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SPECIAL REPORT

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Genetic literacy series: Primer part 2—Paradigm shifts in epilepsy genetics

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Summary

This is the second of a 2-part primer on the genetics of the epilepsies within the Genetic Literacy Series of the Genetics Commission of the International League Against Epilepsy. In Part 1, we covered types of genetic variation, inheritance patterns, and their relationship to disease. In Part 2, we apply these basic principles to the case of a young boy with epileptic encephalopathy and ask 3 important questions: (1) Is the gene in question an established genetic etiology for epilepsy? (2) Is the variant in this particular gene pathogenic by established variant interpretation criteria? (3) Is the variant considered causative in the clinical context? These questions are considered and then answered for the clinical case in question.

KEYWORDS

epilepsy, epilepsy gene, genetics, genomics, heritability, recurrence risk, seizures, twins

Clinical Scenario

You are seeing a young boy with an unexplained epileptic encephalopathy with normal magnetic resonance imaging and unremarkable metabolic testing. Prior to taking a full history, you are reviewing the medical records with the family. The parents point out that genetic testing had been performed in the past. They state that the test was for "all the epilepsy genes" and came back with several findings, including variants in *SCN9A*, *EFHC1*, *SPTAN1*, *KCNQ1*, *SCN2A*, and *TBC1D24*. Parental testing has not been performed. The parents were told the identified variants are of uncertain significance. What can we tell the family about the role of the reported variants? What additional information about the variants do we need?

*A full list of International League Against Epilepsy Genetics Commission members is given in Appendix 1.

1 | INTRODUCTION

The landscape of clinical genetic testing for patients with epilepsy has changed dramatically in the past several years, primarily due to the introduction of massively parallel or "next generation" sequencing (NGS) technologies into the field of human genetics. In the research laboratory, NGS has led to an unprecedented rate of gene discovery across many genetic disorders.[1,2] These discoveries are rapidly translated to the clinical context, where the technology is used for diagnostic testing of several, many, or all genes simultaneously.

In contrast to traditional Sanger sequencing, which analyzes individual fragments of DNA and has been used successfully for >30 years, NGS technologies permit simultaneous or parallel sequencing of billions of fragments of DNA, enabling an entire genome to be sequenced in a single experiment. The first human genome was successfully sequenced using traditional methods; it took >10 years and cost as much as $1 billion.[3] Today, a whole genome can be sequenced in a few days for approximately $1000.

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In the clinical setting, NGS is most commonly used for gene panel or whole exome sequencing (Box 1). This technology has changed the approach to diagnostic testing, especially in patients with disorders that are genetically heterogeneous. Rather than testing one gene at a time, which can add up to considerable time and cost if the first guess is not correct, many genes can be tested at once. As a result, the use of gene panel or exome sequencing in patients with epileptic encephalopathy has increased the likelihood of discovering a causal genetic variant from single digits to >30%.[4–6] As novel genetic etiologies continue to be discovered, the diagnostic rate is virtually certain to increase further. Conversely, these technologies increase the risk of uncertain results when a rare sequence variant is detected in one or more genes. Finding a variant of uncertain significance (VUS) can lead to additional testing in the patient or family members to determine whether the variant is causative.

BOX 1 Terminology box

Epileptic encephalopathy. A severe type of epilepsy that usually begins in early childhood, typically associated with intellectual disability.

ExAC. A database of genetic variants detected from the exome sequencing of >60 000 individuals sequenced as part of a range of genetic studies. The database, available at exac.broadinstitute.org, provides a list of variants and how many people have those variants in their genome. As of February 2017, the ExAC database has been extended to the gnomAD with 120 000 exomes and 15 000 genomes (http://gnomad.broadinstitute.org).

Exome. The portion of the genome that encodes proteins.

Gene panel. A diagnostic test that sequences genes known or believed to have variants that are responsible for a particular disease.

Gonadal mosaicism. A condition that can give rise to a mutation in the genome of a child that is not detectable in the parent (de novo mutation). *Online Mendelian Inheritance in Man (OMIM).* An online database cataloging human genes and their role in diseases. The database provides information about the biological role of the proteins encoded by the gene, a summary of the evidence supporting the role of a gene in a disease, and where available, a list of variants reported in the literature to cause the disease (http://omim.org).

