A genomics revolution

Epilepsy genetics and precision medicine

Prof Deb Pal and epilepsy genetics counsellor Stephanie Oates describe the way genomics has enriched our epilepsy understanding. They look at possible future treatments for genetic epilepsy and discuss the importance of careful counselling for patients and families

In the year that Nelson Mandela was released from Robben Island and before the world-wide web had even launched, the international Human Genome Project was announced. Its intention was to sequence the entire human genetic code. Not since the NASA mission to the moon had there been a project of such unparalleled ambition, and to date, it remains the world's largest collaborative biological project.

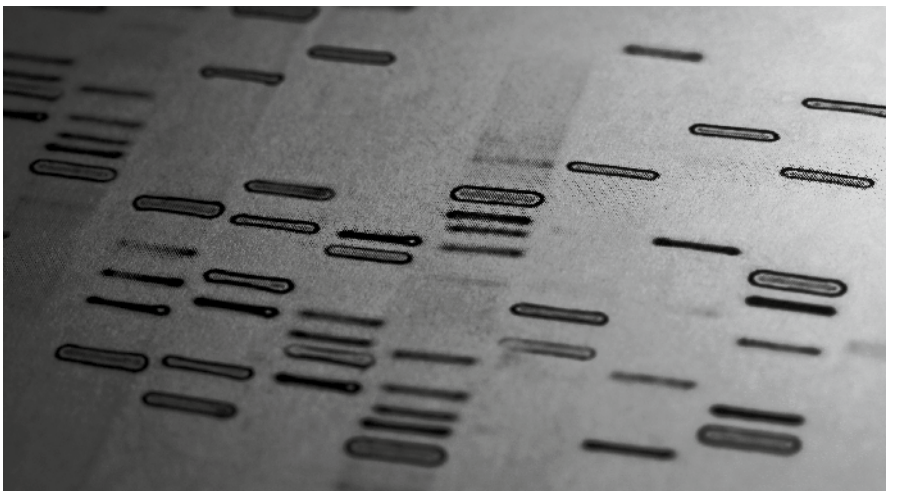
The impact of genomics on our knowledge of the underlying causes of epilepsy has been revolutionary and will continue to grow over the decades to come. Thirty years ago, epilepsy classification was crude. Almost all infantile onset epilepsies were lumped together while the vast majority of common epilepsies were labelled as 'idiopathic', which at that time meant 'unknown, presumed genetic'. Now, thanks to technology and international research collaboration, we recognise almost 200 separate genetic causes of rare and severe epilepsies caused by single genes. Progress is also being made in working out the more complex genetics of the common epilepsies too.

Fortunately, most professionals working with epilepsy won't need to remember all 200 genetic epilepsies. Around a dozen genes account for about 70% of genetic epilepsies: SCN1A, SCN2A, SCN8A, KCNQ2, KCNT1, SYNGAP1, STX1B, PCDH19, CHRNA4/B2, SLC6A1, SLC2A1. Nevertheless, every epilepsy professional should improve their genomic knowledge in order to keep up to date with evolving practice.

Health Education England is offering flexible and subsidised training [Genomics Education Training, 2019]. Comprehensive and up-to-date, expert-compiled information on many genetic epilepsies is also freely available at the National Center for Biotechnology Information (NCBI) Gene Reviews [Adam, 2019].

The most prominent impact has been on the diagnosis of infants with severe and rare types of epilepsy. The role and nature of genetic testing has rapidly transformed, and next-generation sequencing, either by targeted panel or whole exome sequencing (WES), is now considered a first-line investigation here. The diagnostic yield has correspondingly shot up to around 30-40% in this group. A genetic diagnosis can provide great comfort to many parents. It can allow them to better understand why their child has developed epilepsy and to know that there was nothing they did or didn't do that could have caused it. However, for others it may intensify feelings of anxiety, guilt, frustration and isolation. Skilful genetic counselling is essential prior to initiating genetic testing. It allows us to talk through the process, potential outcomes and benefits and limitations of genetic testing, and to support families through this part of their diagnostic odyssey. Through this process, you can find out what the patient and family know, what they want to know, manage expectations around genetic testing and identify those who might need extra support at this time. Counselling also allows a basis for calculating recurrence risks and discussing reproductive options in future pregnancies or among relatives, once the parental genetic status is known.

