European Journal of Medical Genetics 65 (2022) 104531

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Defining causal variants in rare epilepsies: an essential team effort between biomedical scientists, geneticists and epileptologists

Amy McTague [a,b,*], Andreas Brunklaus [c,d], Giulia Barcia [e], Sophia Varadkar [b], Sameer M. Zuberi [c,d], Nicolas Chatron [f], Elena Parrini [g], Davide Mei [g], Rima Nabbout [e], Gaetan Lesca [f]

[a] Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, Member of the ERN EpiCARE, London, UK
[b] Department of Neurology, Great Ormond Street Institute of Child Health, Member of the ERN EpiCARE, London, UK
[c] The Pediatric Neurosciences Research Group, Royal Hospital for Children, Member of the ERN EpiCARE, Glasgow, UK
[d] Institute of Health and Wellbeing, University of Glasgow, Member of the ERN EpiCARE, Glasgow, UK
[e] Department of Pediatric Neurology, Centre de Reference Epilepsies Rares, Hôpital Necker-Enfants Malades, Assistance Publique-Hôpitaux de Paris, Member of the ERN EpiCARE, Paris, France
[f] Department of Medical Genetics, Lyon University Hospital, Université Claude Bernard Lyon 1, Member of the ERN EpiCARE, Lyon, France
[g] Pediatric Neurology, Neurogenetics, and Neurobiology Unit and Laboratories, Meyer Children's Hospital - University of Florence, Member of the ERN EpiCARE, Florence, Italy

ARTICLE INFO

Keywords:
Epilepsy
Gene
Phenotype
Genotype
Multidisciplinary meetings

ABSTRACT

In the last few years, with the advent of next generation sequencing (NGS), our knowledge of genes associated with monogenic epilepsies has significantly improved. NGS is also a powerful diagnostic tool for patients with epilepsy, through gene panels, exomes and genomes. This has improved diagnostic yield, reducing the time between the first seizure and a definitive molecular diagnosis. However, these developments have also increased the complexity of data interpretation, due to the large number of variants identified in a given patient and due to the phenotypic variability associated with many of the epilepsy-related genes.

In this paper, we present examples of variant classification in "real life" clinic situations. We emphasize the importance of accurate phenotyping of the epilepsies including recognising variable/milder phenotypes and expansion of previously described phenotypes. There are some important issues specific to rare epilepsies – mosaicism and reduced penetrance - which affect genetic counselling. These challenges may be overcome through multidisciplinary meetings including epileptologists, pediatric neurologists, and clinical and molecular geneticists, in which every specialist learns from the others in a process which leads to for rapid and accurate diagnosis. This is an important milestone to achieve as targeted therapiesbased on the functional effects of pathogenic variants become available.

1. Introduction

Recent advances in genetic testing techniques have enabled the unprecedented discovery of hundreds of epilepsy genes and transformed the diagnostic journey for patients (Helbig and Lowenstein, 2013; Lesca and Depienne, 2015; McTague et al., 2016). These include *de novo* variants as well as somatic mosaicism and recessive disorders. NGS (next generation sequencing) is a powerful tool to perform a huge number of genetic tests in a large number of patients with epilepsy, including gene panels and exomes (Mei et al., 2017). This has had major consequences in improving the diagnostic yield, reducing the time between the first seizures and the definitive molecular diagnosis. Early testing has been shown to be particularly beneficial in young children with therapy resistant seizures, up to 50% of whom may have a single-gene cause identified (Berg et al., 2017; Symonds et al., 2019). More importantly, genetic diagnoses provide guidance towards specific treatment approaches in up to 80% of individuals with genetic diagnoses (Berg et al., 2017; Symonds et al., 2019). The value of genetic testing is not limited to diagnosis and treatment but also shortens the diagnostic odyssey for patients, prevents the performance of unnecessary investigations, assists

* Corresponding author. Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, Member of the ERN EpiCARE, London, UK.
E-mail address: a.mctague@ucl.ac.uk (A. McTague).

https://doi.org/10.1016/j.ejmg.2022.104531
Received 28 December 2021; Received in revised form 1 May 2022; Accepted 19 May 2022
Available online 23 May 2022

genetic counselling and provides families with an "answer" enabling them to readjust their goals and expectations (Brunklaus et al., 2013)

However, these developments have also greatly increased the complexity of data interpretation, due to the large number of variants identified in a given patient and due to the phenotypic variability associated with many of the epilepsy-related genes. Some cases are also difficult to interpret due to scarce literature available for recently identified genes. To allow more rational interpretation of genetic data, some guidelines for variant interpretation are now available such as those proposed by the American College of Medical Genetics (Richards et al., 2015). These include guidance on the numerous *in silico* tools which can be used to classify variant pathogenicity. However, except for obvious cases, data interpretation is usually complex in patients with epilepsy and there are many pitfalls including multiple variants of unclear significance and non-specific or variable phenotypes. These issues may be overcome using multidisciplinary meetings including epileptologists, pediatric neurologists, and clinical and molecular geneticists, in which every specialist learns from the others in a more formal and on-going process. In this paper, we will share the views and experiences of geneticists and epileptologists involved in the research and diagnosis of Mendelian epileptic disorders from different European countries.

2. Experience of the authors' teams

In this section, we will describe the respective experiences of the centre of each of the authors participating in this paper. This illustrates the evolution in parallel of these groups and their processes and development towards a common use of new diagnostic tools. Our experience demonstrates the importance of multidisciplinary teams for discussion of complex cases, before and after genetic results.

2.1. University Hospital of Lyon

At the University Hospital of Lyon, France, we began multidisciplinary team (MDT) working on genetic testing in patients with epileptic disorders in 2010. The MDT includes medical geneticists (clinical and laboratory), neuropediatricians and electrophysiologists. Meetings occur on a monthly basis and are attended by 8–15 people from the three departments (including students and residents). EEGs and video EEGs are shown during the meetings. At the beginning, we only performed molecular testing for certain genes in our lab: *CSTB, EPM2A, NHLRC1, MECP2, CDKL5, STXBP1, SCN2A, KCNQ2, KCNQ3, LGI1* and *GRIN2A* and karyotype with FISH for some microdeletion syndromes with rapid replacement by array CGH. In 2015, in coordination with the other groups participating in the French EPIGENE Network, we started our gene panel (including 86 genes) that has since been updated four times. As a result single gene testing was abandoned immediately. The current panel includes 144 genes and is today performed with a rapid turnover for patients with early-onset epilepsies. Trio-based whole exome sequencing(WES) was performed in a subset of patients, mainly with early-onset epileptic encephalopathies. These patients now have access to whole-genome sequencing by the AURAGEN platform (https://www.auragen.fr/). After fragmentation with a Covaris L220plus, fragments are prepared with TruSEq DNA PCR-free (Illumina) and sequenced on a NovaSEq 6000 (Illumina). Alignments are performed with BWA-MEM, data calling with HaplotypeCaller (GATK) and filtering and annotation with Variant Effect Predictor. *CSTB* dodecamer expansions are still studied by specific methods.

2.2. Great Ormond Street Hospital, London

At Great Ormond Street Hospital, London, UK, since 2011 we have undertaken NGS panel testing in our epilepsy patients with diagnostic rates of 20–40%, with higher rates in patients presenting before two months of age . From 2021 trio (patient and parents) -based whole genome sequencing (WGS) has been available on a diagnostic basis for patients with early onset epilepsy. In addition, we have had access to rapid trio-based WES for a small number of children in the neonatal or paediatric intensive care setting. From 2018 we have delivered a multidisciplinary clinic with a paediatric epileptologist, clinical geneticist with an interest in epilepsy and a developmental paediatrician. In this clinic we see patients with a possible genetic diagnosis and aim to understand the significance of the variant in the context of the patient's epilepsy and developmental phenotype. In 2020 we launched the Epilepsy Genomics MDT, which is co-chaired by a paediatric epileptologist and clinical geneticist and attended by a clinical scientist from the genetics laboratory, paediatric neurologists, clinical nurse specialists and trainees. We aim to help clinicians with interpretation of the variants including pathogenicity, need for parental testing and referrals for formal clinical genetics opinion.

