Clinical exome sequencing in neurologic disease

Brent L. Fogel, MD, PhD; Saty Satya-Murti, MD; Bruce H. Cohen, MD

Abstract
Purpose of review: The landscape of genetic diagnostic testing has changed dramatically with the introduction of next-generation clinical exome sequencing (CES), which provides an unbiased analysis of all protein-coding sequences in the roughly 21,000 genes in the human genome. Use of this testing, however, is currently limited in clinical neurologic practice by the lack of a framework for appropriate use and payer coverage.

Recent findings: CES can be cost-effective due to its high diagnostic yield in comparison to other genetic tests in current use and should be utilized as a routine diagnostic test in patients with heterogeneous neurologic phenotypes facing a broad genetic differential diagnosis. CES can eliminate the need for escalating sequences of conventional neurodiagnostic tests.

Summary: This review discusses the role of clinical exome sequencing in neurologic disease, including its benefits to patients, limitations, appropriate use, and billing. We also provide a reference template policy for payer use when considering testing requests. Neurol Clin Pract 2016;6:164–176

Clinical exome sequencing (CES) is a new state-of-the-art molecular diagnostic genetic test. It has the potential to rapidly and efficiently detect disease-causing genetic mutations within any gene in the human genome and is therefore becoming widely used in clinical practice. This template policy addresses the health benefits, limitations, and appropriate use of this type of testing in patients with neurologic disease.

The health benefits of genetic diagnosis
Recent evidence suggests that health outcomes improve as a result of accurate genetic diagnosis. Results of genetic testing in patients have led to identification, initiation, or change of symptomatic and disease-modifying treatments. Other benefits have occurred in reproductive counseling and family planning. In some instances, test findings have extended the diagnostic evaluation to discover unknown significant and actionable systemic involvements and comorbidities. These tests have also facilitated prognostication and selective participation in research or clinical treatment trials.

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Many common disease phenotypes exist that could result from mutations in various different genes. This genetic overlap is particularly true in neurologic disorders, including disorders causing neuropathy, epilepsy, ataxia, special sensory impairment, myopathy, movement disorders, intellectual disability, and others, which creates a particular challenge for accurate genetic diagnosis. In addition, there are many examples where one gene can cause more than one phenotypic, and sometimes disparate, illness. Effective strategies are therefore necessary to achieve accurate genetic diagnosis efficiently within this complex landscape. Broad indiscriminate testing and incorrect interpretation result in inaccurate conclusions. For proper genetic testing, careful patient selection through expert clinical evaluation is an invariant prerequisite. Validation of testing methodology and demonstration of clinical utility are also essential. Failure to follow these sequential steps will limit the realization of the emerging health benefits of genetic testing to patient care.

### Current methods for genetic diagnostic testing

**Single-gene sequencing (Sanger sequencing)** At present, the most common form of genetic testing employed is single-gene sequencing, i.e., testing for a mutation in a specific gene that is known to be associated with a particular disease. In clinical practice, the clinician must determine the most probable disorder and order specific testing of the gene (or genes) that causes the illness. The technique currently employed most widely is Sanger sequencing, a method capable of sequencing portions of DNA in short (<1 kilobase) segments (table 1). Sanger sequencing can detect most common mutations that affect coding sequences (e.g., missense and nonsense point mutations, small insertions/deletions, and mutations involving the splice site junctions). This method would not detect large insertions/deletions, copy number variations, or nucleotide repeat expansions. This form of testing is also not applicable to the testing of all genes, because sequencing large or numerous regions of DNA is prohibitive due to the exponential increases in labor and cost incurred as the method is scaled up to evaluate larger or multiple genes. The cost of Sanger sequencing varies widely based on the amount of DNA sequenced per gene, but relative estimates of ~$1,000 per gene have been suggested, although the commercial pricing of single gene testing may further increase that figure, which can rapidly limit cost-effectiveness if multiple genes must be considered for a clinically heterogeneous disorder.

**Multigene sequencing (Sanger sequencing, panel testing)** In clinically heterogeneous neurologic disorders (e.g., intellectual disability, epilepsy, ataxia, leukodystrophy, polyneuropathy), commercial laboratories have developed genetic panels of variable size for testing multiple genes simultaneously. As discussed above, because such panels typically utilize Sanger sequencing methods, the commercial price can be expensive (e.g., $30,000 or more), with a correspondingly low diagnostic yield in many cases due to phenotyping errors or variability in phenotype across disorders, creating a considerable burden on the health care system and the individual patient. Furthermore, as new genes associated with these broad neurologic disorders are described, previous testing becomes quickly obsolete.

