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*

Lancet Neurol 2016; 15: 304–16

Published Online November 16, 2015 http://dx.doi.org/10.1016/S1474-4422(15)00250-1

*Authors contributed equally

Molecular Neurosciences (A McTague MBChB, M A Kurian PhD) **and Clinical Neurosciences** (Prof J H Cross PhD), **Developmental Neurosciences Programme, UCL Institute of Child Health, London, UK; Department of Neurology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK** (A McTague, Prof J H Cross, M A Kurian); **Department of Neurology, Royal Children's Hospital, Melbourne, VIC, Australia** (K B Howell MBBS, Prof I E Scheffer PhD); **Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia** (K B Howell, Prof I E Scheffer); **Neurosciences Group, Murdoch Childrens Research Institute, Melbourne, VIC, Australia** (K B Howell); **Department of Medicine, Epilepsy Research Centre, University of Melbourne, Austin Health, Melbourne, VIC, Australia** (Prof I E Scheffer); **and Florey Institute of Neurosciences and Mental Health, Melbourne, VIC, Australia** (Prof I E Scheffer)

Correspondence to: Prof Ingrid E Scheffer, University of Melbourne, Austin Health, Melbourne, VIC, Australia **scheffer@unimelb.edu.au**

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.[1,2] 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.[3,4]

Epilepsy is most common in childhood,[5] with an incidence of 70·1 per 100 000 children aged younger than 2 years.[6] 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.[6] The most common epileptic encephalopathies of infancy are West syndrome with an incidence of 25–42 per 100 000 per year,[7] and Dravet syndrome, with an incidence of one per 22 000.[8,9]

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.[10] 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.[11,12]

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

	Genes (and approximate proportion of syndromic cases where known)	Sex affected and incidence	Age at onset	Seizures at onset	EEG	Treatment	Epilepsy evolution and outcome	Syndrome differential diagnosis	Development
Early infantile epileptic encephalopathy (Ohtahara syndrome)	*STXBP1* in ~30%; *KCNQ2* in ~20% (emerging syndrome with discrete features); *SCN2A* in ~10%; *AARS*, *ARX*, *BRAT1*, *CACNA2D2*, *GNAO1*, *KCNT1*, *NECAP1*, *PIGA*, *PIGQ*, *SCN8A*, *SIK1*, *SLC25A22*	Equal; exception: *ARX* is X-linked and mainly affects boys; rare	0–3 months	Tonic seizures; might have seizure types including focal seizures and infantile spasms; myoclonus rare	Interictal: burst-suppression pattern; ictal: diffuse attenuation or low-voltage fast activity; focal rhythmic spiking seen with focal seizures; often evolves to hypsarrhythmia	Limited response to treatments; ketogenic diet	75% evolve to West syndrome ; ongoing seizures in most	Early myoclonic encephalopathy	Severe-to-profound delay
Early myoclonic encephalopathy	*ERBB4*, *PIGA*, *SETBP1*, *SIK1*, *SLC25A22*	Equal; rare	0–3 months	Fragmentary myoclonus; might have seizure types including tonic and focal seizures	Interictal: burst suppression, which is worse in sleep and persists beyond infancy; ictal: myoclonias do not show EEG correlation	Resistant to several antiepileptic drugs	No evolution in most, but persistent myoclonic and focal seizures	Early infantile epileptic encephalopathy	Severe-to-profound delay
Epilepsy of infancy with migrating focal seizures	*KCNT1* in ~50%; *SCN2A* in ~25%; *PLCB1*, *QARS*, *SCN1A*, *SCN8A*, *SLC25A22*, *TBC1D24*, *SLC12A5*	Equal; 1:200 000–400 000	0–6 months (median 7 weeks)	Focal seizures that migrate from one hemisphere to the other	Interictal: can be normal initially, becomes slow with multifocal epileptiform abnormalities; ictal: migrating ictal focus between hemispheres	Resistant to several antiepileptic drugs (most patients); beneficial (some patients): phenytoin, ketogenic diet, levetiracetam, rufinamide, corticosteroids, bromides	Infantile spasms develop later in ~7%; ongoing seizures in some, others have infrequent seizures after first year of life	Early-onset epileptic encephalopathy (starting before 3 months)	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
West syndrome	*CDKL5* in ~10% (emerging syndrome with discrete features); *STXBP1* in ~2%; *ARX*, *ALG13*, *DOCK7*, *DNM1*, *FOXG1* (duplications), *GABRB1*, *GABRB3*, *GNAO1*, *GRIN1*, *GRIN2A*, *GRIN2B*, *MAGI2*, *MEF2C*, *NEDDL4*, *NDP*, *NRXN1*, *PIGA*, *PLCB1*, *PTEN*, *SCA2*, *SCN1A*, *SETBP1*, *SIK1*, *SLC25A22*, *SLC35A2*, *SPTAN1*, *ST3Gal3*, *TBC1D24*, *TCF4*, *WWOX*	Equal; exceptions: *ARX* affects boys, *CDKL5* affects girls more than boys; 1:2 000–6000	2–12 months (peak 6 months)	Infantile spasms	Interictal: hypsarrhythmia; ictal: electrodecremental response or high-amplitude midline slow wave with admixed fast activity	First-line treatment: corticosteroids (vigabatrin first line for tuberous sclerosis); other beneficial treatments: vigabatrin, ketogenic diet	Might evolve to Lennox-Gastaut syndrome or develop other seizure types		Pre-seizure development can be normal or abnormal; most plateau or regression occurs with spasm onset; outcome varies with aetiology; 80% with confirmed aetiology have developmental delay
Dravet syndrome	*SCN1A* in 90% (mutations in >85%, copy number variants in <5%; >90% occur de novo, 5–10% inherited [mostly missense mutations]); *PCDH19*,* *GABRA1*, *GABRG2*, *HCN1*,* *STXBP1*	Equal; exception: *PCDH19* mainly affects girls (>99%); 1:20 000–40 000	5–16 months (peak 5–8 months)	Febrile or afebrile hemiclonic or generalised tonic–clonic seizures, usually as status epilepticus	Normal for first 1–2 years; generalised and multifocal epileptiform abnormalities later; photosensitivity common	Resistant to several antiepileptic drugs; beneficial: topiramate, stiripentol in combination with sodium valproate and clobazam, levetiracetam, ketogenic diet; exacerbating: carbamazepine, lamotrigine†	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	Epilepsy in females with mental retardation, epilepsy with myoclonic–atonic seizures, Lennox-Gastaut syndrome	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-severe delay (rare cases of normal development reported)