2.3. Meyer Children's Hospital, Florence

At Meyer Children Hospital, Florence, Italy, in 2006, we started the neurogenetic service for the genetic diagnosis of patients with epilepsy and cerebral malformations. At the beginning, we only performed genetic testing by Sanger sequencing for a limited number of epilepsy-related genes (<15). Array CGH, karyotype and FISH for microdeletion syndromes were performed by the Genetic Laboratory of our Institution. In 2011, we offered our first gene panel (including 36 epilepsy-related genes) that was since updated six times. Our "in use" trio-based gene panel now includes about 400 genes associated with idiopathic and syndromic epilepsies. In addition, we also perform trio-based WES in a subset of patients, mainly with early-onset epileptic encephalopathies or complex phenotypes including epilepsy and developmental disorders. Since 2011, we established an MDT to systematically discuss the genetic findings from patients with epileptic disorders. These meetings are organized by the head of the Neurogenetics Laboratory and involve neurologists and child neurologists, geneticists, molecular biologists and laboratory technicians. Meetings occur on a monthly or bi-weekly basis, with 8–12 people from the Neurology department usually attending (including students and residents). Molecular/genetic and clinical data, EEGs, video EEGs and brain MRIs are shown during the meetings. Genetic findings are correlated to patient's phenotype in order to improve variants classification, to plan additional testing and to help clinicians in the patient's management and genetic counselling.

2.4. Royal Hospital for Children, Glasgow

At the Royal Hospital for Children in Glasgow, UK, we started the genetic epilepsy service with *SCN1A* testing in 2004/5 and over time added 9 other single genes to our testing repertoire as well as performing karyotype and array CGH analysis. A specific *SCN1A* referral form was developed including key electro-clinical data to aid variant interpretation. The paediatric neurologist met lab scientists weekly to discuss referrals and results. In 2014 we established our 104-epilepsy gene panel and developed an electronic referral form which is mandatory prior to testing and captures clinical data to aid variant interpretation. The genetic epilepsy MDT meetings now include genetic scientists, clinical geneticists, paediatric neurologists and a genetic epilepsy nurse specialist meeting every 2 weeks. Scientists prepare the variant information and collate them according to ACMG criteria in an excel spreadsheet. We have developed an Access database including key clinical features at presentation for each gene and hyperlinks to relevant literature. Before each meeting clinical and genetic data on class 3, 4 or 5 variants are reviewed by the clinical geneticist and paediatric neurologist to prioritize cases for MDT discussion. During the meeting the clinical phenotypes and genetic data are presented, followed by a discussion about clinical significance, classification and further steps. If indicated, patients, paediatric and adult, are offered an appointment in a

joint genetic epilepsy clinic that is attended by the child neurologist, clinical geneticist and genetic epilepsy nurse specialist. We have access to developmental exome testing and whole genome sequencing on a research basis. In 2021 we expanded our panel testing to around 450 genes associated with epilepsy.

2.5. Necker -enfants Malades University Hospital, Reference Centre for Rare Epilepsies

In 2009 we began to perform Sanger sequencing for a limited number of epilepsy-related genes (*SCN1A, STXBP1, KCNQ2, SLC25A22, KCNT1*) in the Molecular Genetics Laboratory. Array CGH, karyotype and FISH for microdeletion syndromes were performed by the Cytogenetic Laboratory of our Institution for the preceding 15 years. In 2015, we introduced our gene panel (including 150 epilepsy-related genes) that was updated once in 2018. This trio-based gene panel now includes about 200 genes associated with early onset epilepsies (including subgroups of developmental and epileptic encephalopathies, self limited epilepsies in neonates and infants, myoclonic epilepsies and progressive myoclonic epilepsies, idiopathic and generalized epilepsies). Single gene testing was abandoned in 2015. We tested about 650 patients with a diagnostic rate of 25–40% depending on the electro-clinical phenotype and the epilepsy syndrome. We perform trio-based exome sequencing in a subset of patients, mainly with early-onset epileptic developmental encephalopathies or complex unclassified phenotypes including epilepsy and developmental disorders. We also initiated rapid trio exome sequencing for a small number of children in the setting of "urgent" genetic counselling for couples having a child with pharmacoresistant epilepsy and developmental and epileptic encephalopathies (DEEs) of unknown aetiology.

Since 2010, we established an in-house multidisciplinary bi monthly team meeting involving child neurologists, geneticists, molecular biologists, neuroradiologists, electrophysiologists, laboratory technicians, engineers, and residents to discuss the phenotype before the patient inclusion in the panel and to discuss the results obtained. Molecular/genetic and clinical data, EEGs, and brain MRIs are presented during the meetings. Genetic findings are correlated to the patient's phenotype in order to improve variant classification, to plan additional testing and to direct patient's management and genetic counselling, within a large program of genetic counselling at our institution. Some cases need further discussion at the national reference centre for rare epilepsy network (CRéER).

Since 2020, the "French Plan for Genomic Medicine 2025" provides access to whole-genome sequencing by two national platforms (Sequoia, https://www.seqoia.fr/, and Auragen, www.auragen.fr) for patients with early onset (<2 years) DEE and pharmacoresistent epilepsy with negative "first step" analyses (NGS panel and CGH-array). The inclusion of the trio is discussed in a national multidisciplinary virtual meeting to confirm the complete phenotyping and exclude any diagnosis that might be easily made by a biochemical or radiological test. The result of the genetic testing is delivered to the family by the referring physician and the geneticist in charge of the epilepsy genetic program.

3. Variant classification in "real life" clinic situations

In the last years, with the advent of next generation sequencing, we have been able to perform a huge number of genetic testing in patients with epilepsy including gene panels and exomes (Mei et al., 2017). This has also increased the complexity of data interpretation. While some guidelines for variant interpretation are now available, such as those proposed by the ACMG, a multidisciplinary approach for a meaningful interpretation is essential. For example, the only criterion designated with Very Strong strength level for pathogenicity in the ACMG guidelines was PVS1, which was defined as "null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss-of-function (LoF) is a known mechanism of disease" (Richards et al., 2015). This criterion has been refined by the ClinGen Sequence Variant Interpretation (SVI) Workgroup to provide a decision tree with specific considerations for the different types of loss of function variants, the evidence for the likelihood of a true null effect, and criteria identifying the genes for which PVS1 can be applied (Abou Tayoun et al., 2018).

The first example is a 10 year old boy with language delay, dysmorphic features, and, since the age of six-years, seizures, who was referred for epilepsy gene panel analysis. We identified a heterozygous stop gain variant in the *GNAO1* gene (NM_020998.2). The variant was inherited from the heterozygous healthy father and was absent from the gnomAD dataset. *De novo GNAO1* mutations were initially identified in children with early-onset epileptic encephalopathy and severe developmental delay, with later development of dyskinetic movement disorders, and in children exhibiting developmental delay and severe dyskinesia without seizures (Schirinzi et al., 2019). In particular, *GNAO1* loss of function (LoF) variants have been linked to epilepsy (Feng et al., 2018). The *GNAO1* LoF variant we observed was challenging since it was inherited from a healthy father while *GNAO1* pathogenic variants are often observed as *de novo* events. In addition, the patient's phenotype was different from those described in *GNAO1* clinical spectrum. The *GNAO1* gene is considered highly intolerant to LoF variants (pLI = 0.99). However, looking at the pext score, a transcript-level annotation metric based on GTEx v7 dataset known as the "proportion expressed across transcripts" and summarizing isoforms expression status of exonic regions across tissues (Cummings et al., 2019), we observed that the exon harbouring the LoF variant we identified was not expressed in brain (gnomAD) and that two gnomAD individuals harbour a canonical donor site variant affecting the same exon. These observations prompted us to reclassify the variant from likely pathogenic to variant of uncertain significance.

