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REVIEW

Diagnostic Considerations in the Epilepsies—Testing Strategies, Test Type Advantages, and Limitations

Wei-Liang Chen[1] · **Heather C. Mefford**[1,2]

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Abstract

The role of genetics in epilepsy has been recognized for a long time. Over the past decade, genome-wide technologies have identified numerous genes and variants associated with epilepsy. In the clinical setting, a myriad of genetic testing options are available, and a subset of specific genetic diagnoses have management implications. Furthermore, genetic testing can be a dynamic process. As a result, fundamental knowledge about genetics and genomics has become essential for all specialists. Here, we review current knowledge of the genetic contribution to various types of epilepsy, provide an overview of types of genetic variants, and discuss genetic testing options and their diagnostic yield. We also consider advantages and limitations of testing approaches.

Keywords Genetic epilepsy · Chromosome microarray · Gene panel · Exome sequencing · Inheritance pattern

Abbreviations

ACMG	American College of Medical Genetics and Genomics
CGH	Comparative Genome Hybridization
CMA	Chromosome Microarry (includes array CGH and SNP array technologies)
CNV	Copy number variants. Also called microdeletion or microduplication
GWAS	Genome-Wide Association Studies
LP	Likely pathogenic
LOH	Loss of heterozygosity
NGS	Next-generation sequencing. Also known as massive parallel sequencing
PRS	Polygenic risk score. Quantitative risk score generated by GWAS
SNP	Single nucleotide polymorphism. Single base pair variations seen in population. Usually has allele frequency > 1%
VUS	Variant of uncertain clinical significance

✉ Heather C. Mefford
heather.mefford@stjude.org

[1] Department of Pediatrics, Division of Genetic Medicine, University of Washington and Seattle Children's Hospital, Seattle, WA 98105, USA

[2] Current Location: Center for Pediatric Neurological Disease Research, St. Jude Children's Research Hospital, Memphis, TN 38105, USA

Introduction

Ever since the discovery of pathogenic variants in the *CHRNA4* gene in families with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) [1], searching for genetic etiologies of epilepsy has been a major endeavor for the epilepsy research community. Earlier studies primarily focused on candidate genes that encode ion channel proteins, ushering in the so-called channelopathy era [2]. Indeed, those early studies represented a major advance in our understanding about genetic epilepsy and opened the door for improved diagnostic testing and precision treatment in epilepsy. Over the past decade, our knowledge of the genetic basis of epilepsy has rapidly increased; with it, expanded options for genetic testing in the clinical setting have emerged. Especially for the severe pediatric epilepsies, the availability of genetic testing has changed the diagnostic approach to the patient.

A phenotype-based approach has been the traditional way for clinicians to determine a testing strategy. For example, a significant family history of febrile seizures may prioritize genetic testing for *SCN1A* or gene panels for febrile seizures; whereas a focal-onset seizure will more likely prompt a comprehensive neuroimaging study. With the discovery over the past decade that variants in any one of dozens of different genes can predispose to or cause epilepsy, it has become clear that phenotype-based approaches have inherent limitations. Testing one or a few genes based on the phenotype

at presentation can cause a significant delay in molecular diagnosis, and serial testing can add up to significant cost over time. Increasingly, a genetic diagnosis not only provides an answer regarding etiology but influences seizure management. Especially in epilepsy with neonatal presentation, timely diagnosis of the underlying genetic etiology is crucial for choosing appropriate treatments. Therefore, comprehensive and efficient "genome-first" approaches using large gene panels and exome sequencing are becoming the mainstream strategies and play an important role in shortening the diagnostic odyssey.