(Table continues on next page)

	Genes (and approximate proportion of syndromic cases where known)	Sex affected and incidence	Age at onset	Seizures at onset	EEG	Treatment	Epilepsy evolution and outcome	Syndrome differential diagnosis	Development
(Continued from previous page)									
Epilepsy with myoclonic–atonic seizures	*SLC2A1* in 5%; *SLC6A1* in 4%; *CHD2*, *GABRA1*, *GABRG2*, *SCN1A*, *SCN1B*, *KCNA2*	2:1 (boys:girls) when onset at age >1 year; equal when onset at age <1 year; 1:10 000	7 months–6 years (peak 3–4 years)	Several seizure types: myoclonic–atonic with or without myoclonic, absence, or tonic–clonic seizures, and episodes of non-convulsive status epilepticus	Interictal: hypersynchronous theta or delta slowing; generalised spike-wave or generalised polyspike-wave activity, increasing in sleep; photosensitivity in some	Most patients resistant to several antiepileptic drugs; beneficial: ketogenic diet (>50% improve), corticosteroids	Remission in most within 3·5 years of onset; persistent seizures in more severe cases, usually as nocturnal tonic or tonic vibratory seizures	Benign myoclonic epilepsy of infancy, Dravet syndrome, Lennox-Gastaut syndrome, atypical benign rolandic epilepsy, late-onset epileptic spasms, other myoclonic epilepsies	Early development normal in most; regression often occurs with epilepsy onset; outcomes vary from normal intellect (26–67%) to severe intellectual disability
Lennox-Gastaut syndome	*ALG13*, *CACNA1A*, *CDKL5*, *CHD2*, *DNM1*, *FLNA*, *GABRB3*, *GRIN2B*, *HNPRNU*, *HNRNPH1*, *IQSEC1*, *iQSEC2*, *KCNQ3*, *MTOR*, *SCN1A*, *SCN2A*, *SCN8A*, *STXBP1*	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, atonic, myoclonic, or generalised tonic–donic seizures, spasms, focal seizures, episodes of tonic or non-convulsive status epilepticus	Interictal: slow background, slow (<2·5 Hz) spike-wave, generalised paroxysmal fast activity in sleep; ictal: electrodecrement 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–atonic 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 synchronous 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 sodium valproate, sulthiame, ethosuximide, levetiracetam; exacerbating: carbamazepine	Epilepsy is 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 $Na_v1·1$. Voltage-gated ion channel subunits such as $Na_v1·1$ 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.[13] Mutant $Na_v1·1$ channels affect development, as shown by patients with autism spectrum disorder with *SCN1A* mutations.[14] Animal models of Dravet syndrome provide initial evidence that cognitive dysfunction might be mediated by loss of $Na_v1·1$ function in neuronal networks, independent of seizures.[15] 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 $Na_v1·1$ 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.[23–29] 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.[30] 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).[31] CNVs in the form of microdeletions and microduplications exist in every human molecular karyotype, and many represent normal human variation.[32] 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.[33]

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.[34] 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.[23] 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,[29,30,33,35,36] which probably occur in the gamete or early in embryogenesis.[37] Most mutations are exonic, but interest is increasing in interrogating the regulatory and non-coding regions of DNA, including microRNAs, for mutations.[38,39]

X-linked inheritance might occur with recessive (eg, *ARX*), dominant (eg, *DCX*),[22] and male-sparing patterns (eg, *PCDH19*).[36] 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.[40]

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.[41,42] 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.[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.[43]

See Online for appendix

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.[41] Mosaicism refers to two populations of cells in a human being, one with the gene mutation and the other with the normal allele.[20] 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.[44–46]

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.[20] 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.[21,47] In other cases, deep sequencing (high-coverage targeted sequencing) of leucocyte DNA has identified somatic mutations in patients with cortical malformations.[48] 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.[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 0·04–85·00% of cells.[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.[49–52]