The consequences of a genetic diagnosis on treatment can be significant and will continue as a major area of growth and development over the next two decades. At present, a precise genetic diagnosis can suggest particular classes of treatment and in some cases, experimental therapies. The oldest examples include Glut-1 deficiency syndrome (SLC2A1 loss-of-function mutations) indicating ketogenic diet, and SCN1A loss of function in Dravet syndrome suggesting avoidance of sodium channel blocking agents. A pilot trial of transdermal nicotine patch is currently underway for sleep-related hypermotor epilepsy (SHE) [Epilepsy Research UK].



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As we learn more, we may be able to make reliable genotype-phenotype correlations, providing parents and health professionals alike with more certainty regarding prognosis and tailored treatment. But, at the moment, the correlation between specific genetic variants and particular clinical features and prognosis is variable and a matter for expert interpretation.

There are currently no published national practice guidelines on genetic testing for epilepsy. However, we suggest that, particularly for neonatal and infantile onset epilepsy or brain malformations, targeted panels or WES should be part of their first-line investigations [Sánchez Fernández *et al*, 2019]. Chromosome microarray yield is low and more relevant for patients with intellectual disability, autism spectrum disorder (ASD), dysmorphism or other neurological comorbidities. Single gene tests are best avoided due to extensive phenotypic and genetic heterogeneity, unless there is a strong clinical indication such as Dravet syndrome. But even in Dravet syndrome, several genes may mimic SCN1A while co-occurring sodium channel variants may modify the phenotype [Steel *et al*, 2017]. Whole genome sequencing (WGS) is not commonly used yet, but will likely be the next step in epilepsy genetic testing, in line with the new NHS Genomic Medicine Service [NHS.england.uk, 2019].

Aside from diagnostic purposes, genetic testing can also inform who not to give certain medications to. This is the field of pharmacogenetics. For example, HLA-B*15:02 testing should be carried out prior to commencing carbamazepine therapy for patients from high-risk populations (South and South-East Asia) to minimise the risk of adverse reaction [Amstutz *et al*, 2014]. In 2019, genetic testing is as quick and cheap as it has ever been, with some laboratories able to turnaround a WGS within one week. The technology only continues to improve. Unfortunately, our ability to interpret the results produced is still lagging somewhat behind, something that data gleaned from the 100,000 Genomes Project may help to improve in the future. Nevertheless, currently available genetic testing, appropriately selected after thorough patient phenotyping, has great potential. It can save time, money and reduce the number of other tests necessary to make a diagnosis [Oates *et al*, 2018]. This is a win for the health service and the family.

The Epilepsy Genetics Service, based at Kings Health Partners, has been running since 2015. It has two components: a specialist outpatient clinic and a molecular diagnostic service. The service is run by Deb Pal and Stephanie Oates. Deb Pal is a professor of Paediatric Epilepsy at Kings College London and an honorary consultant paediatric neurologist at Kings College Hospital and Evelina London Children's Hospital. Stephanie Oates is the first specialist genetic counsellor for epilepsy in England.

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The service accepts referrals from local and regional paediatric neurologists and paediatricians specialising in epilepsy. It sees patients and families diagnosed with complex, early-onset, intractable epilepsy, with

or without a family history. The service will also soon accept referrals for adults with a history of drug resistant or comorbid epilepsy since childhood. As well as evaluating children and families for genetic testing, the service offers pre and post-test counselling in accordance with American College of Medical Genetics and Genomics (ACMG) guidelines.

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For clinicians more experienced in offering their patients genetic testing, it also offers assistance post-test with interpretation and advice for the clinician. Post-test counselling for the patients and families is also available on request. The molecular diagnostic service is currently provided in collaboration with Amplexa Genetics, an ISO certified clinical laboratory in Odense, Denmark. However, it will likely move over to the NHS Genomic Medicine Service, in line with the rest of the country, when it officially opens.