Although comprehensive genetic testing approaches have greatly accelerated the diagnostic process, they also increase the likelihood to encounter variants of uncertain clinical significance (VUS). Counseling families about potential outcomes of genetic testing (pre-test) as well as results (post-test) is a critical part of the diagnostic process that requires knowledge about types of human genetic variations and their molecular mechanisms, basic principles of genetic testing, classifications of clinical validity and variant pathogenicity, and inheritance pattern. Disease-causing genetic variants can range from a single base pair change to aneuploidies involving entire chromosomes (Table 1). Furthermore, epilepsy itself is a very heterogenous diagnosis and many times it is a part of a more complex genetic diagnosis. Therefore, to select appropriate testing strategies for suspected underlying genetic etiologies first requires some fundamental knowledge about molecular genetics and genomics. Second, one needs to understand the advantages and limitations of modern genome-wide testing, including chromosome microarray and next generation sequencing (NGS). Lastly, result interpretation is a process that must integrate both genotype and phenotype information.

This paper will discuss our recent understanding about the genetic basis of epilepsy, the molecular mechanisms of genetic and genomic variants, and the advantages and limitations of common genetic tests. Lastly, we will summarize the important concepts in correlation between genotype and phenotype and standard tools to classify variants.

Genetic Basis of Epilepsy: Current Knowledge

Epilepsy is a common neurological disorder with a prevalence of 5 to 8 per 1000 individuals and an individual lifetime risk of 2–3.0% [3, 4]. Although the underlying causes of epilepsy are heterogenous, early genetic epidemiology studies suggested its heritability and genetic basis [5–8]. However, the genetic architecture of the major classes of epilepsy—developmental and epileptic encephalopathies (DEEs), generalized epilepsy, and focal epilepsy—differ, which has implications for clinical testing.

In many syndromes associated with high risk for epilepsy, the genetic bases are clear. Syndromes associated with malformation of cortical development such as lissencephaly (*DCX* and *ARX* genes) and Tuberous Sclerosis (*TSC1* and *TSC2* genes) or disorders causing epileptic encephalopathy such as Angelman syndrome (*UBE3A*) are examples. The introduction of genome-wide chromosome

Table 1 Types of pathogenic genetic variants

	Size range	Basis of the variation	Example in epilepsy
Single base pair variation	1 bp	Substitution of one base at a particular location in the genome	Pathogenic missense or nonsense variants in monogenic epilepsies (such as *SCN1A, KCNQ2, UBE3A,* etc.)
Insertion/deletion (indels)	1 bp to several hundred bp	Addition or deletion of a short segment of DNA within a gene	Indels may cause a frameshift and premature truncation in a single gene
(Micro)deletion and (micro)duplication	10 kb to > 1 Mb	Deletion or insertion of a long segment of DNA that typically involves several contiguous genes	15q13.1 and 16p.13.11 deletion syndromes; Wolf-Hirschhorn (4p deletion) syndrome
Aneuploidy	Entire chromosome	One or more chromosomes have extra or missing copy	Trisomy 21
Structural rearrangement	Part of chromosome, usually > several Mb	Abnormal chromosomal combination as a result of chromosome breakage, recombination or exchange	Ring chromosome 20; large inversion
Imprinting disorder	1 bp to entire chromosome	Abnormal parent-of-origin gene expression caused by deletion, single nucleotide variants, uniparental disomy or imprinting defect	Prader-Willi and Angelman syndromes (15q11.3-q13)
Mosaicism	1 bp to entire chromosome	Mutation occurs post-zygotic and is therefore present in only a subset of cells	Focal cortical dysplasia and hemimegalencephaly due to mosaic variant in (primarily) brain tissue

arrays and then NGS approaches facilitated the discovery of potentially pathogenic variants in an unbiased fashion. Efforts through large consortia such as Epi4K and EuroEPINOMICS [9, 10] as well as many individual labs exponentially increased the rate of gene discovery, and there are now > 100 genes in which de novo variants (new mutations) are known to cause developmental and epileptic encephalopathies and many novel recessive and X-linked causes as well. Given the severity of these disorders, the affected individuals generally do not have offspring, and genetic variants are seldomly passed down in a family. Therefore, even though these rare genetic variants have high effect size, the heritability is low.

