Chapter 5

Genetics of the epilepsies

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Introduction

Genetics is a huge and growing area across human biology and medicine, providing information about basic processes from birth to death, from development to degeneration, and in some instances revealing enough about disease biology to lead to rational new therapies or better use of existing treatments. There is much hope that the power of genetics to provide insights into how diseases start and develop will lead to smarter therapies that in turn will make care not only more effective, but also more cost-efficient. This is particularly an issue for healthcare systems in developed economies where chronic conditions are becoming key issues. One can only hope that genetics will provide benefits also for developing regions, in the way that mobile telephony bypasses the need for more expensive infrastructure. Whether these hopes will be realised remains to be seen and answers are likely to be forthcoming in the next few years.

Genetics can empower all sides in the healthcare setting, from the person with the condition to the physician delivering care. Much has already been written about the experience and perspective of people with epilepsy, and there will be more to come, but this will form the focus of the current chapter. Physicians are excellent at seeing patterns in their patients: genetics can produce biological explanations for such patterns and for unusual deviations from such patterns. Syndromes become explicable diseases, and clinicians will remain the key element in the translation of genetic discovery to clinic for the benefit of people with epilepsy. The pace of such discovery and the magnitude of the challenge ahead will come as a surprise to most of us. It is therefore important that everyone involved in treating epilepsy should have some grounding in genetics, and in epilepsy genetics. By providing information, probably the most available from any single test, genetics will change our thinking about epilepsy, at least as much as did EEG and MRI. People with epilepsy themselves, and their families and carers, are already pushing ahead. Genetics offers real possibility for meaningful collaboration not just between scientists and clinicians, but also between all parties, including funders, providers and, most of all, those with the conditions.

We have long known that ‘epilepsy’ is not one condition. Progress in the genetics of the epilepsies is providing a factual landscape for this established diversity of the epilepsies. Today’s challenges are not to acquire genetic data, but to interpret the vastness of the data emerging from genetic work in the epilepsies in the context of the even larger universe of genetic data across the life sciences. In this context, this chapter will focus more on concepts than on individual genes. Any printed list of genes linked with an epilepsy is soon dated.

Background

As is well known, a role for genetics has long been postulated in the epilepsies, derived from observations in families. More broadly, the ‘neurological trait’ is a phenomenon talked about by clinically-astute neurologists for many years (see, for example, Gowers 1881) – it is,
incidentally, interesting that recent research efforts in genetics are exploring the genetic underpinnings of such phenomena, seeking shared genetic susceptibility across brain diseases (http://www.ashg.org/2014meeting/abstracts/fulltext/f140123198.htm), and soon also across somatic co-morbidities. More formal heritability studies, mainly based on twin cohorts, began to define and quantify aspects of the genetic contribution to the epilepsies\textsuperscript{2,3}, but have inevitably been limited because we have not really known if the input phenotypic mixes are biologically correct. On this background of belief in a role of genetics in causation, newer technologies have made possible real advances not only in discovery of causes but also discovery in other domains where genetics might have a role – such as susceptibility, specific phenomenological traits, pharmacogenomics and outcomes, as well as co-morbidities and the definition of new syndromes and new categorisation of the epilepsies based on a better understanding of causation.

**Current understanding of genetics of the epilepsies**

The reorganisation of the epilepsies promulgated in 2010 by the ILAE Commission on Classification and Terminology was predicated on the belief that genetic information in the epilepsies was informing a new understanding based on biological discovery underpinning clinical pattern recognition\textsuperscript{4}. The spate of publications in epilepsy genetics over the last few years bears witness to this. The reorganisation was of course controversial, and it is not the case that all the beliefs enshrined in the original reorganisation have been underpinned by actual genetic discovery\textsuperscript{5}. Nevertheless, there has been enormous progress, with discoveries in a number of major domains: syndromic epilepsies, epileptic encephalopathies (which may have an overlap of course with ‘syndromic’ epilepsies), progressive myoclonic epilepsies, and a small group of generalised epilepsies and some focal epilepsies. There has also been much more limited progress in other areas, such as treatment genomics. Most other aspects of the epilepsies, such as outcomes and co-morbidities, have not yet been addressed. Recent progress is reviewed here in terms of concepts, rather than in terms of every gene that has so far been linked to epilepsy.