Pathogenic variant. A genetic variant found in a patient that is believed to be responsible for a disease based on current knowledge.

Variant of unknown significant (VUS). A genetic variant found in a patient where it is unclear if the variant is responsible for a disease based on current knowledge.

Key Points

- Next generation sequencing (NGS) technologies are commonly applied in clinical practice, including gene panels and whole exome sequencing
- A gene's clinical validity (ie, if the gene is an established cause of disease) should be considered when interpreting clinical results
- Additional familial testing, often both biological parents, may be helpful in determining the pathogenicity of variants
- Genetic testing results should be interpreted in the appropriate clinical context

In Primer Part 1, we introduced the building blocks for understanding types of genetic variation, inheritance patterns, and their relationship to disease. Here, in Part 2, we will begin to apply these basic principles to navigate some of the challenges that come with increasingly comprehensive genetic testing in the clinical setting.

In brief, we address the 3 main questions that arise in the context of gene and variant interpretation: (1) Is the gene in question an established genetic etiology for epilepsy? (2) Is the variant in this particular gene pathogenic by established variant interpretation criteria? (3) Is the variant considered causative in the clinical context? (Box 2).

Coming back to the case vignette, the parents wonder whether there is any new information about the test results in their child and whether any more testing can or should be done. They also want to know whether genetic testing can help them figure out how to better treat their child's epilepsy. The questions raised by the parents address many of the issues surrounding genetics. These questions are relevant in an epilepsy clinic today, including the role of genetic testing and interpretation of uncertain results.

2 | THE NOVEL GENOMIC TECHNOLOGIES—WHAT ARE THESE NEW TESTS?

Clinical tests that employ NGS include gene panels and whole exome sequencing. Although both tests use NGS and evaluate multiple genes at once, there are important

BOX 2 Three main questions to consider in the context of gene and variant interpretation in epilepsy

1. Is the gene in question an established genetic etiology for epilepsy?
2. Is the variant in this particular gene pathogenic by established variant interpretation criteria?
3. Is the variant considered causative in the clinical context?

MCQ test

1. Regarding epilepsy gene panels versus exome sequencing for genetic testing in epilepsies, which of the following is true?
   A. Gene panels provide a greater depth of coverage compared to exome sequencing.
   B. Exome sequencing adequately covers all epilepsy genes, especially important genes like *ARX*.
   C. Gene panels can miss small deletions, whereas exome sequencing can detect them.
   D. Exome sequencing predominantly assesses introns.
2. When deciding if a genetic variant is benign or pathogenic, which of these considerations is important to bear in mind?
   A. Clinical validity
   B. Variant pathogenicity
   C. Clinical correlation
   D. All of the above

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differences. On the technological side, one of several "capture" technologies is used. These technologies pull out selected regions from the human genome, and the captured DNA is sequenced to a high depth, meaning that each fragment is sequenced several dozen to several hundred times depending on the design. For gene panel sequencing, an array of selected genes is chosen including exons and exon-intron boundaries of the genes of interest. When the selected regions include the majority of the protein-coding regions of the human genome, it is referred to as exome sequencing.

2.1 | Epilepsy gene panels

Gene panels have been developed for many disorders, including epilepsy, for which simultaneous testing of several genes is clinically meaningful. This is typically the case if there are multiple genes in which pathogenic variants can cause the disorder (genetic heterogeneity) and if the phenotypes caused by different genes are similar enough that it is difficult to predict a specific gene in a given patient. In these cases, it can become exceedingly time consuming and expensive to test each gene one by one until the causative variant is identified. Although some genetic epilepsies such as Dravet syndrome (Online Mendelian Inheritance in Man database [OMIM] #607208) have a very strong connection between the clinical presentation and a single gene (*SCN1A*), most epilepsies for which testing is performed typically have a clinical presentation that is compatible with several causative genes. For example, patients with infantile spasms or Lennox-Gastaut syndrome may have causative pathogenic variants in one of >20 genes that have been identified in these conditions.