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.[53] 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 $K_v7·2$.[54] In-vitro studies implicate haplo-insufficiency as a likely disease mechanism.[55] *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.[56–58] These patients often have de-novo *KCNQ2* mutations, which might be dominant

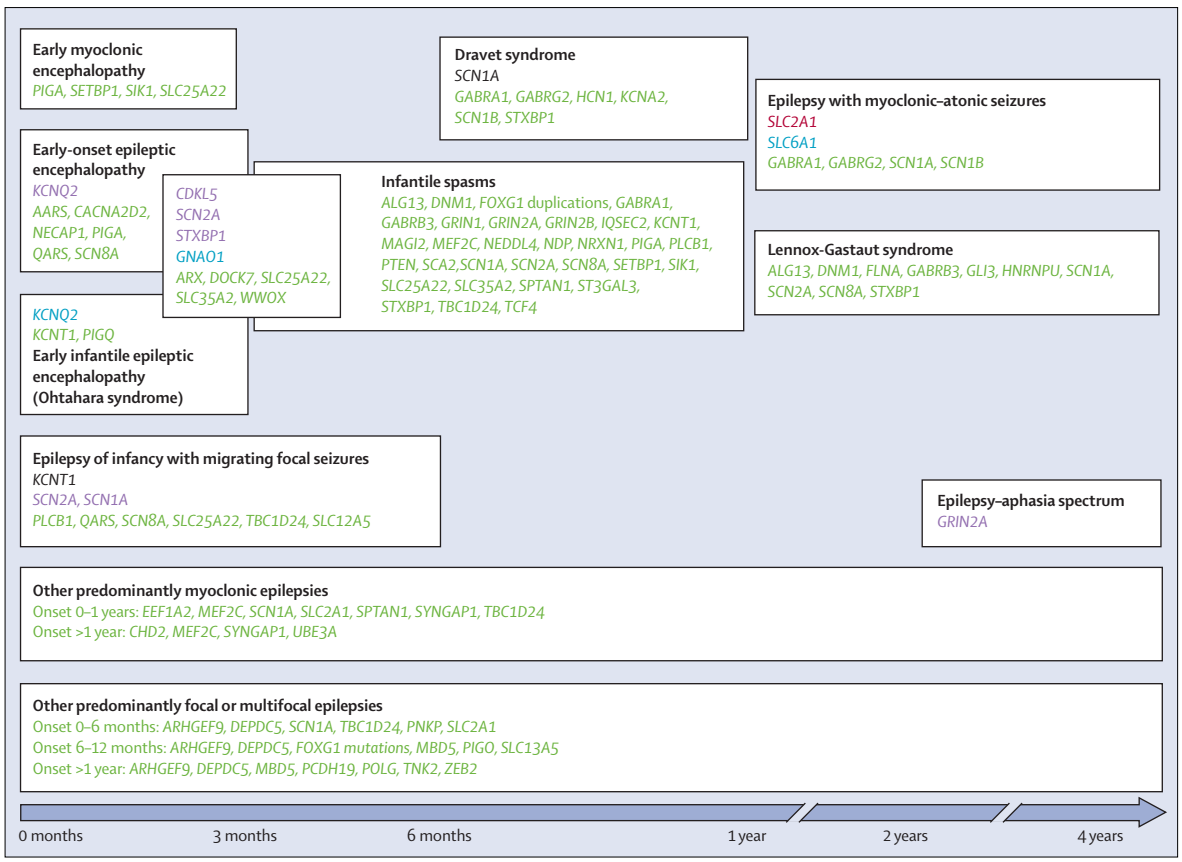

Figure 1: **Genetic causes of epilepsy syndromes**
Genetic causes, and proportion of cases caused by each gene, including only non-chromosomal, non-malformative, and non-metabolic disorders. Only genes with more than one case reported are included. Black font denotes genes that account for at least 50% 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.

negative, with far more profound overall negative effect on channel function than those that cause benign familial neonatal epilepsy.[49,50]

In the case of *SCN1A*, mutations are identified in most patients with Dravet syndrome, with half having missense and half having truncation mutations;[59] 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.[60] 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.[29,61–64] 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.[64–66] Additionally, rare cases of Ohtahara syndrome and West syndrome have *KCNT1* mutations.[63,64,67] Functional studies show a possible phenotype–genotype association, with a 13-times increase in channel amplitude in Ohtahara syndrome.[67,68] 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.[69,70]

Genetic heterogeneity

Genetic heterogeneity occurs in every epilepsy syndrome. Even in the prototypical genetic epileptic

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.[71] 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,[72] 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.[3,73] 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,[27,74] Lennox-Gastaut syndrome,[27,75] and Ohtahara syndrome,[33,51,67,76–81] 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.[3,73,82] 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.[83]

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.[64] 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.[42,84]

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.[85] Abnormalities of interneuron development, migration, or function are associated with genes such as *ARX* and *SCN1A*.[17,86] 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.[27,29]

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.[24–26,30,51,56]

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.[15] Such mechanisms contribute to cognitive impairment, and are unlikely to be rescued with conventional antiepileptic therapies.[14] 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.[87] 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.[88] Diagnosis enables planning for their child's long-term care and early access to services and therapies.[89]

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).[90] Optimisation of antiepileptic drugs improves seizure control, enabling improved cognitive function, seen even late in adult life.[88,89] 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.[91] 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.

