Neurology Individualized Medicine: When to Use Next-Generation Sequencing Panels

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Abstract

Next-generation sequencing (NGS) is increasingly being applied to clinical testing. This practice is predicted to grow especially in neurology clinics because many of their patients have monogenetic causes for their “diagnostic odyssey.” The cost of sequencing has been steadily decreasing, but the cost of DNA sequencing is a minor part of the total cost. Downstream data analysis, storage, and interpretation account for most of the total expense. In patients with nonspecific neurologic disorders in which an extensive number of genetic differential diagnoses exist, whole-genome sequencing (WGS) or whole-exome sequencing (WES) has shown promise in the identification of genetic causes. However, both WGS and WES have incomplete coverage and produce a large number of rare variants of unknown importance. In addition, ethical dilemmas are often created by unexpected findings in genes unrelated to the initial sequencing indication. Targeted-panel NGS starts with the capture of a set of disease-focused genes, followed by massive parallel sequencing. For many genetically heterogeneous neurologic disorders, a genetic panel that is disease focused yet inclusive of a large genetic differential diagnosis can be defined to reduce cost, increase turnaround time, and optimize performance. Targeted-panel NGS is currently the preferred first-tier approach because it provides a reliable clinical application while eliminating unexpected ethical dilemmas. Targeted-panel NGS is leading to
A paradigm shift in the diagnosis of many neurologic disorders, enabling individualized precision medicine. In this review, we provide an overview of WGS, WES, and targeted-panel NGS in consideration of their utility in clinical testing for neurologic diseases.

One of the most notable medical innovations in the past decade is next-generation sequencing (NGS). This revolutionary technology makes it feasible to comprehensively consider the genetic component of any medical illness. In neurology clinics where Mendelian-inherited “diagnostic odyssey” cases are common, this technology has been transformative. The complex biology that defines the nervous system particularly benefits from the remarkable technical capabilities of this application. Specifically, diverse pathogenic pathways disturbed by different monogenetic sequence variations frequently result in the same clinical presentations. For example, the genetic pathologic causes of peripheral axonal injury are extremely diverse, but sensory loss or weakness is a common symptom. Acquired etiologies may also perturb the same pathogenic pathways and lead to similar symptoms, making it difficult to differentiate genetic from acquired causes. The genetic and phenotypic heterogeneity combined with clinical presentations that can mimic acquired etiologies makes comprehensive genetic testing especially appealing. Historically, neurologists have been frustrated by low diagnosis rates with use of Sanger sequencing of selected candidate genes. During recent years, the application of NGS in genetic testing has substantially increased the genetic diagnosis rate. The impact of a positive genetic finding is often profound. Establishing a specific neurologic genetic diagnosis allows for (1) avoidance of improper therapies and additional costly and invasive testing such as biopsy, (2) specific prognostic and disease management information, (3) family genetic counseling, and (4) opportunities to participate in drug clinical trials and/or emerging therapies.

Three NGS approaches are currently available: whole-genome sequencing (WGS), whole-exome sequencing (WES), and targeted-panel sequencing (Figure 1). Clinicians, patients, and insurance providers are wrestling to provide genetic testing recommendations. This article provides an overview of sequencing technologies and emphasizes the importance of the laboratory geneticist and clinician working together in designing, implementing, and updating clinical genetic testing. Currently, for many categories of neurologic diseases, targeted panels are the most efficacious choice, and emerging evidence indicates that application of targeted NGS can improve the quality of patient care and reduce health care expenditures.

