REVIEW

# Genetic variations and associated pathophysiology in the management of epilepsy

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**Abstract:** The genomic era has enabled the application of molecular tools to the solution of many of the genetic epilepsies, with and without comorbidities. Massively parallel sequencing has recently reinvigorated gene discovery for the monogenic epilepsies. Recurrent and novel copy number variants have given much-needed impetus to the advancement of our understanding of epilepsies with complex inheritance. Superimposed upon that is the phenotypic blurring by presumed genetic modifiers scattering the effects of the primary mutation. The genotype-first approach has uncovered associated syndrome constellations, of which epilepsy is only one of the syndromes. As the molecular genetic basis for the epilepsies unravels, it will increasingly influence the classification and diagnosis of the epilepsies. The ultimate goal of the molecular revolution has to be the design of treatment protocols based on genetic profiles, and cracking the 30% of epilepsies refractory to current medications, but that still lies well into the future. The current focus is on the scientific basis for epilepsy. Understanding its genetic causes and biophysical mechanisms is where we are currently positioned: prizing the causes of epilepsy "out of the shadows" and exposing its underlying mechanisms beyond even the ion-channels.

**Keywords:** array CGH, copy number variants, epilepsy, ion channels, massively parallel sequencing, next generation sequencing, susceptibility genes

## Introduction

A rare gene variant in a patient associated with a diagnosed clinical condition, or detected from a control population, is defined as one where the allele frequency is less than 1%. This may or may not be a pathogenic mutation. A "polymorphism" has an allele frequency greater than or equal to 1%; or more precisely (to take into account the possibility of multiple rare alleles), a frequency of the major allele less than or equal to 99%. Strictly speaking, single nucleotide polymorphisms (SNPs) have alleles that occur at polymorphic frequencies, though the term SNP has often been applied inappropriately to rare variants. A low frequency polymorphism is more common than a rare variant but with a major allele frequency of greater than or equal to 95%.

Pathogenic mutations of large effect size, which segregate with disease in families, lie within the rare variant range. More frequent variation, if pathogenic, is generally in the susceptibility range. Although susceptibility variants contribute to common diseases, such as the genetic generalized epilepsies, they do not segregate with the disease because on their own they do not cause disease. Furthermore, susceptibility variants are expected to be found in control individuals, since they cause disease only when in combination with rare variants in certain other genes.

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The proportion of heritability accounted for by common variants in diseases with complex genetics is relatively low.[1] The answer to "where is the missing pathogenic variation causing the common diseases?" probably lies within about three million rare variants we all carry when compared with the reference sequence,[2] many of which are unique to the individual when compared with other individuals. These are now detectable with whole genome sequencing, or exome sequencing if we accept that most single base change pathogenic variants reside within coding exons. Superimposed upon coding variants affecting amino acid substitutions is the copy number variation (CNV), often affecting more than one gene, which also differs markedly between individuals.[3] Rare base changes and CNVs are now readily identifiable in the laboratory but many of their pathogenic associations are not yet established.

## Human genetic variation and its medical implications

When weighing up the evidence in support of a rare variant being the causative mutation in a Mendelian epilepsy, we consider the nature of the amino acid change (conservative or not in the context of impact on the protein structure or function), evolutionary conservation across species and across members of the same gene family within the human (a change at a conserved site is less likely to be tolerated without ill effect, because conservation suggests functional significance for that protein domain) and the absence of the variant in an acceptable sample size of controls. Segregation of the variant with the disease phenotype provides circumstantial support, because if causative the two will cosegregate with absolute fidelity, barring phenocopies and allowing for incomplete penetrance. The problem is that every rare variant in every gene within the linkage interval and on the same homolog as the true mutation will cosegregate with the disorder. It can therefore be unclear if one particular variant is the causative mutation or not. This analysis is useful however, in discarding rare variants that "marry-in" to the pedigree, on the other homolog of the chromosome pair. Substantial support in the research context then needs to be assembled from in vitro expression studies and, ultimately, study of the pathogenesis of the amino acid substitution in vivo in an animal model.[4] For diagnostic application, tools such as Polyphen-2[5] and Condel[6] can be helpful to assess novel variants but such approaches may need to be customized to genes responsible for specific disorders tested in each diagnostic laboratory.[7] As knowledge grows, validated pathogenic mutations will be deposited in publicly accessible databases.[8] That has already

begun for epilepsy-related *SCN1A* mutations[9] (http://www.molgen.ua.ac.be/SCN1AMutations/Home).

