetectable in parental leucocyte DNA, as reported in Dravet syndrome.\textsuperscript{41}

Somatic mosaicism occurs in embryogenesis, resulting in mutations confined to a proportion of all cell types, or to specific regions of the body or tissue lineages.\textsuperscript{8,38} Somatic mosaicism is emerging as an important mechanism for brain disorders. For example, brain malformations such as hemimegencephaly show mosaicism with mutations in different genes relevant to the mammalian target of rapamycin (MTOR) pathway only in the malformation but not in peripheral DNA.\textsuperscript{24} In other cases, deep sequencing (high-coverage targeted sequencing) of leucocyte DNA has identified somatic mutations in patients with cortical malformations.\textsuperscript{24} Somatic mosaicism is likely to have a role in epileptic encephalopathies even in the setting of normal imaging, with mutations potentially limited to the CNS or even to just one cell lineage or region of the brain, but discovery is hindered by the scarcity of available brain tissue, because many children are not candidates for epilepsy surgery.\textsuperscript{20,42}

Different tissues might show differing levels of mosaicism such that low levels of an abnormal allele can be challenging to detect and might be missed on routine analysis. This difficulty was elegantly shown in a study of Dravet syndrome, in which an inherited SCN1A mutation was present in 12 cases in parental blood DNA, with mosaicism in 0–85·00% of cells.\textsuperscript{41} The higher the level of mosaicism, the more severely affected the parent, showing a clear relation between mutation load and affected status.

**Phenotypic heterogeneity**

A crucial issue underpinning gene discovery in epileptic encephalopathies is that each gene shows phenotypic pleiotropy, and that each epilepsy syndrome shows genetic heterogeneity (figure 1). This heterogeneity or pleiotropy means that clinical phenotyping is central to interpretation of the relevance of a genetic finding in a patient to understand pathogenesis, guide therapy, and improve outcomes.

Phenotypic heterogeneity or pleiotropy, in which mutations in a single gene cause different phenotypes, is increasingly recognised in epilepsy and across many neurological disorders (appendix). Many factors contribute to phenotypic heterogeneity, including the following: type and timing of mutations during development; timing and location of physiological gene expression; epigenetic factors; and modifier genes. Examples of postulated mechanisms for phenotypic heterogeneity in epileptic encephalopathy genes are provided in the appendix.\textsuperscript{8–12}

The epilepsy syndromes associated with a gene might range from a benign seizure disorder to an epileptic encephalopathy, exemplified by several of the ion channel genes (eg, KCNQ2, SCN1A, SCN2A). For example, KCNQ2 mutations cause the self-limited syndrome benign familial neonatal epilepsy, in which a neonate develops seizures on day 2–3 of life and, after a flurry of seizures, has an excellent outcome with only a small proportion of patients having later epilepsy.\textsuperscript{51} This disorder results from autosomal dominant inheritance with high penetrance. 80% of patients with this familial epilepsy have a mutation in KCNQ2, which encodes the voltage-gated potassium channel K\textsubscript{\textit{v}}7.2.\textsuperscript{27} In-vitro studies implicate haplo-insufficiency as a likely disease mechanism.\textsuperscript{52} KCNQ2 mutations are also associated with a severe neonatal onset epileptic encephalopathy characterised by tonic seizures and profound developmental impairment, sometimes presenting as Ohtahara syndrome.\textsuperscript{52–58} These patients often have de-novo KCNQ2 mutations, which might be dominant
negative, with far more profound overall negative effect on channel function than those that cause benign familial neonatal epilepsy. In the case of SCN1A, mutations are identified in most patients with Dravet syndrome, with half having missense and half having truncation mutations; functional studies show loss of function. Missense mutations are also reported in genetic epilepsy with febrile seizures plus, a mild self-limited epilepsy that often does not need treatment. Thus, the phenotypic picture is crucial for interpretation of the importance of a SCN1A missense mutation, and frames the therapeutic approach.