GENETIC AND PHENOTYPIC HETEROGENEITY OF NEUROLOGIC DISEASES

Genetic involvement in the pathogenesis of neurologic disease is substantial, not only in various forms of Mendelian disorders such as inherited neuropathies, ataxias, and epilepsies but also in common complex disorders such as many types of migraine, autism, multiple sclerosis, dementias, and the predominance of idiopathic epilepsies. Within the publicly accessible library of inherited human disease (Online Mendelian Inheritance in Man [OMIM]), phenotype entries of neurologic disorders with or without known molecular cause are overrepresented (Table). The genetic testing for neurologic disorders has largely been focused on monogenic forms, but neurologic disorders are known to be extremely genetically heterogeneous. Different sequence variations within the same gene may cause quite different phenotypes, and the same phenotype can be caused by many different mutated genes. This variance is not surprising because the nervous system is complex and intricately regulated with high fidelity. The Allen Institute for Brain Science human brain transcriptome project revealed that over 80% of all known coding genes are expressed in the brain, underscoring the nervous system’s vulnerability. In the peripheral nervous system, single-cell axons may be as long as 1 m and have intricate supporting networks, each of which has genetic susceptibility to injury. Before NGS, Sanger sequencing was the main method for mutation screening,
but clinicians had to pick a candidate gene(s) to sequence, and testing was expensive. Picking candidate genes from a long list of causal genes (often >50) has always been challenging, even for experienced clinicians. This step contributed substantially to the low genetic diagnostic rate and high cost before the availability of NGS. It was anticipated and is increasingly realized that neurologic disorders would have high demand for comprehensive genetic testing, and therefore, neurology would be one of the early specialties to benefit from NGS in routine clinic diagnosis.¹

**THE SEQUENCING REVOLUTION**

The sequencing revolution began about 10 years ago with the invention of massive parallel sequencing techniques.²⁻⁴ Fragmented DNA is amplified and sequenced in parallel, then aligned to the reference genome and evaluated for nucleotide changes and small insertions/deletions by powerful bioinformatics software. Since then, DNA sequencing capabilities have been increasing at a dramatic rate, with the data output of NGS more than doubling each year, outpacing the Moore law. The first human genome sequencing published in...
Whole-genome sequencing denotes the sequencing and data analysis of an entire genome, which comprises approximately 3 billion base pairs (bp) of DNA sequence, whereas WES covers all protein-coding regions of the human genome including 5’ and 3’ untranslated regions, totaling approximately 50 million bp (1.5% of the genome). As its nomenclature indicates, WGS offers the most comprehensive sequencing analysis without any limitation from a capturing step, but the entire genome is not actually being sequenced with current WGS because of technical limitations. A recent study analyzed the WGS of 12 individuals and found that approximately 10% to 20% of variants in genes known to be important in inherited diseases were not adequately covered. Aside from incomplete coverage, several other factors limit wide utilization of WGS in the clinical and research setting. First, the functional implications of variants within introns and intergenic regions are largely unknown; thus, interpretation of these variants is quite challenging. Second, the sequencing cost of WGS is 3- to 5-fold higher than that of WES to achieve more than 100X coverage, the average coverage depth used in current clinical practice; WGS is even more costly when compared with targeted sequencing of gene panels. Third, the processing of sequencing data including bioinformatic filtering, data storage, data analysis, and interpretation, along with the time of bioinformaticians, geneticists, and clinicians, is substantially greater for WGS than for other NGS approaches. Fourth, even in the research setting in which lower coverage of WGS is generally accepted, the focus is largely on rare variants, which require large sample sizes to have statistical power. Therefore, until sequencing and data analysis costs decrease substantially, the clinical application of WGS will remain limited.