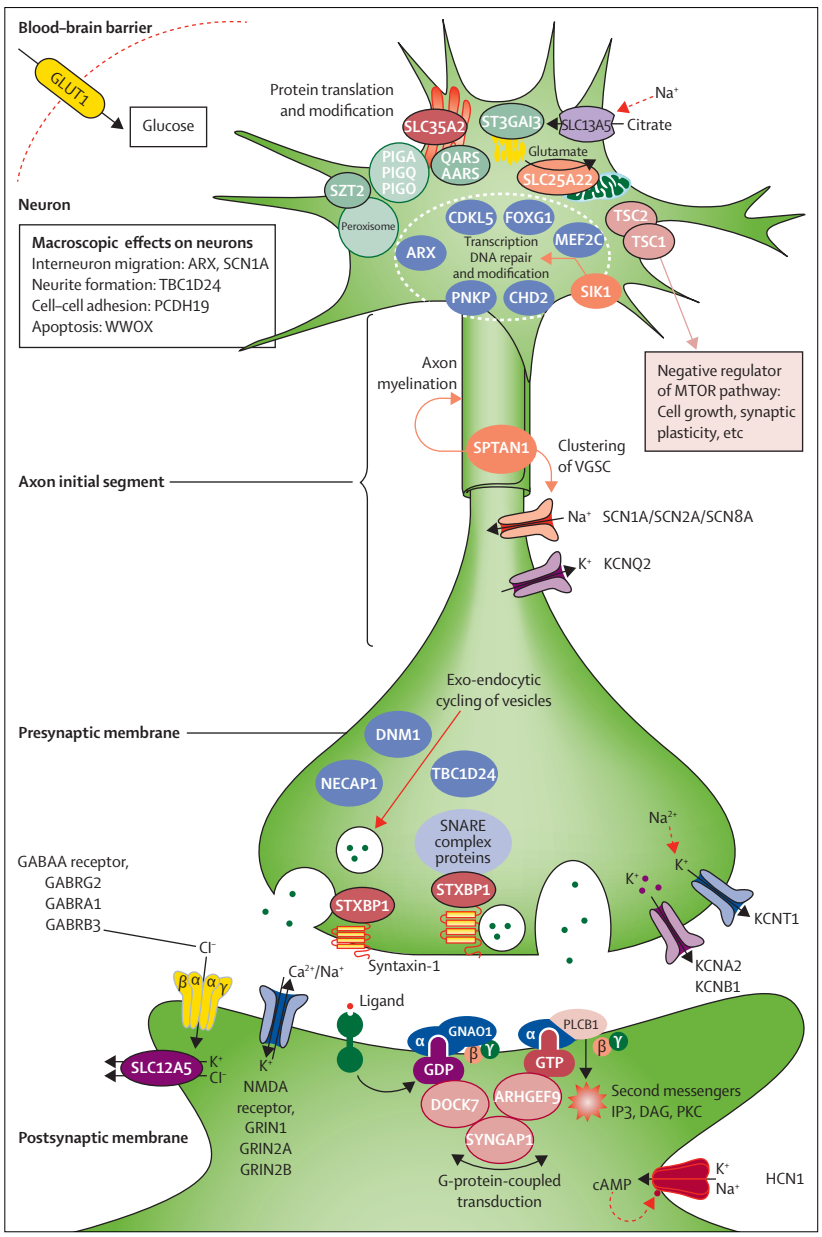

Figure 2: **Disease mechanisms in childhood epileptic encephalopathies**
Neuron, axon, presynaptic, and post-synaptic compartments. Many areas of abnormal neuronal function, including DNA repair, transcriptional regulation, axon myelination, metabolite and ion transport, and peroxisomal function, in 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.[23,27,92]

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.[93] 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.[91] An example is the Epilepsy Genetics Initiative.

For the **Epilepsy Genetics Initiative** see http://www.cureepilepsy.org/egi/

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.[94] Deep sequencing,[47] 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.[96] 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 discolouration in patients taking retigabine have hindered clinical trials.[97] Similarly, NMDA receptor antagonists such as memantine have been suggested for *GRIN2A*-related or *GRIN2B*-related diseases.[98,99] 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.[29,65,68,100,101]

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.[50]

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.

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 astatic 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.

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.

Acknowledgments

AM is funded by a UK Medical Research Council Research Training Fellowship. MAK is funded by a UK Wellcome Intermediate Clinical Fellowship. KBH is supported by the Australian Gustav Nossal National Health and Medical Research Council Postgraduate Scholarship and the Clifford PhD Scholarship. JHC is supported as a co-investigator by research grants from the European Union and from the UK National Institute for Health Research, Action Medical Research, Sparks, and the Great Ormond Street Hospital Children's Charity. IES is supported by an Australian National Health and Medical Research Council Practitioner Fellowship and Program Grant.

References

1 Ohtahara S, Ishida T, Oka E, Yamatogi Y, Inoue H, Kanda S. On the specific age dependent epileptic syndrome: the early-infantile epileptic encephalopathy with suppression-bursts. *No To Hattatsu* 1976; **8:** 270–80.