The same considerations may apply when assessing a rare variant or a variant in a low frequency polymorphism as a susceptibility agent. The challenge for the common genetic epilepsies is determining which variants, among the vast array of variation we all carry, are relevant to seizures. The nature of the amino acid change and the evolutionary conservation of the amino acid site again come into the calculation, but their effects are milder. This makes them more difficult to confirm in vitro and unlikely to be testable in vivo in animal models, because their change in function is much more subtle. The fidelity of segregation with the disease rapidly breaks down when the variant is tracked through families and it may be present without the phenotype in the control population. Theoretically such a variant will be less common in controls but astronomical sample sizes would be needed to demonstrate that statistically. Adding to the challenge, as for pathogenic mutations of large effect in the Mendelian epilepsies, some susceptibility variants may be novel and therefore not amenable to statistical comparison between cases and controls. Most medically relevant genetic variations are likely to be at low frequency (or rare) in the population.[10,11]

## Pathophysiology in human epilepsy

Epilepsy is a common neurological condition involving recurring and unprovoked seizures. Fisher et al[12] defined epileptic seizures as abnormal, excessive, and synchronous neuronal activity in the brain. Their effects can vary from loss of awareness to more obvious and distressing tonic–clonic manifestations. Pathophysiology in the context of epilepsy is the disturbance in neurological processes leading to the expression of seizures. In other words, pathophysiology in epilepsy is about seizure mechanisms. Genetic analysis is no longer confined to gross chromosomal rearrangements.[13] Molecular defects at the gene level are providing glimpses into the pathophysiology; but even with knowledge of the causative gene the complexity of seizure mechanisms needs to be unraveled using painstaking analyses with both in vitro expression systems and in vivo animal models engineered to mimic the human condition. Reid et al[14] comprehensively surveyed the mechanisms associated with the range of genes now known to be related to epilepsy syndromes, in order to assess progress in connecting molecular genetics to the clinic. An understanding of the mechanisms is fundamental to the design and application of efficacious therapeutics.

Numerous syndromes of epilepsy remain poorly understood with regard to pathophysiology due to their

complex genetic basis, the detail of which will remain unresolved for some considerable time into the future. Since the genes for what would be regarded as relatively easily soluble autosomal dominant epilepsies seen across ethnicities in multiple families have not all been identified, the goal of a complete molecular understanding, extended to the autosomal recessive epilepsies and epilepsies with complex inheritance, represents a huge challenge that still lies ahead. Benign familial infantile epilepsy (BFIE) and familial focal epilepsy with variable foci (FFEVF) are two of the monogenic epilepsies which have so far proven refractory to molecular investigation despite well-established gene localizations and ascertainment of multiple families. We use the revised syndrome nomenclature for the epilepsies as recommended by Berg et al[15] throughout this review.

## Pathophysiology of acetylcholine receptor subunit mutations

Recurrent and novel mutations, both of which are rare, were first described in *CHRNA4* for autosomal dominant frontal lobe epilepsy.[16–18] Reports of pathogenic mutations in the β2 and α2 subunits followed.[19,20] The first 1995 result heralded the era of gene discovery and molecular genetics for epileptologists. Around 80%–90% of frontal lobe epilepsy does not have a *CHRN* mutation,[21] hence, at least those which are sporadic cannot be referred to as autosomal dominant. If genetic, these must be considered to be polygenic until proven otherwise. Of the mutations in the autosomal dominant families, these reduce the calcium dependence of the acetylcholine response or increase sensitivity to acetylcholine.[20,22–23] Hirose and Kurahashi[24] provide a comprehensive review of genetic testing and counseling for nocturnal frontal lobe epilepsy.

## Pathophysiology of potassium channel subunit mutations

Defects in the genes encoding the neuronal potassium channel subunits *KCNQ2* and *KCNQ3* are responsible for one of the benign epilepsies of infancy, benign familial neonatal epilepsy (BFNE).[25–27] These are heritable lateralized motor seizures typically with onset at 3 days of age with offset by 3 months of age. The transient nature of seizures in BFNE might be attributable to developmental progression of *KCNQ* expression patterns, compensation through the activation of redundant components of a neural network, or expression of compensatory mechanisms such as gamma aminobutyric acid (GABA) receptors which mature as the neonate develops.[28] Potassium channels mediate potassium ion permeability that establishes the inward–negative membrane potential.[29] We

describe the role of *KCNQ2* and *KCNQ3* but recognize that potassium channel involvement now goes well beyond these two genes[30] with their full potential as agents for epileptogenesis not yet realized.

## KCNQ2

The total number of known pathogenic mutations in *KCNQ2* is approaching 100, making it currently the second most commonly mutated gene for monogenic epilepsies after *SCN1A*. Few sites within *KCNQ2* have been detected as mutated more than once. Mutations include missense, nonsense, splice site, and frameshift changes as well as copy number mutation being recently recognized as significant contributors both at the exon and whole gene level[31] and beyond.[32] The size of the molecular defect does not generally amplify the clinical severity, which is uniformly benign in 85% of cases of BFNE. Truncating mutations are distributed across the gene; however, the clustering of missense mutations, which comprise around one-third of the pathogenic mutations, defines regions of clinical significance in the protein. They are localized in the transmembrane domains especially in the S4 voltage sensor and the pore loop, and the portion of the C-terminal domain that binds to calmodulin has been shown to be involved.[33] Missense mutations significantly reduce the potassium current[34,35] while truncation mutations lead to nonfunctional protein. Taken together, these data indicate haploinsufficiency of *KCNQ2* as the general mechanism for relaxation of seizure inhibition.