Discovery in epilepsy genetics, inevitably, has followed technological advances\textsuperscript{6}. In the current era, the first new genetic technology that became widely available was array comparative genomic hybridisation (aCGH), which permits comparison of segments of a patient’s DNA with, typically, pooled DNA from a group of controls without the same condition, usually healthy individuals. The technique highlights segments where the number of copies of that segment is different to that seen in controls (copy number variation), down to a certain size resolution, usually of the order of a few hundred kilobases, but occasionally with higher resolution. aCGH is now offered by many clinical genetics laboratories and, because of its higher resolution and reliability, has replaced karyotyping as the first-line test in complex epilepsy phenotypes that do not implicate an obvious candidate gene. aCGH is indicated when the presenting epilepsy is syndromic, being associated with other features, such as facial or somatic dysmorphism, intellectual disability, autism spectrum disorder or multiple co-morbidities. Microdeletions and microduplications (together falling within the category of copy number variants, CNVs) have been increasingly reported in association with complex epilepsies\textsuperscript{7–11}, and have sometimes pointed to novel candidate epilepsy genes\textsuperscript{12}. Depending on case series and criteria for inclusion, about 12% of people with complex epilepsy might have a CNV considered relevant. In the current NHS setting, aCGH is the first-line genetic test that should be considered in a patient with a complex phenotype for which there is no obvious candidate gene(s). aCGH retains a place in genetics research, as other current technologies do not always reliably pick up relevant CNVs.

Interestingly, aCGH has also proved informative for some groups of ‘genetic’ (idiopathic) generalised epilepsies. The paper that many consider to herald the current era of genetic discovery in epilepsy reported the first CNV associated with a common epilepsy, 15q13.3
microdeletion, seen in 12 patients in the classic 2009 report by Helbig\textsuperscript{13}. The same CNV has now been studied in a 246-case series, 28\% of whom had seizures\textsuperscript{14}; neuropsychiatric manifestations were common, major congenital malformations were not. Further genotype-phenotype correlation with long-established CNVs associated with epilepsies will become possible as increasing numbers of cases are reported. Such phenotypic delineation will facilitate genetic screening and interpretation for future practice. CNVs have now also been identified in genetic generalised epilepsies with intellectual disability\textsuperscript{15}.

At the leading edge of current research in epilepsy genetics, and the most productive tool in terms of gene discovery, is whole exome sequencing (WES). With falling costs and increasing availability, an ever wider range of epilepsies, more or less homogeneously grouped, have been subjected to WES. Discoveries are being reported at a pace too great to meaningfully list each individually. Progress has been most dramatic for the epileptic encephalopathies. Though individually rare, the encephalopathies account for an important part of the burden of the epilepsies. Their genetic tractability is probably because they are often caused by variants of large effect, which is perhaps not surprising considering the severity of the phenotype. A set of genes and related pathways responsible for a number of epileptic encephalopathies was reported using trio exome sequencing\textsuperscript{16} – several known ‘epilepsy genes’ were identified, and a number of novel candidates were proposed. One candidate, DNM1, was then confirmed by merging data from consortia\textsuperscript{17}, illustrating the frequent need for large numbers of patients to formally declare involvement of a given gene. Mutations in many genes have been identified in the epileptic encephalopathies, including for example AARS\textsuperscript{18}, KCNA2\textsuperscript{19}, STX1B\textsuperscript{20}, PURA\textsuperscript{21}, WWOX\textsuperscript{22}, SLC13A5\textsuperscript{23}, DOCK7\textsuperscript{24} and SZT2\textsuperscript{25} among many others. Some of these conditions have distinctive features, but many do not.