Unlike the syndromes caused by rare, high-penetrance variants, genetic (idiopathic) generalized epilepsy (GGE) has been more puzzling in terms of its genetic etiologies. As the name implies, genetic contribution has been long suggested, due to heritability estimates. The recurrence risks of generalized epilepsy for the first-degree relatives are 5 to 10 times higher than the background risk [5, 6]. Twin studies first suggested high heritability given the higher concordance rate in monozygotic compared to dizygotic twins [7, 11]. Prior to the availability of NGS, investigations for genetic etiology were mainly based on family studies (linkage analysis) when there was a clear pattern of inheritance. However, the genes identified in such families rarely explained GGE cases more broadly. In most families with a history of GGE, the pedigree suggests a complex inheritance pattern likely due to a combination of genetic and non-genetic factors. Genome-wide chromosome microarray studies highlighted several large copy number variants (CNVs) as risk factors for GGE [12], albeit with variable and incomplete penetrance; these CNVs are more frequently found in individuals with GGE and intellectual disability [13]. Exome analysis using NGS has accelerated discovery of new susceptibility genes. In a cohort with familial GGE, ultra-rare variants in any of 43 known monogenetic epilepsy genes (such as *KCNQ2, SCN1A*, and *GABRG2*) were twice as likely to be observed in cases compared to population controls. However, no single gene was significantly enriched in that cohort [14]. In a similar study, an excess of rare missense variants in the family of genes encoding $GABA_A$ receptor subunits was identified in individuals with familial GGE; again, no single gene predominated, and variants did not always segregate with disease in families [15].

In the focal epilepsies, there have been some successes in identifying causative genes, including *LGI1* in autosomal dominant epilepsy with auditory features [16], *KCNT1* in autosomal dominant nocturnal frontal lobe epilepsy [17], and *DEPDC5* in various forms of familial focal epilepsy [18, 19]. A study using exome sequencing in 525 individuals with familial focal epilepsy and 3877 unaffected controls identified five known epilepsy genes (*DEPDC5*, *LGI1*, *PCDH19*, *SCN1A*, *GRIN2A*) in which ultra-rare sequence variants were enriched [20]. As discussed in a later section, somatic mosaic variants have been found to play an important role in focal epilepsy, especially when focal cortical dysplasia or more extensive brain abnormalities are present.

Finally, genome-wide association studies (GWAS), which investigate the role of common sequence variants as risk factors for disease, have been performed in large cohorts of individuals with generalized and focal epilepsy. A mega-analysis combining several studies identified 16 genome-wide significant loci, some of which are in or near known epilepsy genes [21]. Polygenic risk scores (PRS) integrate multiple findings from GWAS to produce a quantitative risk score and have been developed for focal and generalized epilepsy as well [22]. Although testing for susceptibility genetic markers might become available for risk assessment in the near future, the target of currently available clinical genetic testing is primarily focused on causative variants.

Types of Genetic and Genomic Variants

Human genetic variations can occur on a broad spectrum ranging from single base pair variation to large aneuploidies involving entire chromosomes (Table 1). Understanding how each type of variations can cause disease is important for choosing genetic testing. This section will discuss common types of genetic disorders and some examples of genetic epilepsy in each category.

Copy Number Variants (CNVs)

CNVs are defined as losses or gains of chromosomal segments of DNA and can range in size from ~ 1 kb to an entire chromosome (aneupoloidy). CNVs can include any number of genes, and not surprisingly, some CNVs are associated with CNS manifestations including epilepsy. For example, epilepsy is more common in individuals with trisomy 21 (entire chromosome) [23] and Wolf-Hirschhorn syndrome (partial deletion of the short arm of chromosome 4). Another distinctive chromosomal syndrome highly associated with epilepsy is Ring Chromosome 20 syndrome; the phenotype is variable in part due to the fact that many cases exhibit mosaicism for the ring chromosome [24]. These and other chromosomal syndromes have been recognized for decades, as they can be diagnosed by traditional karyotype analysis.