Approximately 15% of cases of BFNE due to *KCNQ2* mutation suffer later neurological complications, including seizures. Rare cases associate with myokymia[36] and progress to severe encephalopathies with intellectual disability.[37–39]

## KCNQ3

BFNE cases due to mutation of *KCNQ3* are much rarer, with only four described so far. There is no evidence for neurological complications beyond seizure offset, but with only four known families the data are limited. Together with *KCNQ2* and other accessory subunits, *KCNQ3* forms the pore of the M-channel. Since the sequences in the transmembrane domains of the two subunits are highly homologous, it isn't clear why there is such a disparity between the apparent mutability of the two genes, or if *KCNQ3* does mutate as frequently, why these are not pathogenic in live births. No systematic study of genetic variation in ion channels from spontaneous abortions has been carried out to assess the role of channelopathies in addition to the well-established range of chromosomal abnormalities[40] in recurrent miscarriages. The four known mutations are in the transmembrane

domain or near the pore loop. None have been investigated electrophysiologically, so one can only surmise a similar mechanism of haploinsufficiency as recognized for the *KCNQ2* partner in forming the pore for the M-current.

## Pathophysiology of sodium channel subunit mutations

Defects in genes encoding the voltage-gated sodium channel subunits *SCN1A*, *SCN2A*, to a lesser extent *SCN3A*, possibly *SCN9A*, and the accessory subunit *SCN1B*, encompass several epileptic conditions. These range from benign epilepsy in infancy (benign familial neonatal-infantile epilepsy; BFNIE) to the full generalized epilepsy with febrile seizure plus (GEFS+) spectrum of mild febrile seizures right through to Dravet syndrome. Mutations in voltage-gated sodium channels are presently the most significant of the known genetic determinants for epilepsy with *SCN1A* the major contributor. They transiently increase plasma membrane conductance to sodium ions in response to depolarizations.[41] Mutations that reduce sodium currents affect electrical excitability of GABA-ergic interneurons.[42] *SCN1A* is the only known epilepsy gene with sufficient sporadic cases to enable determination of the parental origin of the mutations. Of 44 cases examined, 33 were of paternal origin and 11 were of maternal origin.[43] De novo mutagenesis at least for *SCN1A* can occur any time from the premorula stage of the embryo up to maturity, where only germ cells may be affected.[44] Mosaicism of parental tissues can lead to unaffected parents having more than one affected child.

## *SCN1B*

*SCN1B* is an accessory subunit for α-subunits and opened up the field of defective neuronal sodium channels as triggers for the periodic epilepsies.[45] Few mutations are known, with the original one being recurrent.[46,47] They are associated with GEFS+ with the phenotype extended to temporal lobe epilepsy in some cases. Loss of function through mutation interferes with the degree of control exercised over the movement of sodium ions through the pore forming α-subunits. Interestingly, homozygosity of a functional null *SCN1B* variant has recently been found as a rare autosomal recessive cause of Dravet syndrome.[48]

## *SCN1A*

Around 700 *SCN1A* mutations are now documented as being associated with seizures,[9] making this the most commonly mutated of the known genes for monogenic epilepsy conditions. The degree of allelic heterogeneity for the pathogenic variants at this locus is extraordinarily high. Broad phenotypic expression is the hallmark of mutations in this gene[49] ranging from febrile seizures and GEFS+[50] to Dravet syndrome.[51] Variation in other, as yet undefined genes, is suspected of modifying the expression of *SCN1A* mutations. Many of the missense mutations have been analyzed for their electrophysiological effects. Dravet syndrome mutations generally lead to loss of function whereas GEFS+ mutations (all missense mutations so far) change expression of the sodium channel through a variety of electrophysiological mechanisms suggesting either loss of function in some situations, or gain of function in other situations.[14,52] This indicates that it is the change to the status quo in either direction that leads to hyperexcitability that causes the epilepsy. A human GEFS+ mutation introduced into the orthologous mouse *SCN1A* gene suggests that a general outcome of deleterious *SCN1A* mutations is decreased activity of GABAergic interneurons.[53]

Most cases of *SCN1A*-associated Dravet syndrome are due to de novo mutations but 5% appear in GEFS+ families where the associated phenotypes in other family members are less severe. *SCN1A* mutation in Dravet syndrome has significant clinical impact from the treatment and genetic counseling viewpoint. Molecularly unsolved Dravet syndrome comprises about 20% of the clinically diagnosed cases[54,55] and although some have been accounted for by *SCN1A* copy number variation detected most efficiently by multiplex ligation-dependent probe amplification,[56] the majority of the residual, by exclusion of other hypotheses, are likely to be polygenic.