One consequence of this observation is that candidate gene selection in the epileptic encephalopathies is a challenge, making gene panels for clinical genetic diagnosis of limited value, compounded by the rapid pace of gene discovery: a gene may not be considered a candidate for the panel, or not be included because it was not linked with epilepsy at the time of panel design. A further implication is that genotype-phenotype correlation is needed, but will also be challenging, and may need newer phenotyping tools accessing data not typically used in clinical phenotyping\textsuperscript{26}. Moreover, given the richness of the emerging data, there is considerable scope for data mining and novel analytic methods, some to predict new genes for epileptic encephalopathy\textsuperscript{27}, with methods also to prioritise genes\textsuperscript{28,29}. The greatest promise lies perhaps in the identification of pathways implicated across sets of epileptic encephalopathies, such as the mTOR pathway\textsuperscript{30}, that may already have possible treatments or repurposable drugs, or that might point the way to new generic treatments relevant across epileptic encephalopathies linked by shared mechanisms\textsuperscript{16,31}.

WES, and other methods, have also been successful in identifying the cause(s) of some rare conditions which may feature epilepsy as part of a phenotype. Examples include alternating hemiplegia of childhood (due in 80\% of cases to \textit{de novo} mutation in the ATP1A3 gene\textsuperscript{32}), in which rare condition perhaps 50\% of affected individuals have seizures; DOORS syndrome, which is very rare, due in about 50\% of cases to mutation in TBC1D24\textsuperscript{33}; and for which genotype-phenotype correlation may yet show it can be considered in some cases an epileptic encephalopathy; and epilepsies with other comorbid features such as migraine or movement disorders, for which implicated genes include SCN1A, CACNA1A, ATP1A2, SLC2A1, PRRT2, STXBP1 and FOXG1\textsuperscript{34-38}. Of considerable interest are the epilepsies with associated language or speech disorder – these are broad summary terms for aspects of the phenotype that have often been characterised in great detail, within the epilepsy-aphasia spectrum. Mutations have been identified in the NMDA receptor NR2A subunit-encoding gene GRIN2A in Landau-Kleffner syndrome, electrical status epilepticus in sleep
(ESES)/continuous spike and wave during slow-wave sleep syndrome (CSWSS), and typical and atypical rolandic epilepsies. The progressive myoclonic epilepsies (PMEs) were amongst the most successfully studied from a genetic perspective even before WES. Genetic discovery has proved demonstrably valuable in understanding disease biology, especially for example for Lafora disease and Unverricht-Lundborg disease, though breakthroughs in treatment options are still awaited. There have been further discoveries in the PMEs, some of which could be considered surprising. ‘North Sea’ progressive myoclonus epilepsy has been found to be due to homozygous mutation in GOSR2, and has a distinctive phenotype, with all patients having a progressive and relentless course, and all developing scoliosis by adolescence, sometimes with other skeletal findings. A systematic examination of 84 unsolved PME cases using WES as the discovery tool found causal mutation in 31%. Most interestingly, a recurrent de novo mutation was found in an ion channel gene (KCNC1) and identified as a new major cause for PME, with eleven unrelated exome-sequenced (13%) and two affected individuals in a secondary cohort (7%) carrying this mutation. KCNC1 encodes a subunit of voltage-gated potassium ion channels, which have major influence on high-frequency neuronal firing. The detected recurrent mutation causes a dominant-negative loss-of-function effect. Other cases within this cohort that had not been explained were found to have pathogenic mutations in known PME-associated genes (NEU1, NHLRC1, AFG3L2, EPM2A, CLN6 and SERPINI1), while unsuspected mutations were identified in other genes that had previously not been linked to epilepsy and/or PME, including the TBC1D24 gene. It is fascinating that while WES is increasingly identifying genes that do not encode ion channels in other epilepsies, in the PMEs which have not traditionally been considered channelopathies, WES has revealed the involvement of an ion channel, and other genes for which PME was not considered part of the phenotypic spectrum before. This discovery further compromises the idea of gene panels as currently conceptualised.