Not only might the severity of the epilepsy syndrome differ with mutations in a specific gene, but the nature of the syndrome might be surprisingly different. One of the most severe epileptic encephalopathies, epilepsy of infancy with migrating focal seizures, is associated with de-novo mutations in the sodium-activated potassium channel gene KCNT1 in 50% of cases, leading to a three-times gain in channel function. Simultaneously, inherited and de-novo KCNT1 mutations were identified in severe autosomal dominant nocturnal frontal lobe epilepsy, in which childhood onset of nocturnal frontal lobe seizures might be associated with intellectual disabilities and psychiatric disorders. Additionally, rare cases of Ohtahara syndrome and West syndrome have KCNT1 mutations. Although autosomal dominant nocturnal frontal lobe epilepsy and epilepsy of infancy with migrating focal seizures share focal seizures and a genetic aetiology, their electroclinical pattern is quite distinct.

The phenotypic heterogeneity of epileptic encephalopathy genes might extend to both lesional and non-lesional disorders and depend on the nature and location of the mutation. For example, non-lesional X-linked infantile spasms are associated with an expansion of the polyalanine tract of ARX, which encodes a transcriptional regulator, whereas mutations affecting the DNA-binding domain result in lissencephaly. Genetic heterogeneity occurs in every epilepsy syndrome. Even in the prototypical genetic epileptic

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**Figure 1: Genetic causes of epilepsy syndromes**

Genetic causes, and proportion of cases caused by each gene, including only non-chromosomal, non-malformative, and non-metabolic disorders. Only genes with more than one case reported are included. Black font denotes genes that account for at least 50% of cases, purple font 10–50% of cases, and red font 5–10% of cases. Blue font denotes genes that account for less than 5% of cases, and green font denotes genes that account for an unknown percentage of cases.
encephalopathy, Dravet syndrome, in which more than 80% of patients have a SCN1A mutation, other genes (eg, STXBP1 and GABRA1) account for a small proportion of cases.\textsuperscript{27} Often a few cases of a novel genetic encephalopathy are initially recognised, and further studies are needed to confirm the role of the newly identified gene as causative. Analysis of larger numbers of genetically homogeneous cases could demonstrate clinical features that distinguish the phenotype. For example, epilepsy with myoclonic-atonic seizures, described by Doose,\textsuperscript{22} is associated with mutations in CHD2 or SLC2A1 in a small proportion (4%) of cases; CHD2 is associated with clinical photosensitivity and SLC2A1 is associated with paroxysmal exercise-induced dyskinesia.\textsuperscript{23,24} Each genetic entity might show subtly different phenotypic features that help diagnosis and, in turn, might have treatment implications, such as the ketogenic diet in glucose transporter 1 deficiency due to mutations in SLC2A1.

Many genes have been identified for classic epileptic encephalopathies, such as infantile spasms,\textsuperscript{27,28} Lennox-Gastaut syndrome,\textsuperscript{27,29} and Ohtahara syndrome.\textsuperscript{30,31,32,33,34,35} with most genes associated with only a small number of cases (table). Knowledge of genetic causes is likely to further expand, as WES and multigene panels are applied to epileptic encephalopathies and identification of causative mutations becomes more straightforward from a bioinformatic perspective.

A substantial number of patients with epileptic encephalopathies do not have phenotypes that fit into specific epilepsy syndromes. Perhaps the most complex group are those with infantile onset of several seizure types and frequent multifocal epileptiform activity (and generalised activity in some), with poor developmental progress. Within this heterogeneous group, specific genetic epileptic encephalopathies are emerging through careful phenotyping of cohorts with mutations of the same gene; these findings will enable recognition of the phenotype in the future.\textsuperscript{36,37} Although mutations in several genes might result in the same epileptic encephalopathy, dysfunction of the various genes has been suggested to lead to disruption of common pathways or mechanisms at a specific age that converge to produce a given phenotype.\textsuperscript{38}