The clinical and genetic findings review by the MDT is therefore really important and recommended. Indeed, a variant classified using only a molecular grading or a clinical grading, has a higher risk to be misclassified. One nice example is represented by the *GRIN1*-related neurodevelopmental disorder, a condition characterized by developmental delay/intellectual disability and other additional common features including epilepsy, hypotonia, spasticity, movement disorders, feeding difficulties, and behavioural manifestations (Lemke et al., 2016). The *GRIN1*-related neurodevelopmental disorder has been linked either to an autosomal dominant or autosomal recessive inheritance model. The dominant disorder is caused by *de novo* heterozygous missense *GRIN1* variants whereas the recessive disorder is due to biallelic missense or truncating variants (Platzer and Lemke 1993). For instance, a single heterozygous *GRIN1* truncating variant can be classified as likely pathogenic if only the molecular grading is used. However, the same variant would probably be reclassified as VUS/likely benign if a clinical grading interpretation were added to the whole picture. Indeed, most pathogenic *GRIN1* variants tend to be missense and not truncating. For instance, a *GRIN1* stop gain variant can result in fatal autosomal recessive neurodevelopmental disorder with hyperkinetic movements and seizures in biallelic affected patients but it does not result in any symptoms in the healthy heterozygous parents, thus suggesting that heterozygous truncating mutations and haploinsufficiency for *GRIN1* do not result in a neurologic phenotype (Lemke et al., 2016).

Molecular pathology can be very different from one gene to another. Some genes are affected by missense variants because their pathogenic mechanism is related to a gain of function mechanism, such as *KCNT1*. Heterozygous stop or frameshift variants of this gene can be found in control individuals. Most genes, however, are usually intolerant to loss of function. This is the case for *CDKL5* which encodes a serin-threonine protein kinase causing DEE, usually affecting females, and less frequently but more severely males. In a female patient with early-onset epilepsy and psychomotor regression, a one-base deletion in exon 19 (of 21), expected to lead to a frameshift, was identified using Sanger sequencing. Family testing revealed that it was inherited from the

unaffected mother. Analysis of the chromosome X inactivation showed the absence of bias in mother and daughter. In the same patient, a NGS gene panel for epileptic disorders showed a *de novo* missense variant in the *ARX* gene, involving a highly conserved amino-acid located in the functionally important homeodomain. This latter variant was consistent with the clinical phenotype, which included hypoplasia of the corpus callosum, and was then considered as likely pathogenic. Consultation of the gnomAD database showed that while *CDKL5* is globally intolerant to loss-of-function variants, the rare LoF variants that are present cluster in last four exons, as in this patient. Another challenging interpretation involving the *CDKL5* gene was related to a variant identified by trio-WES analysis in a three-years old girl with developmental and epileptic encephalopathy (DEE), characterized by refractory seizures of multiple types (age at onset 15 months), acquired microcephaly, developmental delay, autistic features with abundant stereotypies, and gastrointestinal problems. The patient harboured a *de novo* intronic heterozygous variant (+19G > A) in the *CDKL5* canonical transcript, absent in the gnomAD dataset, and without additional significative variants in other genes. This variant, although *de novo*, was classifiable as VUS/likely benign. However, in the non-canonical *CDKL5* transcript (NM_001323289.1) the variant resulted in a stop gain substitution. This isoform is the most expressed in brain and another pathogenic variant has been identified in the same region (Bodian et al., 2018). These observations, and the patient phenotype which is consistent with a *CDKL5*-related disorder, prompted us to reclassify the variant as likely pathogenic. This highlights that if we had only used the *CDKL5* canonical transcript, it would have led us to the misinterpretation of the variant and also emphasises the importance of considering the clinical phenotype in variant classification.

These examples demonstrate how genetic findings in "real life" practice are often difficult to interpret in a clear-cut way and how a multidisciplinary framework is helpful in this process.

4. The importance of accurate phenotyping of the epilepsies: variable/milder phenotypes, expansion of previous phenotypes

In this section we will consider the importance of understanding the range of phenotypes associated with genetic variants and the impact on variant interpretation and counselling.

One of the benefits of receiving an accurate genetic diagnosis can be to help parents and carers understand the likely prognosis for their child (Poduri, 2017). However, one of the difficulties is the inherent ascertainment basis of gene discovery in rare diseases such as developmental and epileptic encephalopathies (DEEs), where the most severely affected children may be prioritised for genetic testing. As a result, initial case reports or case series can represent a skewed picture rather than the full phenotypic spectrum. The following case examples illustrate how the understanding of the complete range of associated phenotype can evolve with subsequent testing of wider populations.

The first example is of a patient who presented at 6 months of age with two episodes of pallor and jerking, initially thought to be reflex anoxic seizures. At 10 months of age there were episodes of facial grimacing, opisthotonus, clonic jerks and perioral cyanosis. Initial EEG was normal. During a febrile illness, clusters of these seizures characterised by prominent autonomic involvement with facial reddening, drooling, apnoea and post-ictal bradycardia emerged and the patient was loaded with intravenous levetiracetam. EEG-video monitoring captured a typical seizure with initial tonic phase followed by clonic jerking, then a brief period of apnoea and bradycardia and post-ictal suppression of the EEG. After a 4 month period of seizure freedom, afebrile generalized tonic-clonic seizures with post-ictal cyanosis re-emerged. Despite these frequent seizures, the patient's developmental progress continued to be normal with age-appropriate scores on Bayley III testing at 2 years of age. The patient also developed an intermittent mild tremor and ataxia occurring 2–3 times per week from 2.5 years of age. An 82 gene NGS panel identified a novel heterozygous missense variant in the inactivation gate region between domains III and IV of *SCN8A*. This was predicted to be damaging by most *in silico* tools and was absent from population databases including gnomAD. Parental testing confirmed it was *de novo*. Following the diagnosis, lamotrigine was added with good effect and the patient has now been seizure free since 26 months (current age 4 years). When this variant was identified in 2018, *SCN8A*-associated epilepsy phenotypes had been largely reported in the context of either self-limited familial infantile seizures or severe developmental and epileptic encephalopathy with poor developmental progress and outcome (Larsen et al., 2015), neither of which was the clinical picture in our patient. Otherwise the phenotype in this case was reminiscent of *SCN8A*-related epilepsy with prominent autonomic features including bradycardia (Trivisano et al., 2019; Zawadzka et al., 2020). However in 2019, a further series of patients with "intermediate-spectrum" SCN8A related epilepsy was reported, including children with normal or mildly impaired developmental progress (Johannesen et al., 2019) and including movement phenotypes such as ataxia and paroxysmal dyskinesias. Further reports have now clarified there is a broad phenotypic spectrum including early onset severe DEE, mild to moderate intellectual disability with treatable epilepsy and self-limited infantile epilepsies and even phenotypes without epilepsy (Gardella and Møller, 2019; Zaman et al., 2019). Until now there has been no clear genotype: phenotype correlation to enable prediction of disease course, however there is emerging evidence of a less severe gain of function in intermediate or milder phenotypes (Johannesen et al., 2021; Liu et al., 2019). Better stratification of disease-associated phenotypes will continue to inform counselling for affected children and their families, and may allow earlier appropriate use of sodium channel blocking medications.

In the second example, the patient had onset of seizures at 6 months of age, characterised by brief facial and upper limb myoclonus. On occasion these evolved to bilateral clonic seizures of upper limbs. Over time seizure semiology evolved to include nocturnal seizures with facial grimacing and vocalisation, facial and limb myoclonus and atypical absence seizures. The patient was microcephalic with head circumference below the 0.4th centile and had mild global developmental delay with independent walking from 2 years and delayed speech development. A diagnosis of autistic spectrum disorder was made at 7 years of age. There was no developmental regression but continued slow progress. From 2 years of age, an ataxic, broad-based gait was noted. Interictal EEGs at 2 and 4 years of age were normal. A video-EEG at 10 years of age captured a typical event with eyelid flickering and myoclonus affecting face and hands, associated with generalized spike-wave discharges time-locked to myoclonic seizures. By 12 years of age the patient was stable with infrequent nocturnal seizures on a combination of sodium valproate and ethosuximide. There was steady developmental progress and the patient was independently mobile with subtle ataxia and a limited vocabulary. An 82 gene NGS panel identified a heterozygous variant in *KCNC1*, p.Ala421Val, which was not maternally inherited (paternal sample not available). All *in silico* tools predicted the variant to be pathogenic and it was absent from databases of normal variation. The laboratory report cited this variant as possibly being associated with progressive myoclonus epilepsy type 7 (MIM 616187). In 2015, a recurrent heterozygous variant in *KCNC1* had been identified in a new form of progressive myoclonic epilepsy, myoclonic epilepsy with ataxia and potassium channel mutation or MEAK (EPM7, MIM 616187) (Muona et al., 2015). Clinical features included onset of myoclonus in mid to late childhood and loss of independent ambulation by adolescence or early adulthood (Nascimento and Andrade, 2016; Oliver et al., 2017). While this patient did indeed have a myoclonic epilepsy with ataxia and developmental delay, the epilepsy had been pharmacoresponsive to some degree and there did not appear to be a progressive or neurodegenerative disorder. Following the initial reports, a different phenotype was noted in two studies in association with the p. Ala421Val variant identified in our patient with a DEE-like presentation with earlier onset seizures, a non-progressive ataxia and retention of independent walking and intelligible speech (Cameron et al., 2019; Park

et al., 2019). Recently, the MEAK variant R320H was shown to impair dendrite development and interneuronal function in vitro (Carpenter et al., 2021). Although the p.A421V variant has not been modelled in this system, this reflects a possible developmental impact of *KCNC1* dysfunction beyond a channelopathy and in keeping with an early onset DEE.