2 Ohtahara S. A study on the age dependent epileptic encephalopathy. *No To Hattatsu* 1977; **9:** 2–21.

3 Thomas RH, Zhang LM, Carvill GL, et al, and the EuroEPINOMICS RES Consortium. CHD2 myoclonic encephalopathy is frequently associated with self-induced seizures. *Neurology* 2015; **84:** 951–58.

4 Howell KB, McMahon JM, Carvill GL, et al. SCN2A encephalopathy: a major cause of epilepsy of infancy with migrating focal seizures. *Neurology* 2015; **85:** 958–66.

5 Wirrell EC, Grossardt BR, Wong-Kisiel LCL, Nickels KC. Incidence and classification of new-onset epilepsy and epilepsy syndromes in children in Olmsted County, Minnesota from 1980 to 2004: a population-based study. *Epilepsy Res* 2011; **95:** 110–18.

6 Eltze CM, Chong WK, Cox T, et al. A population-based study of newly diagnosed epilepsy in infants. *Epilepsia* 2013; **54:** 437–45.

7 Cowan LD, Hudson LS. The epidemiology and natural history of infantile spasms. *J Child Neurol* 1991; **6:** 355–64.

8 Hurst DL. Epidemiology of severe myoclonic epilepsy of infancy. *Epilepsia* 1990; **31:** 397–400.

9 Bayat A, Hjalgrim H, Møller RS. The incidence of SCN1A-related Dravet syndrome in Denmark is 1:22,000: a population-based study from 2004 to 2009. *Epilepsia* 2015; **56:** e36–39.

10 Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* 2010; **51:** 676–85.

11 Camfield PR. Definition and natural history of Lennox-Gastaut syndrome. *Epilepsia* 2011; **52** (suppl 5)**:** 3–9.

12 Bureau M, Genton P, Dravet C, et al. Epileptic syndromes in infancy, childhood and adolescence, 5th edn. Montrouge: John Libbey Eurotext, 2012: 157–73.

13 Goldin AL, Barchi RL, Caldwell JH, et al. Nomenclature of voltage-gated sodium channels. *Neuron* 2000; **28:** 365–68.

14 Weiss LA, Escayg A, Kearney JA, et al. Sodium channels SCN1A, SCN2A and SCN3A in familial autism. *Mol Psychiatry* 2003; **8:** 186–94.

15 Bender AC, Natola H, Ndong C, Holmes GL, Scott RC, Lenck-Santini P-P. Focal Scn1a knockdown induces cognitive impairment without seizures. *Neurobiol Dis* 2013; **54:** 297–307.

16 Battaglia D, Chieffo D, Siracusano R, et al. Cognitive decline in Dravet syndrome: is there a cerebellar role? *Epilepsy Res* 2013; **106:** 211–21.

17 Yu FH, Mantegazza M, Westenbroek RE, et al. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci* 2006; **9:** 1142–49.

18 Chieffo D, Battaglia D, Lettori D, et al. Neuropsychological development in children with Dravet syndrome. *Epilepsy Res* 2011; **95:** 86–93.

19 Osborne JP, Lux AL, Edwards SW, et al. The underlying etiology of infantile spasms (West syndrome): information from the United Kingdom Infantile Spasms Study (UKISS) on contemporary causes and their classification. *Epilepsia* 2010; **51:** 2168–74.

20 Poduri A, Evrony GD, Cai X, Walsh CA. Somatic mutation, genomic variation, and neurological disease. *Science* 2013; **341:** 1237758.

21 Poduri A, Evrony GD, Cai X, et al. Somatic activation of AKT3 causes hemispheric developmental brain malformations. *Neuron* 2012; **74:** 41–48.

22 Guerrini R, Dobyns WB. Malformations of cortical development: clinical features and genetic causes. *Lancet Neurol* 2014; **13:** 710–26.

23 Carvill GL, Heavin SB, Yendle SC, et al. Targeted resequencing in epileptic encephalopathies identifies de novo mutations in CHD2 and SYNGAP1. *Nat Genet* 2013; **45:** 825–30.

24 Carvill GL, Regan BM, Yendle SC, et al. GRIN2A mutations cause epilepsy-aphasia spectrum disorders. *Nat Genet* 2013; **45:** 1073–76.

25 Lesca G, Rudolf G, Bruneau N, et al. GRIN2A mutations in acquired epileptic aphasia and related childhood focal epilepsies and encephalopathies with speech and language dysfunction. *Nat Genet* 2013; **45:** 1061–66.

26 Lemke JR, Lal D, Reinthaler EM, et al. Mutations in GRIN2A cause idiopathic focal epilepsy with rolandic spikes. *Nat Genet* 2013; **45:** 1067–72.

27 Allen AS, Berkovic SF, Cossette P, et al, and the Epi4K Consortium, and the Epilepsy Phenome/Genome Project. De novo mutations in epileptic encephalopathies. *Nature* 2013; **501:** 217–21.

28 Veeramah KR, Johnstone L, Karafet TM, et al. Exome sequencing reveals new causal mutations in children with epileptic encephalopathies. *Epilepsia* 2013; **54:** 1270–81.