Smaller CNVs (< 5–10 Mb) are not easily identified by karyotype analysis, but the introduction of chromosome microarrays in the early 2000s highlighted their role in a range of neurodevelopmental disorders, including epilepsy.

In the past decade, several breakthroughs were made in discovery of CNVs in epilepsy. As noted above, several large CNVs, most notably recurrent deletions of 15q11.2, 15q13.3, and 16p13.11, were identified as important risk factors for GGE [25], though only in ~ 3–10% of individuals depending on comorbidities. Other recurrent CNVs associated with epilepsy and developmental abnormalities are 1p36 deletion [26], 1q21.1 deletion and duplication [27], 16p11.2 deletion [28], and 22q11.21 deletion and duplication [29]. Non-recurrent deletions and duplications, especially those that involve known epilepsy genes, are important in the etiology of developmental and epileptic encephalopathies. Overall, the diagnostic rate for CNV testing is 5–12% [30–34] with higher yield in patients who also have non-neurological abnormalities and dysmorphic features [29].

Genetic Change at the Sequence Level

With the advent of NGS technologies, our understanding of single-gene disorders has improved substantially. By far, the highest impact has been in the developmental and epileptic encephalopathies, where the most commonly identified pathogenic variants causing epilepsy are highly penetrant alterations of one or a few base pairs in a single gene. Although much emphasis has been focused on de novo variants, recessive and X-linked modes of inheritance are also important in DEE [35]. Taking a careful family history and knowing the most likely inheritance pattern is important for interpretation of potentially pathogenic variants.

Understanding specific types of pathogenic variants and their potential impact on protein function can help when interpreting variants reported by clinical labs [36]. The majority of disease-causing variants cause loss of function of the protein. This can occur via variants that prematurely truncate the protein, such as nonsense, frameshifting, or splicing variants, or through missense variants that abrogate protein function (e.g., amino acid change in a binding pocket or catalytic site). Recessive disorders require loss of function of both alleles. Dominant disorders are often a result of loss of function on one allele (haploinsufficiency); however, depending on the gene, location and type of amino acid substitution, missense variants can cause gain of (or new) function. For some genes, variants resulting in loss of function cause one phenotype while gain-of-function variants cause another. For instance, missense variants in *SCN8A* that cause premature channel opening and persistent current of Nav1.6 sodium channel in excitatory neuron (gain of function) result in DEE [37], whereas loss of function of the same channel causes a milder cognitive impairment with epilepsy [38]. Knowing the mechanism can have important implications for therapy: epilepsy due to gain-of-function variants in *SCN8A* can be treated with high-dose sodium channel blockers [39], whereas the same class of medication may worsen disease due to loss of function of the same channel.

Somatic Mutation

The term somatic (or post-zygotic) mutation refers to variants due to a mutation that takes place after fertilization during the early embryonic stage. The somatic mutation is always de novo and leads to mosaicism, a state in which two or more genetically distinct cell lines in an individual develop from a single fertilized oocyte. The timing (when in development) and location (cell type) determines what tissue type(s) and percentage of cells in each tissue will carry the variant. Somatic mutation has been recognized as an important cause of some focal cortical dysplasias and brain malformations associated with epilepsy [40]. Somatic variants—often restricted to brain tissue—have been identified in several genes that function in the MTOR pathway, including *PIK3CA*, *PIK3R2*, *AKT3*, *MTOR*, and *CCND2*. Associated phenotypes range from focal cortical dysplasia to hemimegalencephaly [41–46]. Importantly, the role of the MTOR pathway raises the possibility to repurpose pathway-specific inhibitors for hyperactive PI3K, AKT, and MTOR kinases [47, 48]. Several recent studies also highlighted the role of somatic mosaic variants in *SLC35A2*, an X-linked gene in radiographically non-lesional focal epilepsy, where up to 15% of individuals undergoing surgical resection had a somatic variant [49–51].