Gene panels can vary greatly in what genes are tested and also in the technology used to perform the test. Both of these factors have important implications for test selection and for interpretation of the results. Currently available epilepsy panel designs range between several dozen to hundreds of selected genes. "Epilepsy" panels can include genes associated with a particular type of epilepsy (eg, childhood onset epilepsy, progressive myoclonic epilepsy, infantile epilepsy) or may include a large set of genes in which pathogenic variants cause a range of disorders, including many for which epilepsy may only be present in a minority of affected individuals.

2.2 | Exome sequencing

Whole exome sequencing is a comprehensive test to analyze the protein-coding sequence of all 20 000 genes in the human genome. Because the exome comprises only 1% of the whole genome, a capture step is used to pull out exons and intron-exon boundaries from the entire genomic sequence. Initial analysis of the data usually focuses on identifying potential pathogenic variants in any of the known 4000 disease genes. In many sporadic, (nonfamilial) cases with severe epilepsies, exome sequencing is performed on a family basis, including the affected patient and both unaffected parents. This strategy allows the diagnostic laboratory to query for genes that have de novo variants. Exome sequencing in the clinical setting broadly leads to a diagnosis in ~25% of cases overall,[4–6] and de novo pathogenic variants account for the majority of cases with positive findings. Importantly, if a variant is found in a gene that is not a known disease gene, it may not be reported.

2.3 | Why choose an epilepsy gene panel?

There are some advantages of gene panels over exomes. First, the higher depth of coverage and the targeted nature

of the test make it more likely that every base pair is analyzed. In addition, the higher coverage increases the likelihood of identifying mosaic variants in the patient that may be missed by other methods. Furthermore, because gene panels are designed to look at a specific set of genes, clinical laboratories will often "fill in" missing sequences using traditional methods. This may be relevant for genes that are insufficiently covered in exomes for technical reasons, such as the *ARX* gene, which is implicated in infantile spasms and brain malformations.[7] Furthermore, many available gene panels include deletion and duplication testing on an exon level, which allows for the detection of small deletions, which are difficult to detect through exome sequencing. For example, in patients with *SCN1A*-related Dravet syndrome, up to 3% of patients may have small *SCN1A* deletions.[8] These small deletions may be missed both by microarrays querying for larger deletions and by exome sequencing. Accordingly, for conditions with a high clinical suspicion for a particular gene, gene panels may be the first choice. It is important to know that there are 2 main technologies used to carry out gene panel testing. The first is to specifically (and only) test the genes of interest; this is accomplished by targeted "capture" of only the genes listed followed by high-throughput sequencing. The second approach is to perform whole exome sequencing, but to perform data analysis for only the genes listed on the "panel." This approach can be described as a gene panel on an "exome backbone," and some companies allow customers to design custom panels using this method. The advantages discussed above refer to the former method.

2.4 | Why choose an exome?

Exome sequencing covers a significant portion of all 20 000 genes in the genome. Exome sequencing is usually performed if the phenotype in question is compatible with a broad range of genetic etiologies or if the disease phenotype is considered genetic, but not clearly compatible with any of the known genes that are routinely tested on gene panels. The question of whether exome sequencing or gene panel analysis should have priority in the genetic workup of patients with epilepsy is unanswered, and there are no reliable data in the epilepsy field that would allow for a comparison of the diagnostic yields of both tests. However, negative results of gene panels typically trigger exome sequencing, which is often performed on a family basis (trio sequencing) to obtain information about de novo variants and to assess whether biallelic pathogenic variants are in *cis* or in *trans* (ie, whether variants in a gene that causes a recessive disorder are on the same chromosome [*cis*] or on different homologs [*trans*]).[9,10] Only in the latter case would the combination of variants be considered pathogenic. In addition to samples from parents, family history is very important for interpretation of test results and should always be provided to the testing company. Exome sequencing is currently considered the most comprehensive test available for genetic diagnostics. In addition to the diagnostic yield, exome sequencing offers the possibility for reanalysis. Most laboratories offer reanalysis of exome data after certain time intervals. In a dynamic field such as epilepsy, a significant number of novel genes are identified each year.[11] Accordingly, exome results that were considered negative at the time of testing may yield additional data after 6-12 months. In addition, research projects such as the Epilepsy Genetics Initiative aim to perform a regular reanalysis of negative diagnostic exome data (URL is given at the end of the article). Of course, one must also exercise caution when performing comprehensive testing. Along with the potential of increased diagnostic yield comes a possible increase in variants of uncertain clinical significance as well as secondary (unrelated to epilepsy) findings.