Challenges and pitfalls

Although valid biological explanations exist for much of the genetic heterogeneity and phenotypic pleiotropy, there is a risk that a variant claimed to be pathogenic is benign and not causative. As 22 000 single-nucleotide variants are identified on WES, and 5 million variants on whole-genome sequencing (WGS) of an individual, whether a variant is causative and of major effect should always be questioned. The gold standard would be for all newly identified variants, even in known genes, to undergo functional assessment in a model system. Understanding whether mutations cause loss or gain of function is essential for design of targeted therapies. For example, KCNT1 mutations in autosomal dominant nocturnal frontal lobe epilepsy show variable penetrance, counter to initial observations, which renders exclusion of variants in healthy transmitting parents of patients questionable.\textsuperscript{39} Another example would be a gene panel result with two novel de-novo variants in known epileptic encephalopathy genes. Genetic counselling without strong evidence for pathogenicity in these situations will be challenging and an increasingly common scenario as WGS is embraced.

Insights into the neurobiology of severe epilepsies

Gene identification has implicated a broad range of disease mechanisms in severe epilepsies, including channelopathies, synaptic dysfunction, transporter defects, transcriptional dysregulation, impaired DNA repair and chromatin remodelling, and metabolic defects (figure 2). In many cases, the mechanisms by which gene mutations produce severe epilepsies are poorly understood. However, bioinformatic approaches (eg, computer-generated network maps of interacting genes) and in-vitro or in-vivo models are being used to identify links between apparently disparate disease mechanisms, with convergence of disease pathways.\textsuperscript{40,41}

Patterns of spatial expression of genes and evidence from animal models suggest that dysfunction in some cell types, brain regions, or molecular networks are important in epileptic encephalopathies. On statistical analysis of 356 trios, a substantial enrichment of de-novo mutations in genes involved in regulation of synaptic transmission was reported.\textsuperscript{42} Abnormalities of interneuron development, migration, or function are associated with genes such as ARX and SCN1A.\textsuperscript{43,44} Results of bioinformatics studies also suggest a role for abnormalities in specific molecular networks, including the large protein network associated with the fragile X mental retardation-related protein, which links neuronal firing to activity-driven protein synthesis.\textsuperscript{45,46}

Furthermore, temporal expression of genes often predicts the timing of epilepsy onset. KCNQ2-related, SCN1A-related, SCN2A-related, and GRIN2A-related epilepsies typically present at around the age at which their expression is needed for normal physiological neuronal development.\textsuperscript{46,47,48,49,50,51,52}

However, increasing evidence shows that genetic causes of severe epilepsy also affect neuronal function through mechanisms distinct from the seizure disorder, including aberrant neuronal migration and formation of abnormal neuronal networks.\textsuperscript{52} Such mechanisms contribute to cognitive impairment, and are unlikely to be rescued with conventional antiepileptic therapies.\textsuperscript{53} Despite substantial advances in gene discovery, our understanding of the mechanisms of these disorders is still in its infancy, with many neurobiological complexities yet to be elucidated.
Translation to improving patient care
Effect of a diagnosis
The importance of making a definitive diagnosis in a patient cannot be overemphasised. Diagnosis changes people’s lives. Once a cause is established, the fraught and often lengthy, painful, and time-consuming diagnostic journey ends. Most patients will have had many investigations, including brain imaging and neurophysiological, blood, CSF, and urine testing, and sometimes more invasive tests such as liver and muscle biopsies. Some might even have faced epilepsy surgery with variable benefits and later received a genetic diagnosis, which, had it been known earlier, might have affected the decision to proceed to surgery. Finding a cause can save the family from further anguish and the child from ongoing investigations.\(^6\) Often parents have been convinced that they were at fault for their child’s illness (eg, because of alcohol intake, illness, or a car accident in pregnancy), and finding a cause finally lays their fears to rest.