<table>
<thead>
<tr>
<th>Technology</th>
<th>Chromosomal microarray analysis</th>
<th>Sanger sequencing</th>
<th>Next-generation sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current clinical use</strong></td>
<td>Copy number variation</td>
<td>Single gene mutation analysis; multigene panel testing</td>
<td>Exome, genome, and targeted gene mutation analysis</td>
</tr>
<tr>
<td><strong>No. of genes sequenced</strong></td>
<td>Not applicable</td>
<td>1 (single gene), 2-30+ (multigene)</td>
<td>21,000 (exome), 21,000 + noncoding sequences (genome)</td>
</tr>
<tr>
<td><strong>Estimated clinical cost per gene sequenced</strong></td>
<td>Not applicable</td>
<td>$1,000 or more</td>
<td>$0.25 or more for exome; $0.50 or more for genome</td>
</tr>
<tr>
<td><strong>Estimated overall clinical price</strong></td>
<td>Varies per laboratory, $2,000 or more</td>
<td>Varies per laboratory and number of genes tested, $1,000-$30,000</td>
<td>Varies per laboratory, exome $5000 or more (includes analysis)</td>
</tr>
</tbody>
</table>
Chromosomal microarray analysis

Chromosomal microarray analysis (CMA), an array-based form of comparative genomic hybridization, is a technique for detecting clinically significant structural changes, particularly deletions or duplications in the genome, termed copy number variation (table 1). Based on diagnostic yield, this method of testing is considered a first-line test for patients with unexplained developmental and intellectual disabilities, autism spectrum disorder, or congenital anomalies. Because it cannot detect sequence variation, CMA is used complementary to CES to detect structural variation that exome sequencing cannot. For certain phenotypes or disorders with diverse genetic causes, their use would not be mutually exclusive, and in some circumstances CMA and CES may potentially be performed sequentially or even simultaneously.

Benefits of clinical exome sequencing

CES utilizes a method commonly referred to as next-generation sequencing, consisting of a massively parallel sequencing strategy enabling rapid genome-scale sequencing of DNA at a significantly reduced cost relative to the Sanger method, yet capable of detecting all the same types of mutations (e.g., point mutations, small insertions/deletions, and splice site mutations). Because of the associated reduction in cost and time, next-generation sequencing allows for sequencing of the entire exome, i.e., the 1%–2% of the human genome representing all protein-coding regions. This technology involves isolating genomic DNA from a patient, fragmenting this DNA into small nucleotide segments, coupling these fragments to artificial linkers, isolating the specific fragments corresponding to exomic sequences, and then sequencing those fragments in parallel on a slide using an in situ amplification method involving fluorescent dyes that are read by computer using a laser in conjunction with a microscope and a digital camera. The resulting sequence reads are next assembled as a series of overlapping fragments and aligned to a reference sequence of the human genome so differences between the patient and the reference sequences can be noted. These differences are collectively termed variants because they can (and in the majority typically do) represent benign polymorphisms (genetic variants found in a single person or in a percentage of the population but not associated with any primary disease), as opposed to pathogenic mutations. In this manner, the same classes of mutation can be identified as for Sanger sequencing (i.e., missense and nonsense point mutations, small insertions/deletions, and mutations involving splice site junctions). An important consideration for next-generation sequencing is depth of coverage, which refers to the average times each nucleotide is viewed and provides an estimate of certainty in how likely a mutation at a specific position would be detected, if present, reducing the risk of false-negative results. For example, a depth of coverage of 10-fold means that each nucleotide has been examined 10 times on average. In addition, there are numerous metrics available to calculate the quality of next-generation data. Routinely attainable minimum read depths and quality levels can be determined where the false-positive rate of next-generation sequencing is less than the false-negative rate of Sanger sequencing, suggesting that this method will likely become the next gold standard in diagnostic testing. The commercial price is approximately $5,000 for sequencing and bioinformatic variant analysis (which includes personnel and computing costs) of the entire exome, approximately 21,000 genes, translating into roughly $0.24 per gene. Because of this significant pricing advantage, CES is rapidly becoming the new standard for sequencing in genetic diagnostics, as all 21,000 genes in the human genome can be evaluated in an...

Results of genetic testing in patients have led to identification, initiation, or change of symptomatic and disease-modifying treatments.
essentially unbiased manner.\textsuperscript{11–13} This limits the effect of phenotyping errors or variability as testing is not solely limited to genes associated with one particular symptom or phenotype.