Coming back to the case vignette, you wonder how to interpret the results. You quickly review the genetic testing report that the parents brought to their child's appointment. The patient had a gene panel test in April 2013 that included >300 genes considered to be related to human epilepsies. The report does not list variants that would be considered pathogenic but lists 6 VUSs (Table 1). You acknowledge and share the parents' frustration with the lack of definitive results. What additional information or testing might add some clarity?

3 | HOW TO INTERPRET THE NEW GENETIC TESTS

In some cases, genetic test results are clearly positive, identifying a causative genetic change, or clearly negative. However, in many cases, the results derived from genetic testing provide uncertain results. The situation described in the case vignette is common and can be frustrating for both the family and the physician. There are several considerations that will help interpret these results in the clinical context of our patient and add certainty to the initial genetic findings. These considerations relate to the gene itself, the type of identified variant, the date of the initial report, and the availability of parental information. The following 2 sections of this review discuss the current criteria that address both the issue of defining disease genes and the pathogenicity of specific variants within these genes.

3.1 | Does the gene make sense—the ClinGen criteria

Prior to the era of large-scale genomic sequencing, the identification of variants in selected epilepsy candidate

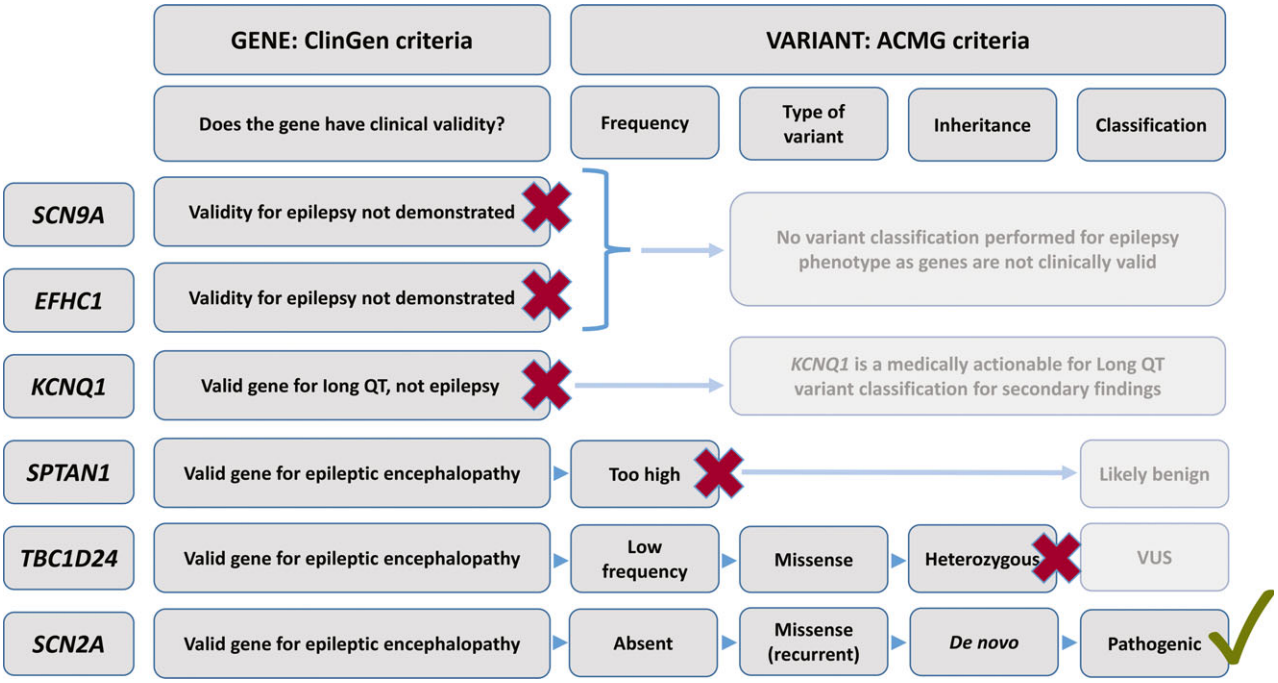

FIGURE 1 Example of interpretation process for determining the clinical relevance of genetic variants described in the vignette (recurrent variant refers to variants previously seen in individuals with a similar phenotype). ACMG, American College of Medical Genetics and Genomics; VUS, variant of uncertain significance