In the diagnostic setting, affected tissue is often required to identify a somatic mutation via sequencing. In some cases, low levels of the variant may be detectable in the blood, but dedicated sequencing approaches such as ultra-deep sequencing with NGS may be required. In following sections, NGS and genome-wide tools will be reviewed in detail.

Clinical Genetic Testing, When to Choose Chromosome Microarray, Gene Panel, Exome Sequencing, or Genome Sequencing?

Chromosome Microarray

Although karyotyping is still used in some clinical settings, it has largely been replaced by chromosome microarray analysis (CMA), which is more precise (Table 2). Two types of technologies are commonly used for CMA: comparative genome hybridization (CGH) and single nucleotide polymorphism (SNP) array. The main difference between SNP and CGH arrays is the ability to detect copy-neutral variants such as isodisomy with SNP array. For the most part,

clinical laboratories determine which type of CMA to use, so the clinician may be unaware of the specific type of CMA employed; the diagnostic yield of each is similar. In rare situations, the SNP array has advantages. For example, if the recessive disorders are suspected due to the family history of consanguinity, SNP array analysis can determine regions of loss of heterozygosity (LOH), where recessive variants may reside; additional sequencing (gene, panel or exome) would be required to identify causative variants. Another advantage is that SNP arrays provide more precise estimation of copy number than CGH and can also detect moderate levels of mosaicism for copy number variants. Neither SNP nor CGH can detect balanced rearrangements, such as balanced translocations, inversions, and some ring chromosomes; karyotype is the appropriate test if such a rearrangement is suspected.

Importantly, copy number changes are increasingly detected using NGS assays, especially exome or genome sequencing. Other than SNP and CGH arrays, many labs that offer exome or genome sequencing today are able to identify copy number variants based on sequencing data. Although CMA remains the gold standard given its relative low cost and well-established reliability, recent research has showed that modified workflow for exome sequencing [52] and newer technologies using PCR-free library preparation [29] make sequencing equally accurate in CNV detection. Therefore, NGS platforms might be considered as equally reliable methods as CMA in detecting CNVs in the near future.

Soon after its introduction into the clinical setting, CMA emerged as a first-line test for a range of neurodevelopmental disorders [53]. However, NGS approaches (discussed below) that allow sequencing of multiple or all genes simultaneously *as well as* CNV detection are moving earlier in the diagnostic process. Even without CNV detection, exome sequencing has a better diagnostic yield than CMA in the epileptic encephalopathies. CMA testing should still be considered early in the diagnostic process for individuals with early-onset epilepsy and dysmorphic features or congenital anomalies; individuals with GGE and intellectual disability or other neurodevelopmental abnormalities; and individuals with severe early-onset epilepsy for which NGS does not identify a causative variant.

Next-Generation Sequencing (NGS)

NGS is a method of sequencing of millions of small fragments of DNA in parallel, also referred to as massively parallel sequencing. Using bioinformatics, these small fragments can be mapped to and compared with the human reference genome to identify variants. NGS can be utilized to sequence the entire genome or can be targeted to specific genes of interest, ranging from a selected gene set (gene panel) to all ~ 22,000 coding genes (exome sequencing). NGS has drastically changed clinical genetic testing. In the clinic, the phenotype-based single-gene test using traditional Sanger sequencing is rarely appropriate and has largely been replaced by gene panels, which are usually designed to include genes that share a similar predominant phenotype, such as epilepsy. Some gene panels are more specific to a given diagnosis and only include a few genes; others might include hundreds to thousands of genes to cover a non-specific diagnosis, such as neurodevelopmental delay. Exome sequencing is the ultimate gene panel, with all known genes included.