29 Barcia G, Fleming MR, Deligniere A, et al. De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. *Nat Genet* 2012; **44:** 1255–59.

30 Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am J Hum Genet* 2001; **68:** 1327–32.

31 Mefford HC, Yendle SC, Hsu C, et al. Rare copy number variants are an important cause of epileptic encephalopathies. *Ann Neurol* 2011; **70:** 974–85.

32 Itsara A, Cooper GM, Baker C, et al. Population analysis of large copy number variants and hotspots of human genetic disease. *Am J Hum Genet* 2009; **84:** 148–61.

33 Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet* 2008; **40:** 782–88.

34 Rauch A, Wieczorek D, Graf E, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 2012; **380:** 1674–82.

35 Harkin LA, McMahon JM, Iona X, et al, and the Infantile Epileptic Encephalopathy Referral Consortium. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain* 2007; **130:** 843–52.

36 Depienne C, Bouteiller D, Keren B, et al. Sporadic infantile epileptic encephalopathy caused by mutations in PCDH19 resembles Dravet syndrome but mainly affects females. *PLoS Genet* 2009; **5:** e1000381.

37 Vadlamudi L, Dibbens LM, Lawrence KM, et al. Timing of de novo mutagenesis—a twin study of sodium-channel mutations. *N Engl J Med* 2010; **363:** 1335–40.

38 Makrythanasis P, Antonarakis SE. Pathogenic variants in non-protein-coding sequences. *Clin Genet* 2013; **84:** 422–28.

39 Gan J, Qu Y, Li J, Zhao F, Mu D. An evaluation of the links between microRNA, autophagy, and epilepsy. *Rev Neurosci* 2015; **26:** 225–37.

40 Rahman S. Mitochondrial disease and epilepsy. *Dev Med Child Neurol* 2012; **54:** 397–406.

41 Depienne C, Trouillard O, Gourfinkel-An I, et al. Mechanisms for variable expressivity of inherited SCN1A mutations causing Dravet syndrome. *J Med Genet* 2010; **47:** 404–10.

42 Noebels J. Pathway-driven discovery of epilepsy genes. *Nat Neurosci* 2015; **18:** 344–50.

43 Singh R, Andermann E, Whitehouse WP, et al. Severe myoclonic epilepsy of infancy: extended spectrum of GEFS+? *Epilepsia* 2001; **42:** 837–44.

44 Depienne C, Arzimanoglou A, Trouillard O, et al. Parental mosaicism can cause recurrent transmission of SCN1A mutations associated with severe myoclonic epilepsy of infancy. *Hum Mutat* 2006; **27:** 389.

45 Marini C, Mei D, Helen Cross J, Guerrini R. Mosaic SCN1A mutation in familial severe myoclonic epilepsy of infancy. *Epilepsia* 2006; **47:** 1737–40.

46 Morimoto M, Mazaki E, Nishimura A, et al. SCN1A mutation mosaicism in a family with severe myoclonic epilepsy in infancy. *Epilepsia* 2006; **47:** 1732–36.

47 Lee JH, Huynh M, Silhavy JL, et al. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nat Genet* 2012; **44:** 941–45.

48 Jamuar SS, Lam A-TN, Kircher M, et al. Somatic mutations in cerebral cortical malformations. *N Engl J Med* 2014; **371:** 733–43.

49 Miceli F, Soldovieri MV, Ambrosino P, et al. Genotype-phenotype correlations in neonatal epilepsies caused by mutations in the voltage sensor of $K_v7.2$ potassium channel subunits. *Proc Natl Acad Sci USA* 2013; **110:** 4386–91.

50 Orhan G, Bock M, Schepers D, et al. Dominant-negative effects of KCNQ2 mutations are associated with epileptic encephalopathy. *Ann Neurol* 2014; **75:** 382–94.

51 Nakamura K, Kato M, Osaka H, et al. Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome. *Neurology* 2013; **81:** 992–98.

52 Singh NA, Pappas C, Dahle EJ, et al. A role of SCN9A in human epilepsies, as a cause of febrile seizures and as a potential modifier of Dravet syndrome. *PLoS Genet* 2009; **5:** e1000649.

53 Pettit RE, Fenichel GM. Benign familial neonatal seizures. *Arch Neurol* 1980; **37:** 47–48.

54 Singh NA, Charlier C, Stauffer D, et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet* 1998; **18:** 25–29.

55 Biervert C, Steinlein OK. Structural and mutational analysis of KCNQ2, the major gene locus for benign familial neonatal convulsions. *Hum Genet* 1999; **104:** 234–40.

56 Weckhuysen S, Mandelstam S, Suls A, et al. KCNQ2 encephalopathy: emerging phenotype of a neonatal epileptic encephalopathy. *Ann Neurol* 2012; **71:** 15–25.

57 Weckhuysen S, Ivanovic V, Hendrickx R, et al, and the KCNQ2 Study Group. Extending the KCNQ2 encephalopathy spectrum: clinical and neuroimaging findings in 17 patients. *Neurology* 2013; **81:** 1697–703.

58 Kato M, Yamagata T, Kubota M, et al. Clinical spectrum of early onset epileptic encephalopathies caused by KCNQ2 mutation. *Epilepsia* 2013; **54:** 1282–87.