With a diagnosis, the family can move on to learning about the disease, its comorbidities, and prognostic implications. For example, results of studies of older adults (aged 20–66 years) with Dravet syndrome have shown late onset of dysphagia necessitating a percutaneous endoscopic gastrostomy; incontinence; and ongoing cognitive and motor decline.\(^6\) Diagnosis enables planning for their child’s long-term care and early access to services and therapies.\(^3\)

Appreciation that a diagnosis often informs management, even in the absence of curative or disease-specific therapy, is crucial. For example, in Dravet syndrome, specific antiepileptic drugs, such as carbamazepine and vigabatrin, exacerbate seizures, whereas others are beneficial, such as topiramate and stiripentol, often administered with clobazam or valproate (table).\(^6\) Optimisation of antiepileptic drugs improves seizure control, enabling improved cognitive function, seen even late in adult life.\(^2,6\) This improvement suggests some reversibility of the Dravet phenotype.

For families of patients with epileptic encephalopathy, genetic counselling is essential. Parents of babies with epileptic encephalopathy will often be contemplating further children, and the finding of a causative mutation informs precise genetic counselling. With knowledge of the specific mutation, further pregnancies can be tested in utero or prenatally with in-vitro fertilisation and preimplantation diagnosis after reproductive counselling. This knowledge can substantially reduce their risk of having another affected child, and is highly relevant to siblings.

An invaluable benefit that comes with diagnosis of a disease is formation of disease-specific support groups. These groups, mostly online, offer enormous psychosocial support to families in understanding the disease and its effects on daily life. These groups use worldwide social media forums and enable families to promote awareness of the disorder; develop database registries of phenotypes and genotypes to assist in understanding responses to therapies, comorbidities, and prognosis; work collaboratively with clinicians and scientists; and raise funds for focused research on their disease with tangible and rapid outcomes.\(^6\) These support networks are an exciting platform that accelerates development of targeted novel therapies for families desperate to improve the outcome for their loved one.

![Figure 2: Disease mechanisms in childhood epileptic encephalopathies](image)
Neuron, axon, presynaptic, and post-synaptic compartments. Many areas of abnormal neuronal function, including DNA repair, transcriptional regulation, axon myelination, metabolite and ion transport, and peroxisomal function, in addition to channelopathies and synaptic dysfunction, are implicated in childhood epileptic encephalopathies.
Future genetic diagnostic approaches

Despite increasing recognition that many epileptic encephalopathies have a genetic basis, genetic testing is not widely available. Access to testing is dependent on economic and scientific resources and varies around the world. Where testing is readily available, between 10% and 50% of cases can be diagnosed with current molecular techniques.31,32,35

In the future, genetic testing done early in the patient’s disease course will be an invaluable tool for neurologists, who, after careful phenotypic assessment, will need to understand the implications of the molecular findings. With decreasing costs of next-generation sequencing, each patient will undergo WES, which yields about 22 000 variants.31 To simplify the analysis, examination of variants in known epileptic encephalopathy genes might often be done first. This type of screening is already commercially available in the guise of so-called epilepsy gene panels, in which only the genes of interest (which might number in the hundreds) are interrogated for pathogenic variants. The remaining data are not assessed bioinformatically, but can be reanalysed as more genes are discovered, depending on the laboratory.

Clinical WES offers an unbiased opportunity to identify novel genes and expand the phenotypes of known genes. However, often the implications of a variant might be difficult to establish. Support for pathogenicity can be drawn from similar patients with mutations of the same or a related gene or from experimental data showing impaired function, meaning that interpretation might be possible only in a research setting. Collaboration between physicians with access to clinical WES, research laboratories, and families is increasing to move these findings rapidly to clinical practice by contributing results to international databases to enable research collaboration and new discoveries.30 An example is the Epilepsy Genetics Initiative.

With time, pipelines for functional validation of mutation pathogenicity in both newly identified and known genes will be integrated into the diagnostic process. High-throughput laboratory screening of variants identified in patients will inform understanding of the functional effect and provide a platform to study the effect of specific drugs on the function of the mutant protein. Knowledge of the functional effect of a mutation in a known or novel gene might be used to predict the clinical phenotype (appendix), with prognostic and treatment implications for patients, and with potential for advancing understanding of disease mechanisms, which might inform novel treatments.