Gene Panels

Gene panels are a common and important class of NGS tests that have some advantages over more comprehensive exome sequencing. First, gene panels test a set of genes related to a specified phenotype; therefore, unexpected findings—such as a variant in a cancer susceptibility gene—are less likely to be reported. Similarly, the frequency of VUS may be lower, since there is usually some knowledge of the types of variants associated with disease on the gene panel. Secondly, true gene panels, in which only the genes of interest are captured and sequenced, have higher depth of coverage that allows a more comprehensive analysis of each base pair. Additionally, many commercial labs use supplementary methods to enhance the coverage for the missing sequences and frequently include deletion/duplication testing on exon level, which cannot be detected by CMA or exome sequencing. Thirdly, the high depth of coverage also increases the likelihood to identify mosaicism (somatic mutation). Lastly, gene panels are in general less costly. It is important to note, that especially for large gene panels, the laboratory may actually perform exome sequencing but only analyze data for genes listed on the panel; when an "exome backbone" is used, the advantages of high sequence depth are lost. On the other hand, if a panel is performed using an exome backbone does not yield a diagnosis, the entire exome can be reflexively analyzed (usually for additional cost) without the need for an additional sample or test.

Many commercial gene panels for epilepsy are available. The number of genes included in those panels vary. Some of them are designed specifically for certain syndromes such as progressive myoclonic epilepsy and contain only a few genes; others are more comprehensive and include hundreds of genes. In addition, the genes included in each panel are not always overlapping. Unique genes can be seen in gene panels from different labs yet targeting the same diagnosis. Hence, it is difficult to systemically compare the yield of those panels. Nevertheless, the trend of diagnostic yield can be observed collectively (see review [54]). Several recent studies reported a diagnostic yield of gene panel (~ 40–80

Table 2 Technologies used in genetic analysis

	Resolution	Applications	Limitations
Karyotyping	2–3 Mb (high-resolution banding) to 5 Mb (routine banding)	Scanning for aneuploidy, large deletion or duplication and structural variants. Particularly useful in patients with distinctive multiple congenital anomalies indicative of chromosomal abnormalities (trisomies, ring chromosomes)	Cannot identify single nucleotide (sequence) changes, indels, or small deletions and duplications
Chromosome microarray-single nucleotide polymorphism (SNP) array	Few kb or longer	Scanning the entire genome for any copy number variation (microdeletion and microduplication syndromes); can identify regions of loss of heterozygosity (LOH)	Cannot identify copy number neutral variant such as balanced translocation and inversion
Chromosome microarray-comparative genome hybridization (aCGH)	Few kb or longer	Scanning the entire genome for any copy number variation. SNP-array and array-CGH can be considered as first-tier testing in patients with intellectual disability and/or non-neurological congenital abnormalities	Cannot identify copy number neutral variant such as balanced translocation and inversion; cannot identify LOH
Gene panel testing	One to several bp	Next-generation sequencing of several to hundreds of genes of interest. Can be supplemented with other technologies (such as Sanger sequencing) to fill in low-coverage regions and detect small CNV (exonic deletion or duplication). "True" panel testing with deep coverage can detect low-level mosaicism	Cannot detect noncoding or deep intronic changes. Different labs have gene panels with similar targeted phenotypes yet consist of different sets of genes
Exome sequencing	One to several bp	First or second tier testing for patients with intellectual disability, developmental delay and congenital anomalies. Rapid exome sequencing should be considered in critical patients. In some cases, copy number variants can be identified	Often require parental samples (trio exome). Pre- and post-testing counselling are imperative (high likelihood to find VUS or secondary findings). Might miss variants in low-coverage regions and deep intronic changes. Can miss low-level mosaicism (sequencing with standard coverage). Less reliable for copy number calling
Genome sequencing	One to several bp	Scanning for entire genome including non-coding and deep intronic regions. PCR- and capture-free methods make genome sequencing very reliable to detect copy number variants and variants in exonic regions that are insufficiently captured by exome sequencing	Cost remains greater than exome. The predicted effects of most variants in non-coding regions are difficult to interpret

genes) ranging from 15 to 28.5% [55–58]. Higher yield was seen in younger patients (23.2–52%) [55, 58] and patients with epileptic encephalopathy (50.6%) [57]. Therefore, when the clinical phenotype is very compelling, gene panels can be used as the first-tier investigation, especially if a rapid turn-around panel is available in the context of managing neonatal and drug-resistant epilepsy.