59 Zuberi SM, Brunklaus A, Birch R, Reavey E, Duncan J, Forbes GH. Genotype-phenotype associations in SCN1A-related epilepsies. *Neurology* 2011; **76:** 594–600.

60 Scheffer IE, Zhang Y-H, Jansen FE, Dibbens L. Dravet syndrome or genetic (generalized) epilepsy with febrile seizures plus? *Brain Dev* 2009; **31:** 394–400.

61 Coppola G, Plouin P, Chiron C, Robain O, Dulac O. Migrating partial seizures in infancy: a malignant disorder with developmental arrest. *Epilepsia* 1995; **36:** 1017–24.

62 McTague A, Appleton R, Avula S, et al. Migrating partial seizures of infancy: expansion of the electroclinical, radiological and pathological disease spectrum. *Brain* 2013; **136:** 1578–91.

63 Ohba C, Kato M, Takahashi N, et al. De novo *KCNT1* mutations in early-onset epileptic encephalopathy. *Epilepsia* 2015; **56:** e121–28.

64 Møller RS, Heron SE, Larsen LHG, et al. Mutations in *KCNT1* cause a spectrum of focal epilepsies. *Epilepsia* 2015; **56:** e114–20.

65 Heron SE, Smith KR, Bahlo M, et al. Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. *Nat Genet* 2012; **44:** 1188–90.

66 Derry CP, Heron SE, Phillips F, et al. Severe autosomal dominant nocturnal frontal lobe epilepsy associated with psychiatric disorders and intellectual disability. *Epilepsia* 2008; **49:** 2125–29.

67 Martin HC, Kim GE, Pagnamenta AT, et al, and the WGS500 Consortium. Clinical whole-genome sequencing in severe early-onset epilepsy reveals new genes and improves molecular diagnosis. *Hum Mol Genet* 2014; **23:** 3200–11.

68 Milligan CJ, Li M, Gazina EV, et al. KCNT1 gain of function in 2 epilepsy phenotypes is reversed by quinidine. *Ann Neurol* 2014; **75:** 581–90.

69 Strømme P, Mangelsdorf ME, Shaw MA, et al. Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. *Nat Genet* 2002; **30:** 441–45.

70 Shoubridge C, Fullston T, Gécz J. ARX spectrum disorders: making inroads into the molecular pathology. *Hum Mutat* 2010; **31:** 889–900.

71 Carvill GL, Weckhuysen S, McMahon JM, et al. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. *Neurology* 2014; **82:** 1245–53.

72 Doose H. Myoclonic-astatic epilepsy. *Epilepsy Res Suppl* 1992; **6:** 163–68.

73 Mullen SA, Marini C, Suls A, et al. Glucose transporter 1 deficiency as a treatable cause of myoclonic astatic epilepsy. *Arch Neurol* 2011; **68:** 1152–55.

74 Michaud JL, Lachance M, Hamdan FF, et al. The genetic landscape of infantile spasms. *Hum Mol Genet* 2014; **23:** 4846–58.

75 Lund C, Brodtkorb E, Øye AM, Røsby O, Selmer KK. CHD2 mutations in Lennox-Gastaut syndrome. *Epilepsy Behav* 2014; **33:** 18–21.

76 Fullston T, Brueton L, Willis T, et al. Ohtahara syndrome in a family with an ARX protein truncation mutation (c.81C>G/p.Y27X). *Eur J Hum Genet* 2010; **18:** 157–62.

77 Kato M, Das S, Petras K, et al. Mutations of ARX are associated with striking pleiotropy and consistent genotype-phenotype correlation. *Hum Mutat* 2004; **23:** 147–59.

78 Absoud M, Parr JR, Halliday D, Pretorius P, Zaiwalla Z, Jayawant S. A novel ARX phenotype: rapid neurodegeneration with Ohtahara syndrome and a dyskinetic movement disorder. *Dev Med Child Neurol* 2010; **52:** 305–07.

79 Touma M, Joshi M, Connolly MC, et al. Whole genome sequencing identifies SCN2A mutation in monozygotic twins with Ohtahara syndrome and unique neuropathologic findings. *Epilepsia* 2013; **54:** e81–85.

80 Saitsu H, Kato M, Osaka H, et al. CASK aberrations in male patients with Ohtahara syndrome and cerebellar hypoplasia. *Epilepsia* 2012; **53:** 1441–49.

81 Nakamura K, Kodera H, Akita T, et al. De novo mutations in *GNAO1*, encoding a $G\alpha_o$ subunit of heterotrimeric G proteins, cause epileptic encephalopathy. *Am J Hum Genet* 2013; **93:** 496–505.

82 Carvill GL, McMahon JM, Schneider A, et al, and the EuroEPINOMICS Rare Epilepsy Syndrome Myoclonic-Astatic Epilepsy & Dravet working group. Mutations in the GABA transporter SLC6A1 cause epilepsy with myoclonic-atonic seizures. *Am J Hum Genet* 2015; **96:** 808–15.

83 Paciorkowski AR, Thio LL, Rosenfeld JA, et al. Copy number variants and infantile spasms: evidence for abnormalities in ventral forebrain development and pathways of synaptic function. *Eur J Hum Genet* 2011; **19:** 1238–45.