genes was frequently taken as an indication that the particular gene was a disease gene. With the introduction of massive parallel sequencing technologies, the burden of proof has shifted dramatically. Given the enormous variability of the human genome, a significant amount of genetic variation in potential candidate genes is frequently found in patients and control populations. Accordingly, the question arises as to what constitutes adequate criteria to define disease genes and to assess whether a given gene has clinical validity. Clinical validity for a particular epilepsy gene can be demonstrated if a certain class of variants is repeatedly identified in patients, but not in unaffected individuals. For example, this is the case of loss-of-function variants in *SCN1A* in Dravet syndrome or biallelic variants in *ALDH7A1*, the gene for pyridoxine-dependent seizures.[12] Accumulating evidence for clinical validity is an ongoing process. Recently identified epilepsy genes such as *STX1B* or *SIK1* are not established disease genes as of 2017,[13,14] but it will not be surprising if they are eventually confirmed. Curating clinical validity for epilepsy genes is a community effort. For example, the ClinGen Neurodevelopmental Disorders Clinical Domain Working Group is tasked with reviewing epilepsy-related genes for the publicly available ClinGen knowledge base,[15] evaluating the evidence for a large number of suggested epilepsy genes to determine the clinical validity of each and, when possible, genotype-phenotype relationships.

In our case vignette, not all genes mentioned in the initial gene panel report are established genes for human epilepsies (Figure 1). Although commercial gene panels carefully select genes that are offered on epilepsy panels, this selection of genes may sometimes be based on older literature or hypotheses with respect to gene groups. A variant in *KCNQ1* was identified, but despite the apparent similarity to the known epilepsy genes *KCNQ2* and *KCNQ3*, this gene is a causative genetic etiology for long QT syndrome (OMIM #192500),[16] not for epilepsy. It may have been included on the commercial gene panel, as it represents an ion channel gene, but this does not guarantee that this gene plays a role in human epilepsy. Rare variants in *SCN9A* have been discussed as modifiers of an underlying epilepsy phenotype,[17,18] but robust data to establish *SCN9A* as a disease gene itself is lacking. Variants in the *EFHC1* gene had initially been considered to be associated with juvenile myoclonic epilepsy,[19] but the role of *EFHC1* as a monogenic disease gene was recently called into question given the high frequency of alleged disease-associated variants in controls.[20,21] *TBC1D24*-related disease is inherited in an autosomal recessive manner. Although the phenotype of our patient could be consistent, we would expect 2 pathogenic variants: one inherited from the mother and a second from the father. If the patient carried 2 variants, testing the parents to determine whether the variants are in *trans* would be appropriate. If we were highly suspicious of *TBC1D24*-related disease, testing for copy number variants in the gene would be advised. However, de novo pathogenic variants in *SPTAN1* and *SCN2A* have been identified in patients

TABLE 1 Table of variants identified in our patient

	Predicted protein change	ExAC frequency (number of alleles)	Polyphen 2[a]	CADD[b]
SCN9A	p.Ser665Asn	0	0.151	24.3
EFHC1	p.Arg221His	0.0023 (n = 284)[c]	0.099	13.4
SPTAN1	p.Ala1428Gly	0.00064 (n = 78)	0.005	18.5
KCNQ1	p.Thr153Met	0.00018 (n = 22)	0.876	14.0
SCN2A	p.Arg853Gln	0	1	35.0
TBC1D24	p.Arg214His	0.0010 (n = 123)	0.997	23.2

[a]Polyphen 2 scores range from 0 (predicted to be benign) to 1 (likely to be damaging).
[b]Higher CADD scores are associated with higher likelihood of pathogenicity; in general, variants with scores < 10-15 are likely benign.
[c]Including 6 individuals who are homozygous for this variant.

with epileptic encephalopathy.[22–24] Therefore, using clinical judgment that incorporates the patient's phenotype and knowledge about the individual genes, we can narrow the plausible candidate variants from 6 to 2 variants.