Apart from genetic testing for *SCN1A* mutations having counseling and treatment implications, there are medico-legal implications associated with *SCN1A* related mutations in some patients since exposure of the myth of "pertussis vaccine encephalopathy"[57]. Vaccination without prior knowledge of an *SCN1A* mutation can provoke earlier onset of Dravet syndrome,[58] but vaccination is not the cause of what has been erroneously labelled as vaccine encephalopathy.

## *SCN2A*

Missense defects in the gene encoding the sodium channel subunit *SCN2A* are responsible for another of the benign epilepsies of infancy, benign familial neonatal-infantile epilepsy (BFNIE).[59,60] These are lateralized motor seizures typically with highly variable age at onset ranging from three days to 13 months of age.[61] Mutations cause a range of biophysical effects with reduced sodium channel density at the plasma membrane.[62] After seizure offset, normal development proceeds, as determined from what are now a large number of affected cases within the families identified.

Pathogenic *SCN2A* mutations are far less frequent than seen for *SCN1A*, with only 11 documented so far in BFNIE families (one of which is recurrent, having been detected in three unrelated families). These mutations are found spread throughout the protein. Together with *KCNQ2* and *KCNQ3*, *SCN2A* accounts for the remainder of those benign epilepsies of infancy where the molecular basis is known. Due to phenotypic overlap between BFNE and BFNIE, syndrome designations are not always definitive using clinical criteria alone.[63] Therefore we advocate genetic testing, and a molecular-based system of classification with molecular diagnosis where knowledge of the gene involved is necessary to improve patient management or provide more accurate prognosis through genetic counseling.[64] This is especially pertinent for sporadic cases that could have either disorder, where the age of seizure onset falls within the broad overlapping age at onset distribution observed with either BFNE or BFNIE.

Further detail on the molecular genetics of the three benign familial epilepsies of infancy (BFNE, BFNIE and BFIE) is comprehensively reviewed elsewhere.[65] Several more map locations have been reported for BFIE but so far no pure BFIE gene has been identified. More severe mutations in *SCN2A* are extremely rare, causing febrile and afebrile seizures[66] and refractory epilepsy with intellectual disability.[67]

## SCN3A

SCN3A is another of the tandemly repeated subunit-α cluster of sodium channel subunits on chromosome 2 comprising *SCN1A*, *SCN7A*, *SCN9A*, *SCN2A,* and *SCN3A*. Only one known mutation has been reported so far for *SCN3A*, associated with cryptogenic partial epilepsy.[68]

## SCN9A

The *SCN9A* channel arose unexpectedly as one of the likely causes of familial febrile seizures and with an as yet unconfirmed role in Dravet syndrome.[69] This subunit is primarily expressed in the peripheral nervous system so any involvement in what would normally be considered central nervous system mediated disorders, if validated, tells us more about the expression of *SCN9A* than previously realized.

## Pathophysiology of GABA receptor subunit mutations

The $GABA_A$ receptor is the major inhibitor of neuronal transmission in the central nervous system.[70,71] Two of the multiple subunits (*GABRG2* and *GABRA1*) have been implicated in familial epilepsy[72–74] with ascertainment of

additional pathogenic mutations reinforcing the involvement of defects in these two genes. But why are these the only two subunits so far implicated? *GABRD* has a weaker effect and so falls into the category of a susceptibility locus for epilepsies with complex inheritance.[75,76] Mutations interrupting the normal export of chloride ions through the $GABA_A$ receptor affect the capability of the neuron to inhibit hyperexcitability. Syndromes induced by this are childhood absence epilepsy, juvenile myoclonic epilepsy and GEFS+. The vast array of possible combinations of pentameric assemblages from the many known subunits provides enormous potential for both haploinsufficiency and dominant-negative effects as mechanisms evoked by $GABA_A$ receptor subunit mutation.[77]

## Pathophysiology of calcium channel mutations

Voltage-gated calcium channels mediate calcium influx that controls excitability and regulates calcium-sensitive cellular processes.[78] *CACNA1H* is the most thoroughly studied putative susceptibility gene for epilepsy to date.[79–83] Primarily rare variants, but a few polymorphisms as well, have been implicated in genetic susceptibility. The in vitro properties of the variants tested suggest a range of mechanisms leading to the mild pathogenic effects of the variants at this gene. These effects varied but altered channel properties in ways that would predict an increase in calcium current.[83] Characterization of the epilepsy associate variants at this locus provided the earliest experimental support for the rare variant–common epilepsy model which, if extended across epilepsy susceptibility loci, would mean the pursuit of association study designs for epilepsy with complex inheritance is likely to prove futile. The *CACNA1H* gene would however, represent a prime candidate for testing the power of next generation association studies designed to compare the frequency, within genes, of clusters of rare variants in test populations affected with epilepsy against clusters of rare variants from the same genes in controls.[84] Broader involvement of calcium channels is discussed in greater depth by Noebels.[85]