Genetic counselling is defined as a communication process which aims to help individuals, couples and families understand and adapt to the medical, psychological, familial and reproductive implications of the genetic contribution to specific health conditions [National Society of Genetic Counselors' Definition Task Force, 2006]. The process includes, but is not limited to:

- Interpretation of family and medical histories
- Discussion and education to fill in any gaps in patient knowledge about their epilepsy
- Counselling to promote informed choices regarding testing, treatment, and reproductive options, with respect to the patient and family's goals, ethical and religious values
- Support to encourage the best possible future adjustment to the condition

Epilepsy genetics is somewhat different to many other genetic conditions commonly seen by genetic health professionals. The majority of patients who have variants in one or more of the 500 plus epilepsy and neurodevelopmental genes we know about are the only ones in their family to be affected (the variant(s) are *de novo*). The incidence of germline and somatic mosaicism is now understood to be more common than previously thought. However, generally, the option of testing is mainly focused on the benefit of the affected child, not cascade testing for the rest of the family. But in cases where mosaicism is suspected or proven, careful genetic counselling can be extremely valuable for parents thinking of extending their family, particularly if they wish to consider prenatal diagnosis.

As technology improves and the available testing options become more wide-ranging, obtaining fully informed consent is only becoming more challenging. While we still don't know what we don't know, patients need to have some understanding that we might find things that none of us were bargaining on. This is most relevant when considering WES or WGS. The ACMG has provided some guidance around this [REF] but genomics is an ever evolving field so it would be

important to remain informed and cautious and to 'watch this space'.

In the same way that sequencing each human chromosome was internationally distributed across research labs, so too are the efforts to build translational models for different genetic epilepsies. As well as the traditional rodent models, there now exist zebrafish and fruit fly models of genetic epilepsy that offer advantages of higher speed and throughput and lower cost than conventional rodent models. These new models allow scientists to examine individual genomic variants to determine pathogenicity and functional consequence. From there, researchers can try and target the underlying mechanism of these epilepsies, whether that be using already known or repurposed drugs, or developing new agents. In this era of personalised medicine, the focus of research interest is shifting along from gene discovery to the development of gene- or mechanism-specific treatments. Three examples give an idea of what future treatments of genetic epilepsies might look like.

In order to rescue a 'faulty' gene, one can try to replace the gene with a normally functioning one. Or, in the case of a dominant disorder (where only one faulty gene copy or allele is sufficient to cause disease), the expression of product from the normal allele could be increased. In a dominant disorder, we could also try to compensate by increasing the expression of other genes that reduce brain excitability. The first gene therapy trials are already underway for Rett syndrome [Clarke and Abdala Sheikh, 2018] and Duchenne muscular dystrophy [Duchenne UK, 2019]. Meanwhile, preclinical trials have shown that an engineered potassium channel KCNA1 can be replaced in epileptic mouse brains by smuggling the gene through the blood-brain barrier inside a lentivirus vector [Snowball *et al*, 2019]. Other groups are on the verge of introducing anti-seizure agents into the human brain through adeno-associated virus 9 (AAV9) vectors [Noe *et al*, 2012].

The ability to help patients and families to have realistic expectations of new technology, and to cope with uncertainty, is becoming even more important

Genes also have multiple intrinsic regulation systems, one of which involves tiny fragments of RNA known as microRNAs (miRNAs). These bind to the tail ends of genes to increase or decrease expression. A second strategy is to use miRNAs and their antagonists ('antagomirs') experimentally to stop status epilepticus in mice. But the major challenge remains around how to deliver these miRNAs to the brain [Henshall *et al*, 2016]. While there is

plenty of excitement in experimental cellular and molecular approaches, there are still a few practical clinical questions to address. Which part of the brain to treat, when and how often?

Advocacy and support organisations provide patients and families with the opportunity to connect, advocate and collaborate with researchers

Will irreversible treatments be safe in the long term? If they are invasive (such as involving brain injection), will the benefits outweigh the risks?

A third and quite left-field approach is to exploit the diversity of excitatory and inhibitory neuronal populations and redress the balance in over-excitable brain regions. Researchers at University of California San Francisco are perfecting the science of transplanting early stage inhibitory neurons into epileptic brains of mice [Grone and Baraban, 2015]. They will shortly be upscaling the technique in the naturally occurring epileptic sea lion native to the California coast!

How do patients access these new experimental therapies once they have a genetic diagnosis? The first step is to register with research databases and patient registries to contribute as much information as possible about these rare epilepsies [for example with the Epi25 collaborative]. Many advocacy and support organisations have formed to try and push development in treatments farther and faster. These organisations provide patients and families with the opportunity to connect, advocate and collaborate with researchers. This will be an invaluable resource when the time comes for clinical trials of new therapies.

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Further reading

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