### Whole-Exome Sequencing

The purpose of WES is to sequence approximately 1.5% of the total genome, and thus, data analysis is considerably less than that obtained with WGS. Recently, WES has been adopted by many genetic testing laboratories for clinical testing because the exome can be meaningfully interpreted. However,
similar to WGS, WES does not really achieve “whole” coverage, and the exome-capturing step of WES leads to greater variability in coverage depth. Sequencing at an average depth of 150X to 200X generally ensures that most covered regions will have at least 20X coverage. Although the purpose of WES is to cover all the exons and regulatory regions of all genes, in practice, an average of only about 90% of the exome is covered adequately, and sequencing with higher depth (higher cost) still does not lead to complete coverage. In certain problematic regions such as those with high GC or very low GC content, the depth of coverage is much lower or completely missing because of low capture efficiency and sequencing bias. 28 We recently utilized WES to assess patients with idiopathic peripheral neuropathy and found that among known neuropathy genes, 7% of exons had coverage depth of less than 10X, and 2% were not covered at all.29 A recent study analyzed 57 whole-exome data sets with coverage depth of 74X to 120X to evaluate 56 genes that the American College of Medical Genetics and Genomics (ACMG) designated as relevant.30 They found that in 7 of these genes, more than 50% of variant locations listed in the Human Gene Mutation Database (HGMD) had inadequate coverage.31 Thus, using WES to cover all the genes associated with a specific disease category may have major deficiencies if some critical exons are not sequenced or certain known sequence variations are not included in the WES capture. In addition, the cost of WES is still considerably higher than that of targeted-panel sequencing. The sequencing portion of cost for WES is now around $700 per sample for approximately 150X depth, but with the cost of bioinformatic analysis and interpretation, the overall cost of WES is easily 5- to 10-fold higher. Moreover, WES or WGS may identify unexpected pathogenic sequence variations unrelated to the patient’s underlying clinical diagnosis;
this is a delicate and important ethical issue that clinical laboratories cannot take lightly. These types of findings have serious implications for patients and their families; the proper handling is complex and requires additional personnel and infrastructure (leading to increased cost) probably only possible in specialty clinics and at tertiary care referral centers. Guidelines have been created by the ACMG to address these problems. The ACMG requires reporting of any secondary findings found in each of the 56 disease-related genes. These genes were selected because of the clear disease risks associated with sequence variations in these genes as well as the ability to intervene if a mutation is identified. Insurance companies are currently reluctant to pay for WES because of the higher costs and the complex issues of identifying a number of actionable variants by WES.

Nonetheless, WES is a promising genetic testing approach, particularly in patients in whom targeted-panel approaches have not identified causative sequence variations or the clinical localization is diverse, such as in multiple congenital anomalies or autism spectrum disorders. For patients with strong disease inheritance in whom both targeted-panel sequencing and WES has failed, research-based genetic discovery studies utilizing WGS can still be considered (Figure 3).

FIGURE 3. The decision of which genetic sequencing platform to utilize depends on the genetic diversity of a specific neurologic disorder. Next-generation sequencing (NGS) by whole-exome sequencing is best for “diagnostic odyssey” cases in which neurologic localization is not well established or the genetic differential diagnosis is not limited to any specific disease category. Among those with a defined genetic differential diagnosis, targeted-panel NGS has the best test performance metrics. Certain neurologic phenotypes have very specific characteristics, and single-gene testing remains very effective.
TARGETED-PANEL SEQUENCING

Advantages

Targeted NGS panels are best suited for patients who have been diagnosed as having a focused disease category in which high genetic heterogeneity exists but focused genetic panel testing likely yields a reasonable detection rate. Such disease categories include inherited neuropathy, myopathy, neuromuscular junction diseases, and motor neuron and epilepsy syndromes among others. Clinical expertise in these disorders and the relevant genetic knowledge are essential in choosing the appropriate genes for the panels. Several studies have reported that this approach is a robust tool that fully utilizes the customized features of NGS and is effective for genetic diagnosis in neurologic disorders. Targeted-panel sequencing requires a target-enrichment step that selectively captures or amplifies specific genomic regions of interest before massive parallel sequencing (Figure 1). In contrast to WGS and WES, a targeted panel for a specific disease category might only cover several hundred kilobases to several million base pairs (ie, ~0.5%-5% of an exome). Such a panel might include approximately 50 to 300 genes. Assuming that a custom-designed panel covers a total of 2 million bp, the current Illumina HiSeq 4000 can sequence approximately 100 samples per lane and achieve about 400X coverage depth. Current target-enrichment techniques include hybridization-based and multiplex polymerase chain reaction amplification methods. Based on the typical size of targeted panels for neurologic disorders, hybridization-based capture, in which thousands of probes are custom designed to capture all exons and exon-intron boundaries of disease-associated genes, is the most commonly applied method (Figure 3). Samples can be bar-coded by ligating to a unique, approximately 8-bp oligonucleotide, then pooled and sequenced on one lane. After the sequencing run, unique bar codes can assign each sequence read to an individual sample. Compared to WGS or WES, the targeted-panel approach substantially reduces the sequencing cost and downstream bioinformatics expenditure required for data analysis and interpretation while achieving ultrahigh coverage depth. The high coverage depth is especially important in the clinical setting, where accurate variant identification is essential. Clinical laboratories optimize each disease-focused panel to ensure that every underlying variant from these genes is thoroughly assessed. Among all 3 NGS approaches, the targeted-panel approach has the lowest false-negative call rate for a specific set of disease-focused genes.