3.2 | Does the variant make sense—the American College of Medical Genetics and Genomics guidelines

One important finding of the Human Genome Projects and subsequent large-scale sequencing projects was the variability of the human genome. Every human carries hundreds to thousands of unique genetic variants, and most of these variants are not relevant in a disease context. Accordingly, when a large-scale genetic test such as gene panel or exome sequencing is performed, a large number of noncontributory variants may be identified in potential candidate genes. Guidelines have been developed by the American College of Medical Genetics and Genomics (ACMG) to help determine whether a genetic variant is likely to be pathogenic or benign.[25] Some of the criteria used to evaluate potential pathogenicity include the type and predicted impact of the variant, the frequency of the variant in the general population and affected individuals, the inheritance of the variant, and functional evidence if available. The ACMG criteria typically categorize a genetic variant as benign, likely benign, uncertain, likely pathogenic, or pathogenic based on these criteria.[25]

3.2.1 | Type of variant

Genetic variants can result in various predicted effects on the protein, including missense variants that alter the protein sequence, or nonsense variants resulting in predicted loss of function either by truncating the predicted protein by introducing a stop codon (nonsense or stop variant), by disrupting the reading frame (frameshift variants), or through interfering with splicing, disrupting the proper generation of messenger RNA (splice-site variants). In addition, some genetic variants may represent small insertions or deletions that do not interfere with the reading frame, referred to as in-frame deletions or duplications. The interpretation of the variant is gene-specific. For example, a stop variant in the *SCN1A* gene represents a pathogenic variant, given that haploinsufficiency is the known disease mechanism in Dravet syndrome.[26] However, other epilepsies can be due to gain-of-function effects where the disease-causing variants causes an increase in the function of the affected protein or acts in a dominant-negative way. This is the case in *KCNQ2* encephalopathy. Whereas haploinsufficiency is the established disease mechanism in benign familial neonatal seizures, patients with the severe form of *KCNQ2* encephalopathy have missense variants, some of which have been shown to have a dominant-negative effect.[27]

3.2.2 | Pathogenicity scores

Over the past decade, various computational prediction scores have been developed that try to estimate the severity of missense variants on protein functions. Some of these prediction tools use evolutionary conservation, whereas other tools try to assess the impact of the amino acid substitution. For example, when a small nonpolar amino acid is replaced by a large polar amino acid, the predicted change is more severe than a change between 2 nonpolar amino acids. Tools that are used to computationally predict pathogenicity include SIFT, PolyPhen, MutationTaster, and CADD (see URLs at the end of the article). Pathogenicity scores in isolation cannot assess whether a specific variant is disease causing. Variants of various degrees of predicted pathogenicity are found in every individual undergoing exome sequencing. However, these computational scores may help prioritize variants for further follow-up. Unfortunately, some of the prediction tools use terminology (damaging, pathogenic) that is easily confused with the more global ACMG criteria, which include additional information. For example, a predicted damaging or pathogenic variant by prediction algorithms may be considered benign