Gene identification has traditionally been associated with research teams working on patients recruited according to a given phenotype. As in the previous two examples, further studies may show that the phenotype associated with a gene is broader than initially thought. However, when the clinical phenotype is not consistent with those previously reported for a given gene, it is important to review carefully the genetic data before concluding there is a broader phenotype related to mutations in that gene. The third example is a male patient born to unrelated parents at term. He presented with myoclonic jerks when falling asleep at birth, which were first considered benign neonatal myoclonus. The frequency increased and the myoclonus occurred on a daily basis. He had global motor delay with ataxia. At the age of seven, he had no language and no joint attention. Repeated EEG recordings showed polyspike bursts spikes associated with myoclonus, as well as non-epileptic myoclonus. No photoparoxysmal response was observed. Brain MRI was normal. *FMR1* testing showed a normal CGG allele. Array-CGH was normal. A 145-gene panel showed two different missense variants in *PRICKLE1*. Each was inherited from one parent. *In silico* predictions were contrasted. They were reported 6 and 7 times, at the heterozygous state, in the gnomAD database of control individuals, respectively. *PRICKLE1* was included in our gene panel because it has been previously associated with progressive myoclonus epilepsy (Bassuk et al., 2008). Further publications suggested an expanding phenotype, including different conditions, such as early-onset epileptic encephalopathies (Mastrangelo et al., 2018). However, after having carefully reviewed the case in the MDT meeting, we considered that the arguments in favour of pathogenicity for each variant taken separately were not sufficient to make a definite diagnosis. We decided to perform trio exome sequencing which showed a *de novo* recurrent missense variant in *PPP2R5D*, which was considered as pathogenic, explaining the patient's phenotype (Houge et al., 2015).

5. Issues specific to rare epilepsies – mosaicism and reduced penetrance - which affect genetic counselling

In this section, we will consider two of the complex issues that can have a major impact for genetic counselling: mosaicism and reduced penetrance.

Mosaicism can manifest in different ways, depending on the time of the mutation event. The first example is a family with an index case presenting with focal seizures at 8 days of life, followed by West syndrome at 3 months. By gene-panel analysis, we found a heterozygous nonsense pathogenic variant of *STXBP1*. This variant has been recurrently reported in several patients. At the time of diagnosis, the 31-year-old mother has just begun to experience generalized seizures with normal interictal EEG. She was treated with lamotrigine. She had no cognitive impairment and normal neurological examination. The diagnosis of idiopathic generalized epilepsy was considered before genetic testing was performed in her daughter. Sanger sequencing showed the *STXBP1* in the mother's blood in a 25% mosaic state. In this case, the variant has occurred as a *de novo* mutation in the post-zygotic state in the mother. The presence of a normal allele corresponding to >50% was correlated with a milder phenotype compared to her daughter.

The second example is from a family with a male child exhibiting, from his first hours of life, a drug resistant epilepsy characterized by short-lived, self-limiting versive tonic seizures, associated with flushing and desaturation, sometimes followed by focal clonic jerking. His EEG showed a burst-suppression pattern. Brain MRI was normal except for a thin corpus callosum in its anterior third (Spagnoli et al., 2018). An epilepsy gene-panel analysis identified a recurrent heterozygous in-frame deletion of *KCNQ2* resulting in a single amino acid deletion in the 6th transmembrane domain. Sanger sequencing performed in the healthy parents was negative and therefore the variant was deemed *de novo*. A subsequent prenatal analysis performed on the chorionic villus sampling-derived DNA from the mother identified the same variant at a heterozygous state. These findings raised the possibility of a gonadal-limited or somatic mosaicism in one of the parents. The deep sequencing of the *KCNQ2* variant in parental DNA unravelled the pathogenic variant as a low-grade somatic mutation in the mother (8% of the variant allele). This indicated that the variant indeed arose in the mother, at the postzygotic stage, as a *de novo* event. Since Sanger sequencing is unable to detect mosaic alleles below a threshold of 15–20% (Rohlin et al., 2009), this approach missed the variant in the mother and under-estimated the risk of recurrence in further children.

However, the level of mosaicism observed in blood DNA does not always reflect the situation in the brain. Some patients may be asymptomatic with a high level of mutated allele (Milh et al., 2015) while other patients may have a severe disease with a low-grade mosaic variant. This is due to the fact that the percentage of mutated allele may vary from one tissue to another. We observed a male patient exhibiting a severe developmental and epileptic encephalopathy (DEE) with multifocal seizures starting at the age of 11, with autistic features and a missense variant of *GABRB3* in a 20% mosaic state (Møller et al., 2017. This variant was located in the first transmembrane domain, was absent from gnomAD database of control individuals, and was considered likely pathogenic.

Somatic mosaicism in parents (affected or not) is important to consider for genetic counselling as it is associated with a higher recurrence risk. Ideally, parents should be analysed by massive parallel sequencing as a trio (proband and parents) or with methods also able to identify low-grade somatic mosaicism in blood-derived DNA (i.e. amplicon deep sequencing, droplet digital PCR, etc). However, it is important to keep in mind that even in the case of a *de novo* mutation, the recurrence risk for a future child is not null, since we cannot rule out a mosaicism not detectable in blood but present in the germline. Therefore, prenatal diagnosis should be discussed with the parents.

Another complex issue for genetic counselling is related to intrafamilial phenotypic variability. Such a phenomenon is well-known in families with GEFS+ (Generalized epilepsy with febrile seizures plus), usually caused by pathogenic variants in *SCN1A*, in which one family member may have a more severe phenotype, such as Dravet syndrome (Depienne et al., 2010). In some situations, the intrafamilial variability may be more extreme, leading to incomplete penetrance. We will consider two examples. The first one is a patient presenting with Lennox-Gastaut syndrome with severe cognitive regression, self-injurious episodes and hand stereotypies. Gene panel analysis showed a stop variant in exon 7/9 of *GABRB3* that was considered to be pathogenic (Møller et al., 2017). *GABRB3* is expected to be intolerant to loss-of-function variants (pLI = 0.98). However, this variant was also found in the unaffected father and brother. Trio-based WES did not reveal additional pathogenic variants. Although stop and frameshift variants are found in affected individuals, they are likely to have more variable consequences than some missense variants, including variable penetrance and therefore should not be always considered causative. The second example is the case of a 25-year-old female with an history of epilepsy of infancy with West syndrome. Gene-panel analysis showed a missense variant p.Met896Val in *KCNT1* (NM_020822.3). The variant was absent from the gnomAD database of control individuals. The amino acid involved was a hot-spot for pathogenic variants found in patients: p.(Met896Ile), p.(Met896Arg), and p.(Met896Leu) (Møller et al., 2015). Considering these findings, it was classified as a pathogenic variant. However, the segregation analysis performed in the family showed that the variant was inherited from the mother and was also carried by one sister and one brother, who were healthy. Trio-based WES did not find additional pathogenic variants.

There are an increasing number of situations of Mendelian epilepsies

with incomplete penetrance. This could be due to protective genetic factors in the unaffected individuals that remain to be identified. These situations not only make the classification of the variant in question difficult but also makes it difficult to predict the risk for the future child of an unaffected carrier in the family.