The targeted-panel approach limits the variant screening within the scope of the intended clinical investigation by focusing only on known causal genes. This feature not only allows patient samples to be sequenced at low cost but also limits the number of variants with unknown importance, decreasing the complexity of clinical reports and diminishing ethical dilemmas by eliminating discoveries not related to the disease category. In addition, targeted sequencing yields a diagnostic rate similar to that of WES, as documented by recent reports on the efficacy of NGS testing for diagnosing hereditary polyneuropathy and epilepsy. Specifically, among patients with neuropathy who have a family history of the disease and younger age at onset (<40 years), the diagnostic rates are approximately 30% for both WES and targeted sequencing, despite the fact that WES sequences more than 20,000 genes while the neuropathy panel only includes 192 genes. This situation occurs because definitive genetic diagnosis can only be made from the causal genes/sequence variations that have been previously validated. For rare functional variants in novel genes, the conclusive pathogenicity will require further investigation, which often involves considerable additional effort from a research team. Therefore, sequencing genes that have no known disease associations provide little clinical value at the present time. However, this is not to say that those data have no value; rather, the sequencing data obtained from WES will be an invaluable resource for future research studies, especially when we accrue a large data bank that contains sequencing information for a group of well-characterized patients in the same disease category. Future data mining is likely to be very fruitful. For current clinical testing, cost is obviously the major driving factor for choosing targeted panels for various neurologic disorders.
because the cost of thoroughly sequencing all coding exons with high depth coverage remains prohibitive.

Limitations
The main advantage of targeted-panel sequencing over WES and WGS—focusing on a set of disease-specific genes—is also the primary limitation for new gene discovery. Despite the impressive advances in the genetic field, sequence variations in known genes explain only a small proportion of genetic causes of neurologic disease. The diagnosis rate of WES or targeted panels ranges from only 10% to 40%, depending on the specific panel and patient selection. Clearly, more genes contributing to the risk of neurologic disorders remain to be discovered, which is not surprising given the established clinical and genetic heterogeneity of neurologic disorders. When a genetic diagnosis cannot be identified from targeted-panel sequencing, this result suggests 3 possibilities: (1) that disease-causing sequence variations reside outside the region covered by the current targeted panel, ie, a novel gene is involved; (2) the condition is caused by multiple genetic factors; or (3) the disorder is due to an acquired (nongenetic) etiology.