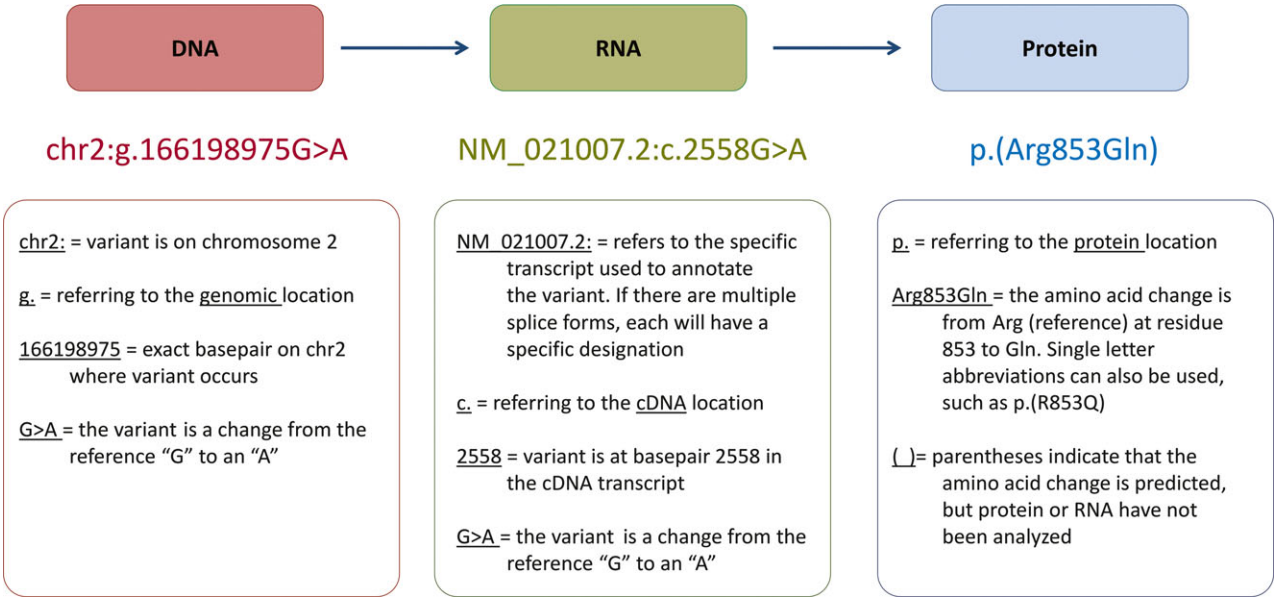

FIGURE 2 Human Genome Variation Society (http://varnomen.hgvs.org/) nomenclature for the *SCN2A* variant identified in the patient

by ACMG criteria as it is unrelated to disease or is seen in a large number of unaffected individuals.

3.2.3 | Variant frequency

The frequency of a variant in available control databases also adds to the assessment of the pathogenicity of the variant. For a severe disorder such as epileptic encephalopathy, pathogenic de novo variants are typically absent from control databases; finding the same variant in unaffected individuals would raise concerns about whether the variant is actually causing disease. If a disorder is rare, we would not expect the variants that cause it to be common in the population. Also, if a disease is severe, we might not expect disease-causing variants to be present in healthy individuals. For variants in recessive disease, a low frequency of heterozygous variants is typically permitted in control populations. Heterozygous individuals would then be considered carriers for this disease. In most circumstances, the population frequency of these variants is expected to be very low. There are several publicly available databases that include exome sequence data from large population studies. One of the largest is the Exome Aggregation Consortium,[28] which houses exome sequence data for >60 000 individuals. As of February 2017, the ExAC database has been extended to the Genome Aggregation Database (gnomAD) with >120 000 exomes and 15 000 genomes (http://gnomad.broadinstitute.org). Of course, there are limitations to using control databases to exclude variants based on the presence in control individuals. Some examples include the inclusion of individuals with related conditions (eg, psychiatric disorders) or who were evaluated prior to the usual age of onset of a given disorder, and the presence of somatic mosaic variants that cause milder or no disease in the control.[29] However, highly penetrant variants causing severe disease should be exceedingly rare if present at all.[30]

In many diseases, recurrent pathogenic variants are found, especially in diseases where a gain-of-function or a dominant-negative effect is the expected mechanism. Recurrent variants often occur at highly mutable CpG dinucleotides and affect critical functional domains in the protein. Epilepsy genes with prominent recurrent variants include *SCN2A*, *SCN8A*, and *KCNQ2*.[31–34] In some diseases, the majority of affected patients carry the same variant, as in progressive myoclonus epilepsy due to the recently identified *KCNC1* gene.[35] In many cases, identification of a well-established variant in a patient with a similar phenotype automatically classifies this variant as pathogenic in our patient.

As with gene validity, variant curation for disease genes is a community effort and a variety of publicly available repositories exist. The most prominent databases include the HGMD database and the ClinVar database (see URLs at the end of the article).