Exome Sequencing

The exome refers to the ~ 1% of the genome that is protein-coding, which includes an estimated ~ 22,000 protein-coding genes, though not all the genes have a known clinical implication. As of December 30, 2020, 6794 phenotypes have known molecular bases; among them, 4368 genes are known to cause phenotypes (https://www.omim.org/statistics/geneMap). Therefore, variant analysis focuses on the genes with known (or predicted) disease associations. The overall diagnostic yield of exome sequencing in epilepsy is between 22 and 59% [59–63], which is higher than gene panels. Selected cohorts such as pediatric patients with intellectual disability or early-onset epileptic encephalopathy seem to have even higher diagnostic rate [60, 63, 64]. A recent study using research exome sequencing to analyze/reanalyze data from a cohort of pediatric patients with undetermined genetic etiology by CMA and/or gene panel shows that exome sequencing identifies a pathogenic or likely pathogenic variant in 25% of these cases. The authors concluded that exome sequencing outperforms gene panels [64]. Sequencing the entire exome also allows for novel gene discovery; novel genes accounted for 7% of genetic etiology in a large cohort study [61]. Though novel gene discovery remains largely a research effort, periodic reanalysis of exome data from clinical exomes is an important contributor, and patients may benefit when updated test results are issued.

There are important factors to consider when deciding whether to send an exome versus a gene panel. Often the most efficient approach for exome sequencing is to perform "trio" analysis, which includes testing the affected individual and both parents. Trio analysis is especially helpful for detecting de novo (new) variants in an affected child or for phasing variants for recessive disorders (to ensure inheritance of one variant from each parent rather than two variants from a single parent). Without parental data in such cases, there is an increased chance for VUS. Compared to true gene panels, where only the genes of interest are captured and sequenced, the genes in exome sequencing have less uniform coverage. In a gene panel with a handful of genes, the non-uniformity can always be corrected with additional methods. However, in exome sequencing, it is impractical to patch all the low coverage regions. Therefore, variants in these low-coverage regions of interest could be missed by exome sequencing.

Pre-test and post-test counseling is an important part of all genetic testing, but especially for exome sequencing. Families should be counseled about the possible test results, which can include positive (diagnostic), negative (nondiagnostic), or VUS, which is also a nondiagnostic result. Research studies have revealed that 10–30% of the variants found by exome sequencing in patients with epilepsy are VUS's [61, 63, 64]. Ideally, a VUS should be treated as a negative result. However, in real world, it is always more complicated. The clinician's comfort level, family dynamics and the interaction between doctors and family all can determine the outcome of counseling for a VUS. Importantly, exome sequencing can identify disease-causing variants that are unrelated to the indication of this genetic testing; families should be counseled about this aspect and allowed to choose whether they receive such results. The ACMG (American College of Medical Genetics and Genomics) published recommendation for reporting pathogenic variants in 59 medically actionable genes, including genes associated with hereditary cancer syndromes and cardiac diseases. In a recent study, 2.7% of patients who underwent exome sequencing for epilepsy were found to have ACMG-classified secondary findings in one of these genes [60].

Exome or Gene Panel?