## Susceptibility genes and the treatment of epilepsy

Apart from *GABRD* and *CACNA1H*, *KCND2*, *KCNA1*, *GABRB3*, and others, have previously been discussed as susceptibility genes.[14,86,87] The most recent one is the potassium channel *KCNV2*.[88] There are likely many hundreds more that remain unidentified. Various oligogenic or polygenic combinations of these loci probably account for the common epilepsies. None of these, not even the

ones identified so far, have clinical application. Their genotype alone, without knowledge of the entire spectrum of alleles at all susceptibility genes contributing to the phenotype, is not predictive of morbidity. Nor is sufficient molecular information available to direct treatment, at this time. Ultimately, not all molecular defects comprising a polygenetically determined epilepsy in a given patient will need to be determined in order to direct treatment. Just enough need to be determined and treated in order to push the phenotype back under the seizure threshold.

Due to the undisputed role of ion channels in neuronal excitability, these are the targets of most antiepileptic drugs, irrespective of whether seizures are triggered by ion channel defects. Most treatments are directed at altering excitation via sodium and calcium channels or GABA and glutamate receptors.[89] Thirty percent of epilepsy cases remain refractory to treatment[90] suggesting that new classes of drugs targeting classes of genes not yet identified are required. That remains a major driver for further gene identification. Treatment strategies are described elsewhere.[91–93] As a general rule, sodium valproate is the first choice for generalized epilepsy, and carbamazepine for partial epilepsies. Specialized treatment is required once Dravet syndrome is established by *SCN1A* testing.[94]

## Comorbidities with the genetic epilepsies

A variety of disorders frequently present with epilepsy, either as an outcome of epilepsy or through genetically based comorbidity (referred to as pleiotropy). *KCNQ2* related comorbidities were discussed above under "Pathophysiology of potassium channel subunit mutations." Mutation of the *ATP1A2* gene has been described as causal in epilepsy with familial BFIE and migraine.[95] Such families are rare. Seriously complicating the search for the BFIE gene are the families which frequently suffer infantile convulsions and choreoathetosis (ICCA) in the form of epilepsy with paroxysmal kinesigenic choreoathetosis or paroxysmal dyskinesia. Gene localizations on chromosome 16 do not necessarily overlap with gene localizations in all reported families.[65]

Intellectual disability is the most frequent condition presenting with epilepsy. Notable examples include *ARX*-related epilepsies, *CDKL5/STK9* Rett-like encephalopathy, *STXBP1*-related West/Ohtahara syndrome, *SRPX2*-related rolandic epilepsy associated with oral and speech dyspraxia and mental retardation, *SCN1A* in Dravet syndrome, and *PCDH19* in epilepsy with mental retardation restricted to females (EFMR). *KCNQ2*-related encephalopathy is a recent addition

where the same mutations associated with benign BFNE can have more sinister consequences.[39]

Discovery of *SCARB2* as the defective gene in action myoclonus renal failure[96] has led to its recognition as a gene for progressive myoclonic epilepsy (PME) alone.[97,98] Mutant genes expressed in a variety of tissues can lead to a variety of comorbidities each of which can have its own incomplete penetrance and variable expression, governed by genetic and environmental modifiers. Not until the gene is identified is it possible to link a range of different clinical presentations to the same molecularly defined disorder. Even mutations identical by descent can be associated with a range of different phenotypes governed by their nonidentical expression in different tissues, each possibly modulated by a plethora of tissue-specific genetic modifiers.

## Pathophysiology beyond the channels: *LGI1*

Mutations in *LGI1* cause autosomal dominant epilepsy with auditory features.[99,100] This was the first non-ion channel gene identified for epilepsy with unknown etiology. This foreshadowed a decade ago the reality that we need to go beyond ion channels in describing the genetic architecture underlying the epilepsies. The mechanism linking this gene to epilepsy is still not understood.

Twenty-five mutations have been described, of which 24 are unique.[101] Their average penetrance is 67%[102] and they account for half of the affected families, indicating genetic heterogeneity for this clinically defined condition. Detection throughout the gene of both missense and truncating mutations associated with the same recognizable syndrome suggests loss of function as the mechanism, and that relates to secretion of the protein.[103] The molecular basis for the other half of the cases remains elusive.