The first 2 possibilities could be considered for research studies searching for novel genes in which WES and WGS are appropriate. WES or WGS should also be employed when clinical presentations are nonspecific and involve multisystem symptoms, a situation in which it is difficult to choose a specific panel. In these cases, copy number evaluations through either traditional chromosomal comparative hybridization techniques or copy number variation (CNV) analysis algorithms using NGS data should also be considered. It should be emphasized that pursuing a genetic diagnosis in undiagnosed patients often requires more than simply sequencing a proband, or trio evaluation by WES or WGS; collaboration with research teams is frequently needed to pursue kindred, linkage, and functional studies for additional evidence to validate the finding and elucidate pathophysiologic mechanisms. The identification of a novel gene will require either a very large pedigree with many affected and unaffected persons or a large number of patients with similar phenotypes. Thus, worldwide collaboration will increasingly become important in genetic studies to look for patients with the same disorders. Increasingly, more initiatives have been introduced to establish and integrate large databases in order to maximize the potential of NGS in the identification of disease-causing mutations and to organize large-scale clinical trials. Substantial progress has been made in recent years. For example, Matchmaker Exchange and GeneMatcher allow clinicians and researchers to share their discoveries on the same genes or similar cases; TREAT NMD and the Inherited Neuropathies Consortium are both large networks enabling concerted research discovery and clinical trials through international collaborations. These efforts have led to important findings.

Clinician Involvement in Targeted-Panel Design and Data Interpretation
With all the technical advancements, clinical NGS testing appears to be a straightforward task, but it also brings a number of challenges and complexities in panel design, data interpretation, and clinical reporting. The importance of integrating clinicians in targeted NGS panel design, test update, and data interpretation needs to be emphasized. First, in the panel design phase, the disease category should be clearly defined by clinicians who have insights into the relevant issues within each phenotypic category and the specific needs of ordering physicians. Importantly, clinicians will be able to utilize clinical testing information (nerve conduction studies, electromyography, electroencephalography, magnetic resonance imaging, and muscle, nerve, and brain pathologic examinations) to establish algorithmic approaches to help establish the most appropriate panel for patients. A well-defined testing algorithm is critical to increase the diagnosis rate and enhance the value of NGS testing, which will also help the reimbursement issue of NGS testing. Creating a practical algorithm along with well-categorized panels is not a trivial task, requiring more than reviewing gene lists in public databases such as OMIM, HGMD, and GeneReviews. It is important to thoroughly review the supporting literature beyond database information in order to ascertain (1) whether the variant or gene has been described in multiple families and linkage/haplotypes established, (2) if full penetrance
of the variant exists in affected families, and (3) whether the genetic defect produces a biochemical defect and if it should be confirmed by a functional assay. Obtaining this information will take a collaborative effort of clinicians who have in-depth knowledge of diseases, laboratory geneticists who understand the specific requirements of clinical testing, and bioinformaticians who can accurately process and annotate variants. For example, the specific algorithms for inherited neuropathy panels and epilepsy panels that we have created highlight the collaborative efforts of neurology specialists, laboratory geneticists, and bioinformaticians. The inherited neuropathy algorithm (Figure 4) utilizes multiple criteria to direct ordering physicians, including age at symptom onset, specific sensory features (large or small fiber involvements), ataxic or pyramidal features, and nerve conduction studies and has been validated to substantially increase the diagnosis rate and reduce overall cost.10 Each disorder has its unique characteristics that require specialty training to interpret. For instance, approximately 70% of demyelinating neuropathy cases have a PMP22 copy number mutation; these patients should first undergo an inexpensive PMP22 duplication test before pursuing NGS. Thus, accurate interpretation of nerve conduction data and electromyographic reports is essential. Epilepsy panels include many genes that have copy number sequence variations, and phenotypic evaluation including electroencephalography and magnetic resonance imaging are helpful in determining whether copy number assessment is a rational first step before applying targeted NGS epilepsy evaluations (Figure 5).38–40