The next major leap forward will be WGS, which offers the opportunity to look beyond the exome at the remaining 99% of the human genome. WGS enables examination of non-coding DNA, regulatory regions, and detailed examination of the ends of the exons and splice sites. WGS remains in the research domain because each individual has in the order of 5 000 000 variants, creating huge bioinformatic demands, and knowledge of the extent of normal human variation in these regions is poor.35 Deep sequencing,35 in which DNA from a tissue is sequenced up to several thousand times, and single-cell sequencing, in which the exome or genome of a single cell is sequenced, are likely to provide further insights into somatic mosaicism at a tissue and cellular level.95

Analysis needs to progress to the next level to understand how a gene, or genes, of major effect interact with modifying genes in an individual to result in an epileptic encephalopathy manifesting with seizures, cognitive regression, and behavioural problems. Use of patient-derived stem cells in which mutated genes are studied in their native context and compared with healthy parental controls will be one approach.42 Other approaches will involve analysis of animal models of many types, in which background genetic and environmental factors can be selectively modified to interrogate their interaction.

Irrespective of the techniques used for genetic diagnosis, investigation of patients as early as possible is crucial. Prompt diagnosis enables early implementation of optimum therapy, hopefully resulting in improved outcomes.

Future treatment approaches

Disease-specific treatments are available for only a minority of severe epilepsies with genetic causes, such as the ketogenic diet in patients with glucose transporter 1 deficiency due to SLC2A1 mutations.95 For the remaining patients, treatment options include the usual range of antiepileptic drugs, which do not address the underlying biological mechanism. Exciting discoveries suggest that directed therapies targeting the gene defect, the abnormal protein, or the dysfunctional pathway are not far away. The aim of targeted therapies is to improve not only seizure control, but also developmental outcome and associated comorbidities, by directly addressing the mechanisms that produce the widespread effects of the disorder, which might be more extensive than those attributed to the epileptic process alone.

Several potential approaches are likely. The most straightforward approach is use of pharmacological agents that directly target the abnormal protein or disrupted pathways. For example, retigabine opens Kv7 potassium channels consisting of KCNQ2 and KCNQ3 subunits, and restores normal channel function of KCNQ2 encephalopathy mutations in vitro.49,50 The effect in patients with KCNQ2 encephalopathy is unknown; reports of blue eye and skin discoloration in patients taking retigabine have hindered clinical trials.7 Similarly, NMDA receptor antagonists such as memantine have been suggested for GRIN2A-related or GRIN2B-related diseases.75,96 Functional experiments might shed light on compounds affecting mutant proteins. For example, an old drug, quinidine, reverses the gain of function caused by the mutant potassium channel KCNT1, suggesting that it could be used in patients with epilepsy of infancy with
migrating focal seizures and severe autosomal dominant nocturnal frontal lobe epilepsy.50,62,63,64,101

Nanotherapies function at an atomic, molecular, or macromolecular level. Development of a protein–protein interaction inhibitor that disrupts the specific α-helical interaction between syntaxin-1A molecules, and thus mimics the action of STXBP1, has been proposed.102,103 α-helical protein–protein interaction inhibitors are under development in cancer and other specialties,104 and might prove to be relevant to genetic epileptic encephalopathies.

The emergence of stem cell technology is enabling understanding of the effects of mutations in neurons and is providing a cell-specific means for testing new drugs. Successful induction of neurons derived from fibroblasts of patients with Dravet syndrome and an SCN1A mutation shows that both excitatory and inhibitory neurons have hyperexcitability.105 Stem cell programmes provide an excellent platform to trial novel compounds and are a conduit to animal studies, patient trials, and wider implementation of new therapeutic approaches.