Despite the clear indications from both epidemiological, and early molecular, genetic studies of probable significant genetic contribution to the genetic generalised epilepsies, such as juvenile myoclonic epilepsy, juvenile absence epilepsy and childhood absence epilepsy, there are still very few genes definitively linked to these phenotypes. In early-onset absence epilepsy, mutations in the SLC2A1 gene, encoding a cerebral glucose transporter and causing GLUT1 deficiency, were reported in one study in about 10% of cases. Subsequently, a review of seven studies identified SLC2A1 mutation in 2.4% (29) of 1110 patients with generalised epilepsies overall, with a higher rate (5.6%) among 303 patients with early-onset absence epilepsy. Clues to a possible SLC2A1 mutation were the additional presence of abnormal movements or a family history of seizures, abnormal movements, or both. As GLUT1 deficiency can be treated with the ketogenic diet, it is important to identify its presence. No other glucose or lactate transporters have been implicated in early-onset absence epilepsy, and no other generalised epilepsies have been shown to be due to SLC2A1 mutation. Mutations or deletions in a variety of genes have been identified in genetic epilepsy with febrile seizures plus (previously known as generalised epilepsy with febrile seizures plus, both GEFS+), including SCN1A, PCDH19, SCN1B, SCN2A, and GABRG2. But most cases of all of these epilepsy types, that is the vast majority of genetic generalised epilepsies, remain genetically unexplained, even with systematic WES. It has also recently been shown that the involvement of EFHC1 in juvenile myoclonic epilepsy needs to be reconsidered, with a number of lines of enquiry raising doubts about the pathogenicity of detected mutations, as nicely outlined in a sobering reminder that the standards for declaring causality must be robust and that supporting evidence should be multidimensional. The genetic generalised epilepsies remain a conundrum, with ‘genetic’ in the currently-recommended name, but little ‘genetic’ in terms of actual genes.
Progress has been made also in the focal epilepsies. Perhaps most interesting, and what may possibly emerge as the most common genetic cause in familial focal epilepsies, is the discovery of mutations in DEPDC5 in several familial and sporadic epilepsy phenotypes, including familial focal epilepsy with variable foci, familial temporal lobe and autosomal dominant nocturnal frontal lobe epilepsies, and rolandic epilepsy. These findings are of especial interest as DEPDC5 is part of the mTOR pathway, activity within which can be manipulated using the existing drug rapamycin. DEPDC5 mutations have also been shown in epilepsies with developmental malformation. Somatic mutations in MTOR itself have been reported in focal cortical dysplasia and hemimegalencephaly. Mutations in the ion channel gene KCNT1 have been reported in malignant migrating partial seizures of infancy and severe autosomal dominant nocturnal frontal lobe epilepsy. Most focal epilepsies, however, remain genetically unexplained.

Finally, there are of course also the epilepsies across the spectrum with well-established genetic causation. These epilepsies include those associated with developmental structural abnormalities, neurocutaneous disorders, chromosomal disorders established well before the aCGH era, several PMEs, neurometabolic disorders, mitochondrial cytopathies, the focal epilepsies, autosomal dominant frontal lobe epilepsy and lateral temporal lobe epilepsy, Dravet syndrome, Rett and related syndromes. Several excellent reviews have been published on these conditions. It is also worth noting that not all cases with phenotypes similar or related to these epilepsies have actually been solved and efforts continue to explain these. Just to give two examples, new genes have been identified for Dravet syndrome (such as CHD2) and for polymicrogyria (such as CCND2) in various settings.