Secondly, clinicians’ input is also invaluable for data interpretation to critically evaluate the variants with unknown importance. Although CLIA (Clinical Laboratory Improvement Amendments)—certified genetic laboratories are well equipped to handle the technical aspects of sequencing and deliver sequencing results from an automated analysis pipeline, the manual interpretive element of the data reporting process will always be essential. A concerted effort from the clinicians, laboratory geneticists, and bioinformaticians is required to generate an accurate and relevant clinical report. Traditional approaches have relied on laboratory geneticists alone to interpret results by evaluating variants on the basis of allele frequency in large databases and previous reports curated by mutation databases. Recently, improved in silico analysis by PolyPhen-2 (Polymorphism Phenotype v2 tool), SIFT (Scale-Invariant Feature Transform algorithm), or GERP (Genomic Evolutionary Rate Profiling method) also provides additional evidence, but these tools have low specificity, tend to overpredict missense changes as deleterious, and cannot deduct the functional impact of copy number changes. As we have learned from the large amount of NGS data, each person can have approximately 400 potentially damaging variants with unknown importance.41–45 Because of the complexity of diseases, there are many false attributions of pathogenicity to a particular variant; many variants labeled as disease causing in the HGMD are later found to be a mutation.46–48 Inasmuch as all variants with unknown importance are from disease-associated genes, phenotype-genotype correlations will be critical to determining the relevance of variants. Sensible judgment should be exercised to avoid false-positive results because they create unnecessary emotional burden and disease management complexity for patients and their clinicians, especially in genes known to be highly polymorphic and to have devastating clinical implications, such as with NFI, the gene responsible for neurofibromatosis type 1.

It is important to understand that NGS data interpretation is more than just listing the known mutation or rare variants; clinical expertise can help the ordering physician understand the report at the patient care level, from recommending additional biochemical testing and muscle or nerve biopsies for tissue-specific testing for confirmation of the pathogenicity of variant to follow-up discussion to obtain the important information regarding genetic diagnosis.49 Validating the pathogenicity of new variants can only be accomplished within the phenotypic context of the patient. For example, if a rare variant is found in MFN2, the most common causal gene for axonal neuropathy, a clinician can critically review electrophysiologic data. If demyelinating features are present, then the potential pathogenicity of this variant is extremely low. However, if the same patient
Chronic slowly progressive length-dependent polyneuropathy*

Ulnar motor forearm nerve conduction velocity <38 m/s with compound muscle action potential (CMAP) test >0.5 mV

<40 y at onset (no obvious cause)

Family history definite

PMP22 duplication analysis

Motor predominant with/without sensory with/without pyramidal features

Small and large fiber sensory predominant with/without motor

Large fiber sensory pyramidal signs with/without motor

Uniform length dependent

Consider spinocerebellar by genetic testing, MRI

Inflammatory-immune, metabolic, toxic, infectious testing

NEGATIVE

Diagnose chronic idiopathic axonal polyneuropathy (CIAP)

Peripheral neuropathy–targeted next-generation sequencing

NEGATIVE OR INCONCLUSIVE RESULTS?

Research kindred evaluation and/or whole-exome/genome sequencing

* All adult patients undergo testing for hemoglobin A1c or glucose tolerance, B12 deficiency, monoclonal proteins.

FIGURE 4. Patients who have polyneuropathy with a suspected genetic cause benefit from an algorithmic approach that combines detailed clinical and electrophysiologic features with best genetic testing platforms. Targeted next-generation sequencing provides the best value in making a genetic diagnosis when PMP22 duplication testing results are either negative or not needed on the basis of nerve conduction studies. MRI = magnetic resonance imaging.
has a history of optic neuropathy, then the likelihood of the variant being causal is increased because optic neuropathy is a rare but specific complication associated with MFN2. The clinical acumen of clinicians in pathology, electrophysiology, and imaging complements the technical competence of laboratory geneticists to generate clinically relevant test reports because genetic findings complement phenotypic presentations to complete the diagnosis. Combining the strengths of geneticists and clinicians is the key to fully utilizing this revolutionary technology to improve the quality of patient care.

CURRENT LIMITATIONS AND FUTURE DIRECTIONS OF NGS IN NEUROLOGIC TESTING

With the expanding application of NGS, we also need to recognize that NGS is not a “magic bullet.” There are genetic abnormalities that NGS has not been optimized to reliably identify. For example, the current read length of 100 to 200 bp precludes efficiently aligning

Epilepsy unexplained refractory and/or familial*

Does the identified epilepsy include congenital anomalies, developmental delay, intellectual disability, autism spectrum, infantile spasms?