6. Working together for rapid diagnosis

The interpretation of putative disease variants is often challenging as different genes can lead to the same epilepsy syndrome whereas one gene might be associated with many different phenotypes (Brunklaus et al., 2020; Steel et al., 2017). The generation of vast amounts of genetic data reveals numerous variants whose pathogenicity has to be carefully determined. Genetic data alone often do not provide a clear answer as many variants are of uncertain significance and have to be interpreted in the context of the clinical information. Clinicians may not have the "genetic literacy" to confidently interpret genetic findings and vice versa genetic scientists and geneticists are often not very familiar with the nuances of the epilepsy phenotype. Prompt and accurate detection and interpretation of genetic testing results therefore poses a challenge. The following clinical examples highlight some of the challenges we encounter in day-to-day practice in pathogenicity interpretation and emphasize how important epilepsy genetic MDT working is to facilitate rapid diagnosis and treatment.

The first case is a term neonate born following an uneventful pregnancy and normal delivery presented on day 3 with tonic posturing associated with focal clonic jerking. Seizures were frequent (10 per day) and difficult to control for several weeks. A 104 epilepsy gene panel was performed and reported as normal, as was array CGH testing. The case was discussed at the epilepsy genetic MDT. Review of the epilepsy phenotype revealed a high suspicion of self-limited familial neonatal epilepsy (SELNE) and *KCNQ2* MLPA was requested revealing a whole gene deletion in keeping with the infant's presentation. The child was started on carbamazepine treatment and became seizure free. This case illustrates that a negative gene panel does not exclude a diagnosis in the genes examined. A significant minority of variants are caused by deletions that cannot be easily detected by current gene panel analysis or Sanger sequencing and require gene dosage (MLPA) analysis. If the phenotype is convincing of a particular genotype, specific dosage testing should be undertaken.

The second case is a patient who presented at 13 months of age with clusters of focal seizures, with eye deviation to the left as the only subtle lateralising feature to the right hemisphere. There was a background of developmental concerns from 8 months of age. Seizures were associated with significant apnoeas and became frequent requiring hospitalisation. The patient had an urgent pre-surgical assessment which revealed possible grey-white matter blurring in the right parietal area. Ictal EEG showed subtle lateralisation to the right hemisphere, although independent left sided inter-ictal discharges were also seen. The patient proceeded to urgent right temporo-occipital disconnection in view of seizure burden. There was a good initial response to surgery allowing discharge but seizures recrudesced. Histology was non-specific. An 82 gene panel for early-onset epileptic encephalopathies had been sent during initial work-up and subsequent to surgery revealed a heterozygous *de novo* nonsense variant in *SMC1A*. *In silico* modelling and absence from gnomAD resulted in classification as a pathogenic variant. Heterozygous truncating variants in *SMC1A* are associated with seizure onset in the first 2 years of life occurring in clusters and with severe developmental delay (Symonds et al., 2017). If the genetic result had been available earlier it may have modified pre-surgical counselling. This case illustrates the importance of rapid availability of genetic results, particularly in complex patients with treatment-resistant seizures.

The last case is an infant presenting with a history of early onset seizures in the first week of life including brief tonic, focal clonic seizures and apnoeas. There was a family history of neonatal onset seizures. Early brain imaging and neurometabolic investigations were normal and ictal EEG revealed focal discharges with secondary generalization. NGS gene panel testing detected a class 3 variant of uncertain significance (VUS) in *KCNQ3*. The phenotype appeared convincing; however, the variant was not. Subsequent array CGH picked up a deletion encompassing *KCNQ2* which explained the phenotype. *KCNQ3* is a well-recognised cause of SELNE and the child's phenotype was entirely in keeping with a *KCNQ3*-related epilepsy. However, epilepsy genetic MDT review revealed that the *KCNQ3* variant was a VUS and did not fulfil the ACMG criteria for pathogenicity. It is important to consider alternative genetic causes, such as the *KCNQ2* deletion in this case, if the genotype is not convincing. This is particularly the case in epilepsy syndromes such as SELNE that can be caused by pathogenic variants in different channel genes (e.g., *KCNQ2* and *KCNQ3*) and different variants in the same gene can cause distinct phenotypes (e.g., *SCN2A*) (Heron et al., 2002; Singh et al., 1998).

7. Conclusion

The cases shown in the previous sections illustrate that the swift detection and correct interpretation of genetic findings requires efficient genetic epilepsy MDT working, facilitating a discussion about variant classification, its clinical significance and potential implications for treatment and genetic counselling. Representation from genetic scientists and clinical geneticists ensures accurate genetic interpretation, whilst the neurologist/epileptologist offers clinical judgement on the epilepsy presentation. Genetic counsellors and genetic epilepsy nurse specialists have an important role in providing patients with accurate information and support. This model of working can be extended to the clinic where patients benefit from a joint clinical review in an epilepsy genetic MDT clinic attended by a neurologists/epileptologist, clinical geneticist and genetic epilepsy nurse specialist.

In some countries, the local MDT groups are integrated in a national network to discuss the complex cases and harmonize the practices in interpretation of clinical and molecular data. This is the case in France with the EPIGENE network and in Italy with the Telethon Undiagnosed Disease Program. The European Rare Diseases Network (ERN) EpiCARE also supports some actions in this field, including: i) a series of teaching webinars delivered jointly by a geneticist and epileptologist focussing on the most frequent genetic epilepsies, ii) on-line multidisciplinary case discussions including genetic findings, and iii) a secure interface to connect the different genotype/phenotype databases of the participants.

As we move from the genetic to the genomic era with the increasing application of whole genome sequencing, it is likely that the number of putative variants, both coding and non-coding, identified per patient and resultant diagnostic complexity will only increase. It is vital that geneticists, epileptologists and clinical scientists continue to work and learn together as one team to provide our patients and their families with the most up to date treatment options, appropriate genetic counselling and accurate prognostic information.

CRediT authorship contribution statement

Amy McTague: Conceptualization, Writing – original draft, preparation, Writing – review & editing. **Andreas Brunklaus:** Writing – original draft, preparation, Writing – review & editing. **Giulia Barcia:** Writing – original draft, preparation, Writing – review & editing. **Sophia Varadkar:** Writing – review & editing. **Sameer M. Zuberi:** Writing – review & editing. **Nicolas Chatron:** Writing – review & editing. **Elena Parrini:** Writing – review & editing. **Davide Mei:** Writing – original draft, preparation, Writing – review & editing. **Rima Nabbout:** Writing – original draft, preparation, Writing – review & editing. **Gaetan Lesca:** Conceptualization, Writing – original draft, preparation, Writing – review & editing.

Acknowledgements

We sincerely thank all of the patients and families that have made this collection of variant classifications in “real life” clinic situations possible. We thank Federazione Italiana Epilessie and Associazione Trenta Ore per la Vita for supporting our research into genetics of epileptic encephalopathy (D.M.). DM is also supported by the Italian Ministry of Health (RF-2019-12370059). AM is supported by grants from the Medical Research Council (MR/T007087/1), the Rosetrees Trust and Great Ormond Street Hospital Children’s Charity (VS0122). AM is also supported by the NIHR GOSH BRC: the views expressed are those of the author and not necessarily those of the NHS, the NIHR or the Department of Health. This work is supported by the ERN for rare and complex epilepsies (EpiCARE).

References

Abou Tayoun, A.N., Pesaran, T., DiStefano, M.T., Oza, A., Rehm, H.L., Biesecker, L.G., Harrison, S.M., ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI), 2018. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. Hum. Mutat. 39, 1517–1524. https://doi.org/10.1002/humu.23626.

Bassuk, A.G., Wallace, R.H., Buhr, A., Buller, A.R., Afawi, Z., Shimojo, M., Miyata, S., Chen, S., Gonzalez-Alegre, P., Griesbach, H.L., Wu, S., Nashelsky, M., Vladar, E.K., Antic, D., Ferguson, P.J., Cirak, S., Voit, T., Scott, M.P., Axelrod, J.D., Gurnett, C., Daoud, A.S., Kivity, S., Neufeld, M.Y., Mazarib, A., Straussberg, R., Walid, S., Korczyn, A.D., Slusarski, D.C., Berkovic, S.F., El-Shanti, H.I., 2008. A homozygous mutation in human PRICKLE1 causes an autosomal-recessive progressive myoclonus epilepsy-ataxia syndrome. Am. J. Hum. Genet. 83, 572–581. https://doi.org/10.1016/j.ajhg.2008.10.003.