Beyond the discovery of genetic causes of specific types of epilepsy, other aspects of the epilepsies are also being investigated. Given the breadth of phenotypic variation seen in some otherwise characteristic epilepsies, there has been much interest in genetic modifiers of phenotype. Identifying modifiers is challenging as many factors other than genetic variation may play a role. Animal models have been explored from this perspective, with evidence for example that mutations in different genes may influence the epileptic phenotype. In humans, SCN9A has been proposed as a modifier of the Dravet and GEFS+ phenotypes. Some have considered microdeletions to be modifiers within genetic generalised epilepsy phenotypes. Taking this concept further, network disruption has been proposed in mesial temporal lobe epilepsies with hippocampal sclerosis. Cause and effect can be difficult to disentangle in such studies, and the standards for proof are yet to be clarified in this area. The provocative effect of intermittent photic stimulation in precipitating seizures has been a topic of genetic research for many years. The area is complex, with definitions and protocols varying between studies, sites and publications. Taking broad common phenotypes into account, and based on the observation that photosensitivity is frequently present in epileptic encephalopathy due to CHD2 mutation or deletion, it was shown that CHD2 mutation was also present in a small proportion of people with photosensitivity and more common epilepsies, and was present in 3/36 patients with the syndrome of eyelid myoclonia with absences.

Treatment genomics in the epilepsies remain a challenge. In keeping with most trials, most studies have been drug-centred. Variants significantly increasing the risk of severe or mild rash on exposure to carbamazepine have been identified in the HLA system, with HLA-B*1502 being a major risk in populations of South Asian extraction and HLA-A*3101 in people of European extraction. Screening for the B*1502 variant has been shown to be cost-effective in a south Asian population. A systematic review has shown that HLA-B*1502 in Asian patients is associated with a pooled odds ratio of 113.4 for severe carbamazepine-induced reactions (Stevens-Johnson syndrome and toxic epidermal necrolysis), and that 461 patients would need to be screened for HLA-B*1502 to prevent one episode of such a severe reaction. For HLA-A*3101, which is more broadly associated with cutaneous rash on exposure to carbamazepine.
hypersensitivity reactions to carbamazepine across multiple ethnicities, this study estimated that between 47 and 67 patients would need to be tested to prevent one episode of hypersensitivity\textsuperscript{73}. For phenytoin, the CYP2C9 nonsense variant rs1057910 (CYP2C9*3) was significantly associated with severe cutaneous adverse reactions, an intriguing finding\textsuperscript{74}. Apart from these few findings for severe skin reactions, there are no other confirmed pharmacogenomic findings in epilepsy currently.

For discoveries beyond severe adverse reactions, and more seizure control genomics, it may be that the strategy will need to change focus from a drug-centred approach to a patient-centred approach, despite the challenges that studies based on small numbers of patients raise, both in terms of proof and regulatory requirements. There are already a few additional examples where genetic findings of course have treatment implications. The best example is the finding of an SCN1A mutation in an appropriate phenotype, such as Dravet syndrome, which should usually lead to the withdrawal of sodium channel-blocking antiepileptic drugs and consideration of valproate, benzodiazepines, and other agents including stiripentol\textsuperscript{75}. In the appropriate clinical contexts, which may be wide, other examples include: identification of an SLC2A1 mutation leading to use of the ketogenic diet\textsuperscript{47,76-78}; in PNPO or ALDH7A1 mutation, supplementation with pyridoxine or pyridoxal 5'-phosphate\textsuperscript{79}; with FOLR1 mutation, use of folic acid\textsuperscript{80}. Novel therapies have been explored at some level in newer genetic epilepsies. KCNT1-associated epilepsies were described in 2012; in 2014, reversal of mutation-associated gain-of-function was reported in a Xenopus oocyte model using a drug (quinidine) previously used in humans, though not one known to be an antiepileptic\textsuperscript{81}. GRIN2A mutations were reported in association with various epilepsies in late 2013; in 2014, functional analysis of one mutation showed that the mutated protein retained sensitivity to a known blocker (memantine) of this channel, which also reduced seizure frequency in the single patient carrying the mutation\textsuperscript{82}. Studies of genetic determinants of response to the ketogenic diet are ongoing. The only known genetic factors predisposing to good response in humans are mutations in SLC2A1 causing GLUT1 deficiency syndrome and some other very rare neurometabolic conditions.