YES

Chromosomal microarray

YES

Diagnostic

STOP

NO

INCONCLUSIVE OR NEGATIVE

Is a specific epilepsy syndrome present?

YES

Focused evaluations
- Epilepsy-PME
- Epilepsy-NeonSz
- Epilepsy-InfSpasm
- Epilepsy-Migraine
- Epilepsy-NeuronMigration
- Epilepsy-FocalSz
- Epilepsy-EncephChronicSz

INCONCLUSIVE OR NEGATIVE

Epilepsy expanded evaluation

YES

Exome (trio)

INCONCLUSIVE OR NEGATIVE

Diagnostic

STOP

NO

*All patients should have brain MRI and EEG.
and screening for nucleotide repeat expansion diseases, which are common in neurologic disorders. Such disorders include the spino-cerebellar ataxias such as Friedreich ataxia or fragile X syndrome and the most common cause of amyotrophic lateral sclerosis with dementia, expansion of C9orf72. Special applications that sequence long-range polymerase chain reaction amplicons on a PacBio instrument that can sequence up to 20-kilobase fragments will allow reliable nucleotide expansion reads but are still in the development stage. In addition, detecting CNVs by NGS, such as larger deletions or duplications (Duchenne or Becker muscular dystrophy, hereditary brachial plexopathy) has not been widely utilized, but newly improved capture baits and CNV analysis algorithms are gradually integrating CNV analysis into NGS testing. Simultaneously detecting CNVs and nucleotide changes can substantially increase the throughput and reduce the cost because a separate screening by microarray-based comparative genomic hybridization or fluorescence in situ hybridization is no longer needed. Our recent investigation has found that targeted sequencing is an efficient and cost-effective approach to detection of copy number changes. The algorithm we used (PatternCNV) can detect as many as a few hundred base pairs (1 exon) or the whole chromosome. This feature is especially important for neuropathy panels, in which PMP22 duplications are the most common cause of demyelinating neuropathy, and will simplify the testing algorithm. The ability to detect CNVs will have even greater impact on epilepsy panels because many causal genes have pathogenic copy number changes. Currently, many targeted NGS panels for epilepsy are still limited to detection of only nucleotide base pairs or small deletion duplications (<50 bp) and for large CNVs, and a microarray method has been employed as illustrated in our current algorithm (Figure 5). The utilization of 2 approaches and 2 sets of data analysis can double the total cost of screening. The CNV detection capability will take the application of NGS in clinical testing to a new level, especially for the disorders that are known to be impacted by a large number of chromosome and copy number changes.

As sequencing technology and analysis algorithms continue to improve and costs continue to decrease, we believe that WES or WGS will eventually become the more standard testing choice in clinical laboratories. This use will also generate greater potential for novel research discoveries. For now, targeted NGS panel approaches have superior metrics across virtually all avenues of clinical testing. Some clinical testing laboratories are currently utilizing WES as the sequencing backbone but only report on genes specified in a disease-focused panel. This approach does not utilize the full merits of targeted sequencing because it does not result in ultra-high coverage of specified genes, and questions of whether the rare variants in the 56 genes mandated by the ACMG should be reported creates ethical complications. As the etiology of more genetic disorders are discovered and additional therapies and treatment options become available, it is anticipated that insurance companies will increasingly support the use of NGS approaches to delineate the underlying genetic etiology and provide treatment options tailored to the individual’s genetic abnormality. The knowledge gained by NGS has provided a foundation for personalized, precision genomic medicine, which will become a standard for neurologic care in the foreseeable future.

Abbreviations and Acronyms: ACMG = American College of Medical Genetics and Genomics; bp = base pair; CNV = copy number variation; HGMD = Human Gene Mutation Database; NGS = next-generation sequencing; OMIM = Online Mendelian Inheritance in Man; WES = whole-exome sequencing; WGS = whole-genome sequencing

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REFERENCES