In the severe epilepsies, gene panel or exome testing have a higher diagnostic yield than CMA. However, there is no clear guideline regarding when to send gene panel testing (or which panel to choose) versus when to select exome sequencing. In the real world, practical issues such as insurance coverage often play a role. Other considerations should include patient phenotype, acuity, turnaround time, availability of family samples, level of support for pre- and post-test counseling, and level of comfort of both the family and ordering provider. If rapid turnaround is needed for an acutely ill patient, a rapid exome may be the most efficient (and costly) route to diagnosis; rapid turnaround gene panels are also available, though a negative result will prompt further testing, extending the time to diagnosis. One recent study showed 52% of critically ill neonates had at least one change in management related to the result of rapid exome sequencing [65]. If it is not possible to include samples from parents or other family members, a gene panel may be less likely to yield VUS. For very specific phenotypes, gene panel testing may be most appropriate. Ideally, in all settings, collaboration with a geneticist or genetic counselor for pre- and post-test counseling is available; if not, the level of comfort of the ordering provider and the family is an important consideration in choosing a test, as the larger the test, the more likely a VUS is reported.

Considerations for Variant Interpretation

Clinical laboratories are responsible for variant interpretation and reporting. However, some familiarity with how variants are evaluated and interpreted in the context of clinical testing is useful for any provider who uses genetic testing. A full discussion of variant interpretation is outside the scope of this review; a comprehensive approach for clinical interpretation of variants was published by the American College of Medical Genetics & Genomics [66] and is implemented by most clinical laboratories. Pathogenicity of a variant is determined by 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 (VUS), likely pathogenic (LP), or pathogenic based on these criteria. Community efforts to evaluate genes and variants specific to epilepsy are ongoing through the NIH-funded ClinGen resource (clinicalgenome.org, [67]). Finally, a recent review focused on gene and variant interpretation in the setting of epilepsy and may be a useful resource for non-geneticists [36].

Final Considerations

Currently, diagnostic genetic testing is most effective in conditions where highly penetrant variants in coding regions of a single gene result in disease. In the epilepsies, this includes the developmental and epileptic encephalopathies, some focal epilepsies, and rare, familial (often autosomal dominant) forms of epilepsy. The more common generalized epilepsies and some focal epilepsies appear to have a more complex genetic basis, with many interacting genetic factors each contributing small risk; current testing is not effective in these cases, though the development of polygenic risk scores [22] may eventually have an impact in the clinic. Even in known single-gene disorders, exome sequencing may not detect some pathogenic variants, including those that lie deep in an intron or in other 'noncoding' regions. Introduction of clinical genome sequencing will facilitate better variant detection, though our ability to interpret disease associations outside of the exome will lag behind our ability to detect variants.

Genetic diagnosis should be a dynamic process, and longitudinal reinterpretation is always indicated. As clinical and research data accumulate, we gain knowledge that affects our understanding of disease-gene associations and variant interpretation. Furthermore, technological advances impact the efficiency and accuracy of variant detection. As a result, reanalysis of clinical testing data can result in an update report that may include an upgrade from uncertain to pathogenic variant, but in rare cases may include a downgrade from pathogenic or uncertain to benign. The clinicians who order genetic testing have the responsibility to counsel their patients that the interpretation of genetic testing may change with advances in medical knowledge and genetic technology.

Conclusion

It is almost impossible these days for a neurologist or epileptologist to care for their patients without some fundamental knowledge about genetics and genomics in the era of precision medicine. Understanding the molecular mechanism of genetic variations and basic principles about modern genome-wide testing are essential to interpret results of the genetic test and treat the underlying disease accordingly. Choosing between different genetic testing such as CMA, gene panels and exome sequencing might not be straightforward. However, several considerations such as the specific phenotype, urgency for the diagnosis, differential diagnosis, psychosocial wellbeing of the family, and provider's comfort level on counseling complex testing should be taken into before ordering a genetic test. Genetic diagnosis is always a dynamic and ongoing process. Patient and family should be informed that further genetic testing and even future re-analysis could be indicated. As the advance in genetic technologies, more comprehensive genetic testing, such as genome sequencing, might become standard care at the cost of identifying more uncertain and incidental findings. Nevertheless, the importance of identifying underlying genetic etiology cannot be overstated as more gene-specific or pathway-specific treatments become available.

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Required Author Forms 16 provided by the authors are available with the online version of this article.

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