Gene therapy, such as modification of transcription of a mutant gene, or delivery or expression of a wild-type gene, is leading to the promise of therapeutic benefit in other neurological disorders. In Duchenne muscular dystrophy, exon skipping restores levels of dystrophin to those seen in the milder Becker muscular dystrophy with concomitant improvements in daily function.106 In the context of epilepsy, aminoglycoside-induced readthrough of a premature truncation codon of a GABRG2 mutation partly rescued the cellular phenotype.107 In view of the range of phenotypes associated with many of the known epileptic encephalopathy genes (eg, SCN1A, SCN2A, KCNQ2, and KCNT1) and the frequent finding of truncation mutations, similar strategies might be beneficial, especially if commenced early in a child’s disease course.

Gene therapy holds much promise for treatment-resistant epilepsies.108,109 However, many obstacles remain: delivery of large molecules and transcripts across the blood–brain barrier and into cells is challenging. Once a gene is introduced, the effect is permanent and might interfere with normal temporal gene expression patterns. These issues of permanency and potential off-target effects might be mitigated by approaches such as optogenetics—in which light-sensitive proteins can be used to activate channels and lasers can be used to induce excitation or inhibition—or locally delivered gene therapy that is targeted to a specific region or population of neurons.110 Allele-specific RNA interference is another targeted gene therapy, shown in long QT syndrome caused by heterozygous mutations of KCNH2, to specifically target the mutated rather than the wild-type allele.111 This approach would be ideal for disorders such as KCNQ2 encephalopathy, in which the mode of pathogenesis is a dominant-negative one.112

Conclusions

The complex genetic landscape of epileptic encephalopathies is emerging with the exciting revelations of the genomic revolution. Several aspects are clear. De-novo mutations are frequently found, especially in genes that encode proteins involved in synaptic function and ion channels. Mosaicism, both somatic and germline, is of increasing importance in understanding pathogenesis, especially in patients for whom exome and panel sequencing is negative. Development of functional pipelines to interpret findings of clinical exome and genome sequencing is essential and will need substantial investment.

However, much of the landscape remains unclear, and needs further investigation. First, the genetic basis of the less well defined syndromes is still unclear but might be uncovered by deeper sequencing and WGS. The importance of regulatory components of the genome, including microRNAs will be, in part, revealed by WGS. Mechanisms of epigenetic regulation including the methylome should also be investigated and will probably contribute to the phenotypic pleiotropy. Moving from identification and interrogation of single genes, to identification of the unique variant profile of an individual and building a bespoke model of the network dysfunction in that patient is key. This step will need development of sophisticated in-silico models in addition to insights from patient-derived stem cells. Identification of further genes, epistatic genes, and novel pathways will lend itself to development of new therapies. Because of wider brain dysfunction and early onset, effective therapy might need neonatal or even prenatal administration to reduce the effects of these devastating disorders.

Contributors

AM and KBH did the literature search, reviewed articles, and wrote the manuscript. AM designed figure 2, and KBH designed figure 1. JHC and MAK wrote the manuscript. IES conceived the review, reviewed articles and figures, and wrote the manuscript.

Declaration of Interests

We declare no competing interests.

Search strategy and selection criteria

References were identified by searching PubMed for articles published from Jan 1, 1969, to Sept 15, 2015, and for references from relevant articles. The search terms “epileptic encephalopathy”, “early infantile epileptic encephalopathy”, “early onset epileptic encephalopathy”, “Ohtahara syndrome”, “early myoclonic epileptic encephalopathy”, “migrating partial seizures of infancy”, “epilepsy of infancy with migrating focal seizures”, “Dravet syndrome”, “severe myoclonic epilepsy of infancy”, “West syndrome”, “infantile spasms”, “myoclonic atatic epilepsy”, “Doose syndrome”, “epilepsy with myoclonic absences”, “Lennox-Gastaut syndrome”, “epilepsy-aphasia spectrum”, “Landau-Kleffner syndrome”, “continuous spike wave in slow wave sleep”, and “atypical benign rolandic epilepsy” were used. The final reference list was generated on the basis of relevance to the topic of this Review.

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