Berg, A.T., Coryell, J., Saneto, R.P., Grinspan, Z.M., Alexander, J.J., Kekis, M., Sullivan, J.E., Wirrell, E.C., Shellhaas, R.A., Mytinger, J.R., Gaillard, W.D., Kossoff, E.H., Valencia, I., Knupp, K.G., Wusthoff, C., Keator, C., Dobyns, W.B., Ryan, N., Loddenkemper, T., Chu, C.J., Novotny, E.J., Koh, S., 2017. Early-life epilepsies and the emerging role of genetic testing. JAMA Pediatr. 171, 863–871. https://doi.org/10.1001/jamapediatrics.2017.1743.

Bodian, D.L., Schreiber, J.M., Vilboux, T., Khromykh, A., Hauser, N.S., 2018. Mutation in an alternative transcript of CDKL5 in a boy with early-onset seizures. Cold Spring Harb. Mol. case Stud. 4 https://doi.org/10.1101/mcs.a002360.

Brunklaus, A., Dorris, L., Ellis, R., Reavey, E., Lee, E., Forbes, G., Appleton, R., Cross, J. H., Ferrie, C., Hughes, I., Jollands, A., King, M.D., Livingston, J., Lynch, B., Philip, S., Scheffer, I.E., Williams, R., Zuberi, S.M., 2013. The clinical utility of an SCN1A genetic diagnosis in infantile-onset epilepsy. Dev. Med. Child Neurol. 55, 154–161. https://doi.org/10.1111/dmcn.12030.

Brunklaus, A., Du, J., Steckler, F., Ghanty, I.I., Johannesen, K.M., Fenger, C.D., Schorge, S., Baez-Nieto, D., Wang, H.-R., Allen, A., Pan, J.Q., Lerche, H., Heyne, H., Symonds, J.D., Zuberi, S.M., Sanders, S., Sheidley, B.R., Craiu, D., Olson, H.E., Weckhuysen, S., DeJonge, P., Helbig, I., Van Esch, H., Busa, T., Milh, M., Isidor, B., Depienne, C., Poduri, A., Campbell, A.J., Dimidschstein, J., Møller, R.S., Lal, D., 2020. Biological concepts in human sodium channel epilepsies and their relevance in clinical practice. Epilepsia 61, 387–399. https://doi.org/10.1111/epi.16438.

Cameron, J.M., Maljevic, S., Nair, U., Aung, Y.H., Cogné, B., Bézieau, S., Blair, E., Isidor, B., Zweier, C., Reis, A., Koenig, M.K., Maarup, T., Sarco, D., Afenjar, A., Huq, A.H.M.M., Kukolich, M., Billette de Villemeur, T., Nava, C., Héron, B., Petrou, S., Berkovic, S.F., 2019. Encephalopathies with KCNC1 variants: genotype-phenotype-functional correlations. Ann. Clin. Transl. Neurol. 6, 1263–1272. https://doi.org/10.1002/acn3.50822.

Carpenter, J.C., Männikkö, R., Heffner, C., Heneine, J., Sampedro-Castañeda, M., Lignani, G., Schorge, S., 2021. Progressive myoclonus epilepsy KCNC1 variant causes a developmental dendritopathy. Epilepsia. https://doi.org/10.1111/epi.16867.

Cummings, B.B., Karczewski, K.J., Kosmicki, J.A., Seaby, E.G., Watts, N.A., Singer-Berk, M., Mudge, J.M., Karjalainen, J., Satterstrom, K.F., ODonnell-Luria, A., Poterba, T., Seed, C., Solomonson, M., Alfoldi, J., Team, T.G.A.D.P., Consortium, T. G.A.D., Daly, M.J., MacArthur, D.G., 2019. Transcript Expression-Aware Annotation Improves Rare Variant Discovery and Interpretation bioRxiv 554444.

Depienne, C., Trouillard, O., Gourfinkel-An, I., Saint-Martin, C., Bouteiller, D., Graber, D., Barthez-Carpentier, M.-A., Gautier, A., Villeneuve, N., Dravet, C., Livet, M.-O., Rivier-Ringenbach, C., Adam, C., Dupont, S., Baulac, S., Héron, D., Nabbout, R., Leguern, E., 2010. Mechanisms for variable expressivity of inherited SCN1A mutations causing Dravet syndrome. J. Med. Genet. 47, 404–410. https://doi.org/10.1136/jmg.2009.074328.

Feng, H., Khalil, S., Neubig, R.R., Sidiropoulos, C., 2018. A mechanistic review on GNAO1-associated movement disorder. Neurobiol. Dis. 116, 131–141. https://doi.org/10.1016/j.nbd.2018.05.005.

Gardella, E., Møller, R.S., 2019. Phenotypic and genetic spectrum of SCN8A-related disorders, treatment options, and outcomes. Epilepsia 60, S77–S85. https://doi.org/10.1111/epi.16319.

Helbig, I., Lowenstein, D.H., 2013. Genetics of the epilepsies: where are we and where are we going? Curr. Opin. Neurol. 26, 179–185. https://doi.org/10.1097/WCO.0b013e32835ee6ff.

Heron, S.E., Crossland, K.M., Andermann, E., Phillips, H.A., Hall, A.J., Bleasel, A., Shevell, M., Mercho, S., Seni, M.-H., Guiot, M.-C., Mulley, J.C., Berkovic, S.F., Scheffer, I.E., 2002. Sodium-channel defects in benign familial neonatal-infantile seizures. Lancet (London, England) 360, 851–852. https://doi.org/10.1016/S0140-6736(02)09968-3.

Houge, G., Haesen, D., Vissers, L.E.L.M., Mehta, S., Parker, M.J., Wright, M., Vogt, J., McKee, S., Tolmie, J.L., Cordeiro, N., Kleefstra, T., Willemsen, M.H., Reijnders, M.R. F., Berland, S., Hayman, E., Lahat, E., Brilstra, E.H., van Gassen, K.L.I., Zonneveld-Huijssoon, E., de Bie, C.I., Hoischen, A., Eichler, E.E., Holdhus, R., Steen, V.M., Døskeland, S.O., Hurles, M.E., FitzPatrick, D.R., Janssens, V., 2015. B56δ-related protein phosphatase 2A dysfunction identified in patients with intellectual disability. J. Clin. Invest. 125, 3051–3062. https://doi.org/10.1172/JCI79860.

Johannesen, K.M., Gardella, E., Encinas, A.C., Lehesjoki, A.E., Linnankivi, T., Petersen, M.B., Lund, I.C.B., Blichfeldt, S., Miranda, M.J., Pal, D.K., Lascelles, K., Procopis, P., Orsini, A., Bonuccelli, A., Giacomini, T., Helbig, I., Fenger, C.D., Sisodiya, S.M., Hernandez-Hernandez, L., Krithika, S., Rumple, M., Masnada, S., Valente, M., Cereda, C., Giordano, L., Accorsi, P., Bürki, S.E., Mancardi, M., Korff, C., Guerrini, R., von Spiczak, S., Hoffman-Zacharska, D., Mazurczak, T., Coppola, A., Buono, S., Vecchi, M., Hammer, M.F., Varesio, C., Veggiotti, P., Lal, D., Brünger, T., Zara, F., Striano, P., Rubboli, G., Møller, R.S., 2019. The spectrum of intermediate SCN8A-related epilepsy. Epilepsia 60, 830–844. https://doi.org/10.1111/epi.14705.