## Pathophysiology beyond the channels: *PCDH19*

Epilepsy with mental retardation limited to females is a remarkable X-linked disorder where heterozygous females are affected but hemizygous males are spared.[104,105] Carrier males transmit the condition to all of their daughters. The gene responsible is protocadherin 19 (*PCDH19*)[106] and the mechanism is loss of function mutations acting very likely via cellular interference.[104,106] Just as remarkable is the clinical spectrum of febrile seizures or afebrile focal or generalized epilepsies, cognitive impairment, and autistic, obsessive and aggressive behavior. Some cases phenotypically resemble Dravet syndrome.[107–111] It is now recognized that mutations

in *PCDH19* are a common cause of epilepsy in females irrespective of family history or mental impairment. *PCDH19* related disorders display a range of phenotypes reminiscent of the situation in *ARX*[112,113] and *SCN1A*.[54] Female patients with encephalopathy who test negative for *SCN1A* mutation need to be tested for *PCDH19*. The differential diagnosis has important genetic counseling implications due to the different modes of inheritance.

## Pathophysiology beyond the channels: GLUT1 deficiency

Another advance "beyond the channels" is associated with the gene *SLC2A1*. Deleterious dominant mutations cause glucose transporter type 1 (GLUT1) deficiency leading to paroxysmal exertional dyskinesia together with epilepsy and intellectual disability in family members. Conversely, early-onset absence epilepsy has mostly been found to be associated with GLUT1 deficiency.[114,115] The molecular diagnosis of GLUT1 deficiency in cases of absence epilepsy has major genetic counseling implications, which also relate to other comorbidities. Diet control may represent a relatively simple therapy.

## A recurring theme

Numerous disorders cataloged in Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/omim/ (OMIM)[116] are associated with epileptic seizures. The studies of Sharp et al [117] linking a recurrent 15q13.3 microdeletion to intellectual disability, including seizures, led Helbig et al [118] to link this CNV to epilepsy alone. Back in 2000, the observation of febrile seizures in Dravet syndrome led Claes et al[51] to the link between *SCN1A* mutation and Dravet syndrome, given the established link between *SCN1A* and GEFS+.[50] Discovery of the gene for action myoclonus renal failure syndrome (AMRF)[96] led on to its role as a gene for progressive myoclonus epilepsy (PME) without renal failure.[97] Similarly, identification of the ARX gene for intellectual disability frequently associated with seizures[112] led to its link to a myoclonic epilepsy with spasticity and intellectual disability.[113] The ARX spectrum disorders proliferated from that point and we refer the reader to recent reviews in that area.[119,120]

## Pathophysiology beyond the channels: entree through copy number variations

Utilization of array comparative genome hybridization (array CGH) means that cytogenetics has now come of age as a genome science.[40] This technology has been translated to

epilepsy.[117,118,121–123] The transformation to a genotype-first approach to diagnosis for clinically heterogeneous disorders with the same mutated gene is now well established.[40,124] The traditional approach to syndrome nosology involved the careful characterization of reasonably consistent phenotypes, reflecting genetic homogeneity. We have now broken out of that plateau in syndrome discovery with the advent of new high resolution cytogenetic and molecular technologies, such as array CGH and massively parallel sequencing (MPS). The same or overlapping micro-chromosomal lesions are now defining new syndrome constellations that could not previously be grouped due to their clinical heterogeneity concealing their identical etiologies.[121]

Any case of epilepsy at variance with classic symptoms of known epilepsy syndromes represents a prime candidate for array CGH as the first tier genetic investigation. The approach is generic: able to detect pathogenic CNVs through the interrogation of the entire genome in one experiment, without prior knowledge of their existence or location. Novel CNVs uncover pathophysiology not related to ion channels and provide an entrée into new gene candidates for epilepsy[125–128] and new insights into mechanisms.

"An epilepsy syndrome is a disorder characterized by a cluster of symptoms and signs occurring in combination."[129] The next highest level is the syndrome constellation,[121] a concept originating from psychiatry where complexity also relates to both the genetics and to the phenotype.[130] CNVs such as the 15q13.3 recurrent deletion can be associated with a constellation of clinically distinct syndromes like intellectual disability, autism, schizophrenia and epilepsy. The same lesion can present with one, two, or even none, of the syndromes that have been recognized as caused by it. The deletion accounts for ~1% of genetic generalized epilepsies without the other comorbidities that are part of this 15q13.3 constellation.[118] Despite the high associated odds risk ratio for the association between 15q13.3 and genetic generalized epilepsy, family studies show that it behaves as a susceptibility allele for a multifactorial epilepsy.[131] Why the same lesion leads to a variety of distinct syndromes on the one hand, and skips generations on the other, has not been established. Other "common" CNVs related to epilepsy have been established at 16p13.11 and 15q11.2 [132,133] and together with the 15q13.3 lesion are major genetic components of about 3% of genetically complex generalized and focal epilepsies.[122]

The recognition of syndrome constellations associated with specific CNV lesions leads on to an alternative conceptualization, the broader concept of epilepsy as part of a spectrum disorder.[134] This view of epilepsy places it as one

symptom of a more general disorder. The challenge is to determine or distinguish between what arises from shared genetic determinants causative for multiple syndromes comprising a syndrome constellation, and what are secondary comorbidities. The seizures caused by structural lesions in the brain arising from excitotoxicity, like hippocampal sclerosis, may be considered secondary comorbidities.