**Current tools, models and problems**

The landscape of epilepsy genetics is changing rapidly – which overall is likely to be to the benefit of people with epilepsy. For the clinician, the tools available for genetic diagnosis are aCGH, candidate gene testing, and gene panels. Array CGH applied in an appropriate clinical setting may identify a pathogenic copy number variant in perhaps 12% of cases, as discussed above. Candidate gene testing requires the clinician to have knowledge of the gene(s) which may be altered to produce the observed phenotype. Some epilepsies have a very characteristic phenotype, and gene selection may be obvious. Candidate gene testing typically uses Sanger sequencing, to which methods such as multiplex ligation-dependent probe amplification may be added for detecting exon-level changes, such as exonic deletions. Dravet syndrome is amongst the best examples: with a typical history, over 80% of cases will have a pathogenic change in the gene SCN1A. Other genes when mutated can cause a Dravet-like phenotype, while having an SCN1A mutation does not mean a patient has Dravet syndrome unless the phenotype is appropriate: recent guidelines for SCN1A testing should help in these situations\textsuperscript{83}, and may be needed for other genes – another challenge for the years ahead. Gene panels partly sidestep this issue of complex and overlapping genotype-phenotype correlation, but have important limitations of their own, and are likely to be a step in the evolution of genetic testing in epilepsy. Next-generation sequencing, reading much more of the available genetic information, is already being applied in a few settings. Next-generation based panels are in use and may be informative, but WES is still to be broadly used in epilepsy genetics. CNVs can be difficult to pick up through WES, and aCGH, or genotyping arrays, may still retain a role in clinical practice even when WES becomes more widely applied. Eventually,
it seems likely that whole genome sequencing (WGS), will become a standard clinical tool, as it can significantly increase yield.

For clinicians, it is important to consider genetic testing as part of the armamentarium that can be used to better understand epilepsy in an individual. Genetic testing should be considered alongside other investigations such as MRI and EEG. WES and WGS are where MRI was 20 years ago – available only in specialist centres if at all, and still presenting important challenges in analysis and interpretation. As with MRI, it seems likely that WES or WGS will become part of the clinical investigation of many more people with epilepsy, to inform understanding of causation, prognosis, treatment, and co-morbidities. The model for genetics should change from its use in occasional cases, to its integration into routine practice as a source of important individual information that alters management. While the current focus is on the genetic code, other aspects of genetic information, such as the control of gene expression through epigenetic regulation, the role of a variety of RNA species and translational modifications, may also eventually prove important, though the need for organ-specific testing makes these avenues hard to explore, at least currently. Moreover, even current and imminent technologies that may advance knowledge will present hurdles. Such issues range from the conceptual, even for familial epilepsies where the condition may be Mendelian, but not necessarily monogenic, to practical considerations such as how the mass of data emerging from genetic testing will be stored, who will have control over its use, how such truly big data will be analysed, how results will be interpreted in the context of the individuals rather than populations, how the relevance of complex gene networks can be judged in an inaccessible organ part way through the natural history of an individual’s epilepsy, and how all this can be managed in an appropriate and just ethical and social environment. At the very least, it seems likely that to realise the full benefits from genomic data in clinical practice will require a multidisciplinary team and changed models of management, which will allow, albeit carefully regulated, individual-level drug repurposing. Among the best outcomes, perhaps we can also hope that epilepsy genomics will also bring better care for the vast majority of people with epilepsy across the world who today do not have access to any care at all. The genome is, after all, our shared heritage.

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