Johannesen, K.M., Liu, Y., Gjerulfsen, C.E., Koko, M., Sonnenberg, L., Schubert, J., Fenger, C.D., Eltokhi, A., Rannap, M., Koch, N.A., Lauxmann, S., Krüger, J., Kegele, J., Canafoglia, L., Franceschetti, S., Mayer, T., Rebstock, J., Zacher, P., Ruf, S., Alber, M., Sterbova, K., Lassuthová, P., Vlckova, M., Lemke, J.R., Krey, I., Heine, C., Wieczorek, D., Kroell-Seger, J., Lund, C., Klein, K.M., Au, P.Y.B., Rho, J. M., Ho, A.W., Masnada, S., Veggiotti, P., Giordano, L., Accorsi, P., Hoei-Hansen, C.E., Striano, P., Zara, F., Verhelst, H., Verhoeven, J.S., van der Zwaag, B., Harder, A.V.E., Brilstra, E., Pendziwiat, M., Lebon, S., Vaccarezza, M., Le, N.M., Christensen, J., Schmidt-Petersen, M.U., Grønborg, S., Scherer, S.W., Howe, J., Fazeli, W., Howell, K. B., Leventer, R., Stutterd, C., Walsh, S., Gerard, M., Gerard, B., Matricardi, S., Bonardi, C.M., Sartori, S., Berger, A., Hoffman-Zacharska, D., Mastrangelo, M., Darra, F., Vøllo, A., Motazacker, M.M., Lakeman, P., Nizon, M., Betzler, C., Altuzarra, C., Caume, R., Roubertie, A., Gélisse, P., Marini, C., Guerrini, R., Bilan, F., Tibussek, D., Koch-Hogrebe, M., Perry, M.S., Ichikawa, S., Dadali, E., Sharkov, A., Mishina, I., Abramov, M., Kanivets, I., Korostelev, S., Kutsev, S., Wain, K.E., Eisenhauer, N., Wagner, M., Savatt, J.M., Müller-Schlüter, K., Bassan, H., Borovikov, A., Nassogne, M.-C., Destrée, A., Schoonjans, A.-S., Meuwissen, M., Buzatu, M., Jansen, A., Scalais, E., Srivastava, S., Tan, W.-H., Olson, H.E., Loddenkemper, T., Poduri, A., Helbig, K.L., Helbig, I., Fitzgerald, M.P., Goldberg, E. M., Roser, T., Borggraefe, I., Brünger, T., May, P., Lal, D., Lederer, D., Rubboli, G., Lesca, G., Hedrich, U.B.S., Benda, J., Gardella, E., Lerche, H., Møller, R.S., 2021. Genotype-phenotype correlations in SCN8A-related disorders reveal prognostic and therapeutic implications. medRxiv 21253711. https://doi.org/10.1101/2021.03.22.21253711, 2021.03.22.

Larsen, J., Carvill, G.L., Gardella, E., Kluger, G., Schmiedel, G., Barisic, N., Depienne, C., Brilstra, E., Mang, Y., Nielsen, J.E.K., Kirkpatrick, M., Goudie, D., Goldman, R., Jähn, J.A., Jepsen, B., Gill, D., Döcker, M., Biskup, S., McMahon, J.M., Koeleman, B., Harris, M., Braun, K., De Kovel, C.G.F., Marini, C., Specchio, N., Djémié, T., Weckhuysen, S., Tommerup, N., Troncoso, M., Troncoso, L., Bevot, A., Wolff, M., Hjalgrim, H., Guerrini, R., Scheffer, I.E., Mefford, H.C., Møller, R.S., On behalf of the EuroEPINOMICS RES Consortium CRP, 2015. The phenotypic spectrum of SCN8A encephalopathy. Neurology 84, 480–489. https://doi.org/10.1212/WNL.0000000000001211.

Lemke, J.R., Geider, K., Helbig, K.L., Heyne, H.O., Schütz, H., Hentschel, J., Courage, C., Depienne, C., Nava, C., Heron, D., Møller, R.S., Hjalgrim, H., Lal, D., Neubauer, B.A., Nürnberg, P., Thiele, H., Kurlemann, G., Arnold, G.L., Bhambhani, V., Bartholdi, D., Pedurupillay, C.R.J., Misceo, D., Frengen, E., Strømme, P., Dlugos, D.J., Doherty, E. S., Bijlsma, E.K., Ruivenkamp, C.A., Hoffer, M.J.V.V., Goldstein, A., Rajan, D.S., Narayanan, V., Ramsey, K., Belnap, N., Schrauwen, I., Richholt, R., Koeleman, B.P.C. C., Sá, J., Mendonça, C., de Kovel, C.G.F., Weckhuysen, S., Hardies, K., De Jonghe, P., De Meirleir, L., Milh, M., Badens, C., Lebrun, M., Busa, T., Francannet, C., Piton, A., Riesch, E., Biskup, S., Vogt, H., Dorn, T., Helbig, I., Michaud, J.L., Laube, B., Syrbe, S., Mendonca, C., Kovel, C.G.F.D.F. De, Weckhuysen, S., Hardies, K., Jonghe, P. De, Meirleir, L. De, Milh, M., Badens, C., Lebrun, M., Busa, T., Francannet, C., Piton, A., Riesch, E., Biskup, S., Vogt, H., Dorn, T., Helbig, I., Michaud, J.L., Laube, B., Syrbe, S., 2016. Delineating the GRIN1 phenotypic spectrum: a distinct genetic NMDA receptor encephalopathy. Neurology 86, 2171–2178. https://doi.org/10.1212/WNL.0000000000002740.

Lesca, G., Depienne, C., 2015. Epilepsy genetics: the ongoing revolution. Rev. Neurol. (Paris) 171, 539–557. https://doi.org/10.1016/j.neurol.2015.01.569.

Liu, Y., Schubert, J., Sonnenberg, L., Helbig, K.L., Hoei-Hansen, C.E., Koko, M., Rannap, M., Lauxmann, S., Huq, M., Schneider, M.C., Johannesen, K.M., Kurlemann, G., Gardella, E., Becker, F., Weber, Y.G., Benda, J., Møller, R.S., Lerche, H., 2019. Neuronal mechanisms of mutations in SCN8A causing epilepsy or intellectual disability. Brain 142, 376–390. https://doi.org/10.1093/brain/awy326.

Mastrangelo, M., Tolve, M., Martinelli, M., Di Noia, S.P., Parrini, E., Leuzzi, V., 2018. PRICKLE1-related early onset epileptic encephalopathy. Am. J. Med. Genet. A 176, 2841–2845. https://doi.org/10.1002/ajmg.a.40625.

McTague, A., Howell, K.B., Cross, J.H., Kurian, M.A., Scheffer, I.E., 2016. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet Neurol. 15, 304–316. https://doi.org/10.1016/S1474-4422(15)00250-1.

Mei, D., Parrini, E., Marini, C., Guerrini, R., 2017. The impact of next-generation sequencing on the diagnosis and treatment of epilepsy in paediatric patients. Mol. Diagn. Ther. https://doi.org/10.1007/s40291-017-0257-0.

Milh, M., Lacoste, C., Cacciagli, P., Abidi, A., Sutera-Sardo, J., Tzelepis, I., Colin, E., Badens, C., Afenjar, A., Coeslier, A.D., Dailland, T., Lesca, G., Philip, N., Villard, L., 2015. Variable clinical expression in patients with mosaicism for KCNQ2 mutations. Am. J. Med. Genet. A 167A, 2314. https://doi.org/10.1002/ajmg.a.37152. –8.

Møller, R.S., Heron, S.E., Larsen, L.H.G., Lim, C.X., Ricos, M.G., Bayly, M.A., van Kempen, M.J.A., Klinkenberg, S., Andrews, I., Kelley, K., Ronen, G.M., Callen, D., McMahon, J.M., Yendle, S.C., Carvill, G.L., Mefford, H.C., Nabbout, R., Poduri, A., Striano, P., Baglietto, M.G., Zara, F., Smith, N.J., Pridmore, C., Gardella, E., Nikanorova, M., Dahl, H.A., Gellert, P., Scheffer, I.E., Gunning, B., Kragh-Olsen, B., Dibbens, L.M., 2015. Mutations in KCNT1 cause a spectrum of focal epilepsies. Epilepsia 56, e114–e120. https://doi.org/10.1111/epi.13071.

Møller, R.S., Wuttke, T.V., Helbig, I., Marini, C., Johannesen, K.M., Brilstra, E.H., Vaher, U., Borggraefe, I., Talvik, I., Talvik, T., Kluger, G., Francois, L.L., Lesca, G., de Bellescize, J., Blichfeldt, S., Chatron, N., Holert, N., Jacobs, J., Swinkels, M., Betzler, C., Syrbe, S., Nikanorova, M., Myers, C.T., Larsen, L.H.G., Vejzovic, S., Pendziwiat, M., von Spiczak, S., Hopkins, S., Dubbs, H., Mang, Y., Mukhin, K., Holthausen, H., van Gassen, K.L., Dahl, H.A., Tommerup, N., Mefford, H.C., Rubboli, G., Guerrini, R., Lemke, J.R., Lerche, H., Muhle, H., Maljevic, S., 2017. Mutations in GABRB3: from febrile seizures to epileptic encephalopathies. Neurology 88, 483–492. https://doi.org/10.1212/WNL.0000000000003565.