84 Oliver KL, Lukic V, Thorne NP, Berkovic SF, Scheffer IE, Bahlo M. Harnessing gene expression networks to prioritize candidate epileptic encephalopathy genes. *PLoS One* 2014; **9:** e102079.

85 EuroEPINOMICS-RES Consortium, and the Epilepsy Phenome/Genome Project, and the Epi4K Consortium. De novo mutations in synaptic transmission genes including DNM1 cause epileptic encephalopathies. *Am J Hum Genet* 2014; **95:** 360–70.

86 Kitamura K, Yanazawa M, Sugiyama N, et al. Mutation of *ARX* causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet* 2002; **32:** 359–69.

87 James S. An American in Oz. Sydney: Allen & Unwin, 2014.

88 Catarino CB, Liu JYW, Liagkouras I, et al. Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology. *Brain* 2011; **134:** 2982–3010.

89 Brunklaus A, Dorris L, Ellis R, et al. The clinical utility of an SCN1A genetic diagnosis in infantile-onset epilepsy. *Dev Med Child Neurol* 2013; **55:** 154–61.

90 Chiron C, Dulac O. The pharmacologic treatment of Dravet syndrome. *Epilepsia* 2011; **52** (suppl 2)**:** 72–75.

91 Might M, Wilsey M. The shifting model in clinical diagnostics: how next-generation sequencing and families are altering the way rare diseases are discovered, studied, and treated. *Genet Med* 2014; **16:** 736–37.

92 Lemke JR, Riesch E, Scheurenbrand T, et al. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia* 2012; **53:** 1387–98.

93 Stitziel NO, Kiezun A, Sunyaev S. Computational and statistical approaches to analyzing variants identified by exome sequencing. *Genome Biol* 2011; **12:** 227.

94 Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* 2008; **456:** 53–59.

95 Cai X, Evrony GD, Lehmann HS, et al. Single-cell, genome-wide sequencing identifies clonal somatic copy-number variation in the human brain. *Cell Reports* 2014; **8:** 1280–89.

96 Klepper J. GLUT1 deficiency syndrome in clinical practice. *Epilepsy Res* 2012; **100:** 272–77.

97 Garin Shkolnik T, Feuerman H, Didkovsky E, et al. Blue-gray mucocutaneous discoloration: a new adverse effect of ezogabine. *JAMA Dermatol* 2014; **150:** 984–89.

98 Lemke JR, Hendrickx R, Geider K, et al. GRIN2B mutations in West syndrome and intellectual disability with focal epilepsy. *Ann Neurol* 2014; **75:** 147–54.

99 Pierson TM, Yuan H, Marsh ED, et al, and the PhD for the NISC Comparative Sequencing Program. GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine. *Ann Clin Transl Neurol* 2014; **1:** 190–98.

100 Bearden D, Strong A, Ehnot J, DiGiovine M, Dlugos D, Goldberg EM. Targeted treatment of migrating partial seizures of infancy with quinidine. *Ann Neurol* 2014; **76:** 457–61.

101 Mikati MA, Jiang Y-H, Carboni M, et al. Quinidine in the treatment of *KCNT1* positive epilepsies. *Ann Neurol* 2015; published online Sept 15. DOI:10.1002/ana.24520.

102 Ma C, Su L, Seven AB, Xu Y, Rizo J. Reconstitution of the vital functions of Munc18 and Munc13 in neurotransmitter release. *Science* 2013; **339:** 421–25.

103 Hussain S. Developing a PPI inhibitor-based therapy for STXBP1 haploinsufficiency-associated epileptic disorders. *Front Mol Neurosci* 2014; **7:** 6.

104 Azzarito V, Long K, Murphy NS, Wilson AJ. Inhibition of α-helix-mediated protein-protein interactions using designed molecules. *Nat Chem* 2013; **5:** 161–73.

105 Liu Y, Lopez-Santiago LF, Yuan Y, et al. Dravet syndrome patient-derived neurons suggest a novel epilepsy mechanism. *Ann Neurol* 2013; **74:** 128–39.

106 Cirak S, Arechavala-Gomeza V, Guglieri M, et al. Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. *Lancet* 2011; **378:** 595–605.

107 Huang X, Tian M, Hernandez CC, Hu N, Macdonald RL. The GABRG2 nonsense mutation, Q40X, associated with Dravet syndrome activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through. *Neurobiol Dis* 2012; **48:** 115–23.

108 Wykes RC, Heeroma JH, Mantoan L, et al. Optogenetic and potassium channel gene therapy in a rodent model of focal neocortical epilepsy. *Sci Transl Med* 2012; **4:** 161ra152.

109 Kullmann DM, Schorge S, Walker MC, Wykes RC. Gene therapy in epilepsy-is it time for clinical trials? *Nat Rev Neurol* 2014; **10:** 300–04.

110 Kätzel D, Nicholson E, Schorge S, Walker MC, Kullmann DM. Chemical-genetic attenuation of focal neocortical seizures. *Nat Commun* 2014; **5:** 3847.

111 Matsa E, Dixon JE, Medway C, et al. Allele-specific RNA interference rescues the long-QT syndrome phenotype in human-induced pluripotency stem cell cardiomyocytes. *Eur Heart J* 2014; **35:** 1078–87.