## Pathophysiology beyond the channels: entree through massively parallel sequencing (MPS)

Next generation sequencing (abbreviated NGS) is a misnomer. It has rapidly evolved into current generation sequencing and continues to evolve – hence our use of the term massively parallel sequencing (MPS). MPS of targeted regions defined by linkage analysis is already being replaced by exome sequencing, with whole genome sequencing approaching a reality as costs fall with the advent of more efficient sequencing technologies.

Two epilepsy genes have already been identified using this approach.[135,136] One of these (*TBC1D24*) is associated with the autosomal recessive syndrome of focal epilepsy, dysarthria and intellectual disability through disruption of primary axonal arborization and specification in neuronal cells. The other gene (*GOSR2*) relates to an autosomal recessive syndrome with ataxia followed by progressive myoclonus epilepsy where mutation affects protein localization. "Navigating the channels and beyond" is now the reality.[87,137] CNVs and in particular MPS have unlocked the door to a new era of epilepsy gene discovery.

## Classification and diagnosis in the management of epilepsy

As our currently limited understanding of the genetics of epilepsy expands, its value as a tool for classification gradually increases.[54–56,64] The molecular basis for a disorder, where known, provides the most fundamental level of understanding for organizing complexity into a logical structured framework which can be easily defined and studied. Molecular genetic testing will inevitably become incorporated into the clinical workup of presenting patients, where definite diagnoses are required to manage them. Clinical examination alone may not be definitive enough to diagnose them into their predetermined genetic classification framework.[54,55,107,138,139] Precise molecular diagnosis can inform patient management decisions where prognosis, counseling, and treatment might vary depending upon knowledge of the causative gene.

The additional expense of molecular genetic testing is generally not warranted for benign disorders, or where prognosis or treatment is not affected. The added precision of a molecular genetics diagnosis, whether benign or not, is always advantageous in the context of patient cohorts that are part of research protocols or clinical trials. Whatever the degree of classification, ultimately the cause of the disorder will need to be communicated in a meaningful and understandable way to those who have been burdened with it.

## Mosaicism and somatic mutation as a mechanism in epilepsy

This phenomenon has emerged as a major consideration for genetic counseling related to sporadic *SCN1A*-precipitated Dravet syndrome and sporadic *PCDH19*-associated EFMR. Mosaicism is where genetically different cell lineages exist within an individual. It can be restricted to the gonads or it can extend to other somatic tissues, depending upon the timing of the mutation.

Gonadal mosaicism (sometimes extending to proven somatic mosaicism) is well established as an important molecular mechanism associated with *SCN1A* missense mutations in Dravet syndrome.[140–143] The phenomenon extends to CNVs containing *SCN1A*.[138] Where detected as somatic mosaicism, it has accounted for milder phenotypic expression, often restricted to febrile seizures, in the parent in whom the new mutation has arisen, postconception. Where more than one affected case occurs within a sibship and neither parent apparently carries the mutation, it is generally assumed that mosaicism occurs in the gonads, at least. Mosaicism is not necessarily looked for in sporadic cases. The frequency among sporadic cases is not known at present, but the possibility has to be conveyed to families contemplating further children that there is a small but indeterminate risk of recurrence, even if neither parent has the mutation detectable in DNA isolated from lymphocytes in their peripheral blood.

Interestingly, for the two cases so far described for EFMR,[144] gonadal mosaicism was inferred due to the presence of more than one affected sibling. Further investigation of their lymphocyte DNA disclosed somatic mosaicism. This highlights gonadal mosaicism of *PCDH19* mutation as an important molecular mechanism associated with the inheritance of EFMR. This knowledge needs to be translated to genetic counseling for couples with no apparent *PCDH19* mutations and just one affected daughter with a *PCDH19* mutation, as they may risk recurrence of affected daughters, and unaffected sons who may then in the future transmit EFMR to their daughters, with the EFMR phenotype skipping

a generation when the pathogenic X-linked mutation is passed through a male.