Muona, M., Berkovic, S.F., Dibbens, L.M., Oliver, K.L., Maljevic, S., Bayly, M.A., Joensuu, T., Canafoglia, L., Franceschetti, S., Michelucci, R., Markkinen, S., Heron, S.E., Hildebrand, M.S., Andermann, E., Andermann, F., Gambardella, A., Tinuper, P., Licchetta, L., Scheffer, I.E., Criscuolo, C., Filla, A., Ferlazzo, E., Ahmad, J., Ahmad, A., Baykan, B., Said, E., Topcu, M., Riguzzi, P., King, M.D., Ozkara, C., Andrade, D.M., Engelsen, B.A., Crespel, A., Lindenau, M., Lohmann, E., Saletti, V., Massano, J., Privitera, M., Espay, A.J., Kauffmann, B., Duchowny, M., Møller, R.S., Straussberg, R., Afawi, Z., Ben-Zeev, B., Samocha, K.E., Daly, M.J., Petrou, S., Lerche, H., Palotie, A., Lehesjoki, A.-E., 2015. A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. Nat. Genet. 47, 39–46. https://doi.org/10.1038/ng.3144.

Nascimento, F.A., Andrade, D.M., 2016. Myoclonus epilepsy and ataxia due to potassium channel mutation (MEAK) is caused by heterozygous KCNC1 mutations. Epileptic Disord. https://doi.org/10.1684/epd.2016.0859.

Oliver, K.L., Franceschetti, S., Milligan, C.J., Muona, M., Mandelstam, S.A., Canafoglia, L., Boguszewska-Chachulska, A.M., Korczyn, A.D., Bisulli, F., Di Bonaventura, C., Ragona, F., Michelucci, R., Ben-Zeev, B., Straussberg, R., Panzica, F., Massano, J., Friedman, D., Crespel, A., Engelsen, B.A., Andermann, F., Andermann, E., Spodar, K., Lasek-Bal, A., Riguzzi, P., Pasini, E., Tinuper, P., Licchetta, L., Gardella, E., Lindenau, M., Wulf, A., Møller, R.S., Benninger, F., Afawi, Z., Rubboli, G., Reid, C.A., Maljevic, S., Lerche, H., Lehesjoki, A.E., Petrou, S., Berkovic, S.F., 2017. Myoclonus epilepsy and ataxia due to KCNC1 mutation: analysis of 20 cases and K+ channel properties. Ann. Neurol. 81, 677–689. https://doi.org/10.1002/ana.24929.

Park, J., Koko, M., Hedrich, U.B.S., Hermann, A., Cremer, K., Haberlandt, E., Grimmel, M., Alhaddad, B., Beck-Woedl, S., Harrer, M., Karall, D., Kingelhoefer, L., Tzschach, A., Matthies, L.C., Strom, T.M., Ringelstein, E.B., Sturm, M., Engels, H., Wolff, M., Lerche, H., Haack, T.B., 2019. KCNC1-related disorders: new de novo variants expand the phenotypic spectrum. Ann. Clin. Transl. Neurol. 6, 1319–1326. https://doi.org/10.1002/acn3.50799.

Platzer, K., Lemke, J.R., 1993. GRIN1-Related Neurodevelopmental Disorder. GeneReviews(®). In: Adam, M.P., Ardinger, H.H., Pagon, R.A., et al. (Eds.). University of Washington, Seattle, Seattle (WA).

Poduri, A., 2017. When should genetic testing be performed in epilepsy patients? Epilepsy Curr. https://doi.org/10.5698/1535-7511-17.1.16.

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W.W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H.L., 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. Genet. Med. 17, 405–423. https://doi.org/10.1038/gim.2015.30.

Rohlin, A., Wernersson, J., Engwall, Y., Wiklund, L., Björk, J., Nordling, M., 2009. Parallel sequencing used in detection of mosaic mutations: comparison with four diagnostic DNA screening techniques. Hum. Mutat. 30, 1012–1020. https://doi.org/10.1002/humu.20980.

Schirinzi, T., Garone, G., Travaglini, L., Vasco, G., Galosi, S., Rios, L., Castiglioni, C., Barassi, C., Battaglia, D., Gambardella, M.L., Cantonetti, L., Graziola, F., Marras, C. E., Castelli, E., Bertini, E., Capuano, A., Leuzzi, V., 2019. Phenomenology and clinical course of movement disorder in GNAO1 variants: results from an analytical review. Park. Relat. Disord. 61, 19–25. https://doi.org/10.1016/j.parkreldis.2018.11.019.

Singh, N.A., Charlier, C., Stauffer, D., DuPont, B.R., Leach, R.J., Melis, R., Ronen, G.M., Bjerre, I., Quattlebaum, T., Murphy, J.V., McHarg, M.L., Gagnon, D., Rosales, T.O., Peiffer, A., Anderson, V.E., Leppert, M., 1998. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat. Genet. 18, 25–29. https://doi.org/10.1038/ng0198-25.

Spagnoli, C., Salerno, G.G., Iodice, A., Frattini, D., Pisani, F., Fusco, C., 2018. KCNQ2 encephalopathy: a case due to a de novo deletion. Brain Dev. 40, 65–68. https://doi.org/10.1016/j.braindev.2017.06.008.

Steel, D., Symonds, J.D., Zuberi, S.M., Brunklaus, A., 2017. Dravet syndrome and its mimics: beyond SCN1A. Epilepsia 58, 1807–1816. https://doi.org/10.1111/epi.13889.

Symonds, J.D., Joss, S., Metcalfe, K.A., Somarathi, S., Cruden, J., Devlin, A.M., Donaldson, A., DiDonato, N., Fitzpatrick, D., Kaiser, F.J., Lampe, A.K., Lees, M.M., McLellan, A., Montgomery, T., Mundada, V., Nairn, L., Sarkar, A., Schallner, J., Pozojevic, J., Parenti, I., Tan, J., Turnpenny, P., Whitehouse, W.P., Study, D.D.D., Zuberi, S.M., 2017. Heterozygous truncation mutations of the SMC1A gene cause a severe early onset epilepsy with cluster seizures in females: detailed phenotyping of 10 new cases. Epilepsia 58, 565–575. https://doi.org/10.1111/epi.13669.

Symonds, J.D., Zuberi, S.M., Stewart, K., McLellan, A., O'Regan, M., MacLeod, S., Jollands, A., Joss, S., Kirkpatrick, M., Brunklaus, A., Pilz, D.T., Shetty, J., Dorris, L., Abu-Arafeh, I., Andrew, J., Brink, P., Callaghan, M., Cruden, J., Diver, L.A., Findlay, C., Gardiner, S., Grattan, R., Lang, B., MacDonnell, J., McKnight, J., Morrison, C.A., Nairn, L., Slean, M.M., Stephen, E., Webb, A., Vincent, A., Wilson, M., 2019. Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. Brain 142, 2303–2318. https://doi.org/10.1093/brain/awz195.

Trivisano, M., Pavia, G.C., Ferretti, A., Fusco, L., Vigevano, F., Specchio, N., 2019. Generalized tonic seizures with autonomic signs are the hallmark of SCN8A developmental and epileptic encephalopathy. Epilepsy Behav. 96, 219–223. https://doi.org/10.1016/j.yebeh.2019.03.043.

Zaman, T., Abou Tayoun, A., Goldberg, E.M., 2019. A single-center SCN8A-related epilepsy cohort: clinical, genetic, and physiologic characterization. Ann. Clin. Transl. Neurol. 6, 1445–1455. https://doi.org/10.1002/acn3.50839.

Zawadzka, M., Matheisel, A., Konieczna, S., Mazurkiewicz-Bełdzińska, M., 2020. Autonomic seizure with prominent apnea in patient with SCN8A-related epileptic encephalopathy. Epilepsy Res. 167, 106440. https://doi.org/10.1016/j.eplepsyres.2020.106440.