3.2.4 | Inheritance pattern

In severe disorders such as epileptic encephalopathy, the disease-causing variant is often de novo; for example, the variant is seen in the patient, but is absent in the parents. If the variant is found in either unaffected parent, it is usually considered benign or likely benign. Gene panel testing is usually performed on the proband only, but follow-up

testing is frequently performed in parents for selected variants to assess whether a variant is de novo or inherited. Exome sequencing is frequently performed on a trio basis including the affected individual and both parents to facilitate the identification of de novo variants. With increasing understanding of the spectrum of genetic variants in patients and controls, the role of the trio approach may diminish over time, but given the large number of unique variants, it currently represents the most comprehensive test available. In some cases, there are multiple affected individuals in a family, suggesting a dominant, recessive, or X-linked pattern of inheritance (see Primer Part 1), and the segregation of the variant is expected to match the suggested inheritance pattern. Another important consideration when there are multiple affected siblings is an apparently de novo dominant disorder with gonadal mosaicism in one parent. In this case, a subset of germ cells (sperm or ova) and often somatic cells carry the disease-causing variant and may approach 50%. In this case, recurrence risk increases with the level of mosaicism in the germline, and the mosaic parent is usually unaffected or only mildly affected. The occurrence of gonadal mosaicism has been well documented in some cases of Dravet syndrome due to apparently de novo pathogenic variants in *SCN1A*.[36]

In our case example, the diagnostic report was issued before the ExAC database was publicly available, so now is a good time to check for updated information. For example, the *SPTAN1* variant reported in our patient is present in 78 individuals (0.06%) in the ExAC database. As this variant was found to be present in healthy individuals, it is very unlikely to be causing our patient's severe, early onset disorder, and this variant would be reclassified to likely benign. Conversely, the *SCN2A* variant (R853Q) is not present in any of the >60 000 individuals in the database (Figure 2). The R853Q variant was found to be one of the recurrent disease variants in the *SCN2A* gene in the ClinVar database, but this information was not present at the time of the initial report. Subsequent testing of the parents demonstrated this variant to be de novo, but even in the absence of segregation data, prior knowledge would have allowed us to classify this variant as pathogenic. In a clinical context, the phenotype of our patient is compatible with *SCN2A* encephalopathy and the variant is therefore considered disease-causing.

4 | CONCLUDING REMARKS

Our vignette provides an optimistic example in which all variants that were initially considered of uncertain significance could confidently be moved into either the benign or the pathogenic category. In many cases, however, many variants do not have sufficient additional evidence to allow for such a classification. Communicating uncertainty to patients is challenging, but the ability to openly discuss our current knowledge and to properly document both clear and uncertain results is critical. The current flood of VUSs is increasingly recognized as the flipside of the increasing diagnostic yield of massive parallel sequencing technologies in the epilepsies. Importantly, the designation of variants as of "uncertain significance" is a placeholder that attests to our current lack of information at the time of testing. In these cases, additional information on both patient cohorts and control cohorts will likely provide more certainty in the future.

In summary, our case vignette provides an example of how considerations about gene validity, variant pathogenicity, and clinical correlation help us interpret a genetic result for a patient with epilepsy. We also used this example to demonstrate that in the era of massive parallel sequencing, genetic data are dynamic and interpretation can change over time. In our vignette, the *SCN2A* variant could be classified as pathogenic given additional patients with the same variant who had been identified after the initial report had been issued. Furthermore, the case vignette demonstrates that given the large variability of the human genome, it is wise to take a conservative approach in the interpretation of genetic results, requiring substantial evidence to implicate specific genes and variants in disease. This conservative attitude toward interpretation of results is particularly important when genetic results will be used for counseling, prognosis, and treatment decisions.

DISCLOSURE

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

URLS

Align GVGD: http://agvgd.hci.utah.edu/
CADD: http://cadd.gs.washington.edu/
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
Epilepsy Genetics Initiative: http://www.cureepilepsy.org/egi/
MutationTaster: http://www.mutationtaster.org/
Polyphen2: http://genetics.bwh.harvard.edu/pph2/
PROVEAN: http://provean.jcvi.org/index.php
SIFT: http://sift.jcvi.org/

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APPENDIX 1

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