## Genetic counseling and prognosis in the management of epilepsy

Genetic testing has prognostic value in the benign childhood epilepsies of infancy since *KCNQ2* mutations are not always benign. Later seizures occur in 15% of cases with *KCNQ2* defects, but not so for cases with *KCNQ3* and *SCN2A*, based on current clinical experience of disorders where these genes have mutated. Severe epileptic encephalopathies are now accepted as an additional risk associated with *KCNQ2* mutations.[39] There is no information for BFIE since the genes have not been found as yet for that benign childhood disorder. When that happens, the reason why a subset of BFIE presents in combination with paroxysmal movement disorders in adolescence might then be predictable. Paroxysmal kinesogenic choreoathetosis and paroxysmal dyskinesia are collectively referred to as infantile convulsions choreoathetosis and represent an area of molecular genetics where progress has been disappointingly slow despite the effort invested. Mutation in *ATP1A2* in a family with BFIE and familial hemiplegic migraine[145] remains uncommon. BFIE is clinically and genetically heterogeneous[146–150] and in the absence of known genes for either the pure or paroxysmal forms, little can be predicted except through linkage analysis where the families are of sufficient size to confidently determine genetic risks using markers mapping across the regional localization.

New technologies mean new interpretative challenges and when applied to humans these have ethical implications. MPS of the exome not only detects any epilepsy mutation we may be seeking but any other pathogenic mutation in the patient's genome, even where it is presymptomatic. Array CGH has its challenges as well. Enough is now known of the common recurrent pathogenic CNVs to be able to convey the genetic risk they pose for their associated syndrome constellation. But predictive counseling regarding which syndrome will be expressed, and whether any of the syndromes within the constellation will be penetrant at all in this or that carrier, remains problematic. The interesting CNVs are the novel ones. If de novo, they are likely to be damaging to their carrier. That assessment can be reinforced once the genes affected by the lesion are taken into account. For familial cases, the phenotype of the carrier parent can be instructive. Evolving principles currently used to interpret CNV results in epilepsy are discussed in greater detail elsewhere.[123]

## Treatment in the management of epilepsy

Treatment in relation to epilepsies with complex genetics was outlined previously in the section "Susceptibility genes and the treatment of epilepsy." Similar principles apply for the monogenic epilepsies. For all epilepsies, there are concerns that while a number of new drugs have proven to be as efficacious as the old ones (eg, Keppra targeting SV2A), none are superior to the old ones.[151,152] That leaves 30% of cases that still remain refractory to effective treatment, despite drug development. More needs to be understood about the molecular basis for this group of patients, and perhaps the newer technologies of array CGH and MPS will enable expansion "beyond the channels" in order to open up new treatment avenues. Dravet syndrome is a special case, where lamotrigine may exacerbate seizures[153] but stiripentol[154] and topiramate[155] can be beneficial. Chiron and Dulac[94] provide the latest treatment options.

## Living with MPS

The discipline of molecular genetics as applied to epilepsy has advanced swiftly since the 1995 finding of a *CHRNA4* mutation in a large Australian family with autosomal dominant frontal lobe epilepsy.[16] Now, the application of massively parallel sequencing has uncovered two more epilepsy related genes as the precursors of a new monogenic avalanche.[135,136] Effectively breaking the shackles of ion channels which have dominated the autosomal dominant epilepsies, MPS is the tool to crack the many autosomal recessive epilepsies that were previously refractory to the molecular approach. The new biochemical pathways that will be identified in the epileptic population, appended to what we now know of the involvement of ion channels, will provide the platform for delving deeper into the common multifactorial epilepsies with complex inheritance than has been possible through the examination of ion channels alone. Only after that can genetic profiles be possibly applied to prediction and prognosis. We say possibly, because not all genetic determinants will likely be additive in their effect, and if not, that will open up another layer of complexity. A challenge waiting is the cure of those 30% of cases afflicted with epilepsy who at the present time are refractory to therapy.

The cost of the technology continues to fall. The rate that DNA sequence data can now be generated is astronomical. Breakpoints of balanced chromosomal rearrangements may affect gene expression, but these were previously cryptic with array CGH. They will soon be detected through wider application of MPS.[156] The bottleneck now becomes making clinical sense out of the results by extracting only what is relevant from the extraordinary level of interpersonal genetic diversity.[157] Will

that end up solving the genetic architecture for the epilepsies? Not any time soon. For the multifactorial epilepsies, capturing the genetic variation is now relatively easy – the problem will be determining which variation relates to epilepsy. There will be a lot of it, but each part will likely be individually rare and existing experimental approaches are probably not sensitive enough to detect what could be a spectrum of very small effects across hundreds or thousands of genes.[158]

## Conclusion

Novel CNVs associated with epilepsy will continuously reveal new candidate genes from microchromosomal regions where combinations of genes might be involved. Lying latent is a reservoir of epilepsies we may not have even seen yet – since not all rare recessive mutations have exposed their associated epilepsies, given low rates of consanguinity in most populations. That should not be a problem for the new previously uncharacterized monogenic epilepsies as they arise since we are now on the verge of more widespread application of MPS through translation to diagnostic laboratories, even to private syndromes. Knowledge of the molecular defect will at some future point in time play a far greater role in guiding treatment than is currently the case.

Finally, "epilepsy" encompasses many clinical and genetic epilepsies.[15] The genetic epilepsies referred to in this review are far from exhaustive.

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## Disclosure

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