**Appropriate use**

Clinical prioritization is a prerequisite before undertaking any test, especially expensive, invasive ones. For instance, in the United States, more than 1 million patients undergo a $9,000 per procedure coronary angiography yearly.\textsuperscript{14} Not all of these procedures lead to appropriate revascularization interventions,\textsuperscript{15,16} and it has been suggested that proper patient selection is the key to reducing inappropriate percutaneous coronary intervention procedures. Similarly, careful patient selection is even more important for exome sequencing (figure). At present, it is imprudent to consider exome sequencing as a primary or first-order endeavor towards establishing a diagnosis when any genetic disorder is suspected. Thorough clinical evaluation by appropriately trained personnel is the essential first step. The decision to proceed should rest on the choice of tests available with established analytic and clinical validity, and with demonstrated clinical utility for the phenotype in question. At this stage, the testing recommendations of a treating health care provider merit serious coverage consideration. Even if an actionable curative decision may not be imminent, there are downstream long-term health benefits.\textsuperscript{17,18} For instance, in situations of diagnostic uncertainty when phenotype alone is unhelpful, an escalating series of diagnostic tests (neuroimaging, biopsies, invasive chemistries, CSF analysis) is frequently employed. Before undertaking these expensive and time-consuming tests, exome sequencing could abbreviate or stop the diagnostic testing cascade by establishing the specific rare diagnosis genetically. A Pediatric Genomic Medicine group found that next-generation sequencing has the potential to truncate the diagnostic odyssey by
The effectiveness of exome sequencing is not limited to specific categories of disease but is widespread across phenotypes resulting from disparate genetic etiologies.

5 years. The average price of testing was over $19,000 prior to establishing a sequencing-based diagnosis, of which 23% were actionable. Exome sequencing can also improve preventive care by alerting physicians to unanticipated comorbidities. An important example of this is in the preventive benefit for family members. Exome sequencing results can alleviate family member concerns regarding potential risk of transmission or identify individuals in need of more specialized care or surveillance to reduce future disease complications.

As described above, CES is not expected to be utilized as a first-line or screening test (figure). Patients would be expected to undergo an initial typical clinical evaluation for common acquired etiologies and high-yield genetic testing (e.g., single-gene or multigene testing, chromosomal microarray) if indicated. For example, it would be more cost-effective for a patient with a specific suspected genetic disorder and the classically described phenotype to receive single-gene testing initially for only that disorder. This would likely be the case for the majority of the most common genetic diseases seen in a typical neurology practice. However, if a genetic etiology with a broad differential basis is suspected, then exome sequencing may be the most high-yield and cost-effective evaluation strategy to pursue. This has been reflected in numerous clinical studies to date. Although much of this early analysis has been performed in pediatric populations, CES is not limited by patient age. It is applicable to both children and adults, and can yield clinically meaningful findings in patients without a clear family history of disease (reflecting either de novo mutation or recessive inheritance). The effectiveness of exome sequencing is also not limited to specific categories of disease but is widespread across phenotypes resulting from disparate genetic etiologies. There are numerous examples in the literature of such effectiveness with diagnosis rates ranging from ~20%–80% for various phenotypes tested. CES and targeted next-generation sequencing focused on large numbers of genes associated with specific phenotypes have shown high diagnostic rates for various disorders (table 2). Clinical experience and best practices will dictate the need for single gene testing vs clinical exome for a specific phenotype, but the above data clearly demonstrate the effectiveness of exome sequencing in clinically heterogeneous conditions.

It must be emphasized that all patients considered for exome sequencing must receive appropriate genetic counseling either from a trained physician or a licensed genetic counselor both before and after the testing is performed. This ensures that the patient has a complete understanding regarding the nature of the test, its benefits and limitations, and the precise clinical interpretation of the results. Additional ethical considerations include variants of uncertain significance and the potential detection of incidental/secondary findings (discussed in detail below). Given the complexity of these counseling issues, we strongly advocate for the use of exome sequencing only in the clinical setting and would not endorse direct-to-consumer testing for any indication.

As of this writing, requests for CES tests will differ from traditional and more familiar diagnostic tests (e.g., neuroimaging, electrophysiology, blood chemistries, or earlier molecular tests such as single-gene tests, chromosomal microarray, or traditional karyotype) in the following manner:

1. They will be very selective and, comparatively, substantially less frequent.
2. Clear clinically reasoned indications must accompany testing requests.
3. The requests will be generated by expertise-based prior evaluations from clinicians familiar with genetic disease and appropriate testing methods.
4. The testing itself will be performed by a few nationally recognized laboratories with extensive experience in this method and correspondingly high technical standards.

While assigning monetary value to a diagnostic test that may not immediately or directly affect the health and well-being of patients is challenging, there is still a need to recognize the indirect benefits. Establishing a genetic diagnosis has value to the patient, the medical care team, the family, and to public health at large by eliminating prolonged diagnostic explorations, improving family counseling, facilitating reproductive decisions, and reducing patient/parental anxiety.

### Challenges and limitations of CES

There are 2 challenges to universal acceptance and policy coverage of CES. The first is more general and the second specific. The first is a conceptual debate surrounding the value of a test

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**Table 2** Diagnostic rates of next-generation sequencing in various neurologic disorders

<table>
<thead>
<tr>
<th>Neurologic disorder</th>
<th>No. of patients or families diagnosed</th>
<th>Total patients or families</th>
<th>Diagnostic rate, %</th>
<th>e-Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Targeted sequencing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ataxia</td>
<td>9</td>
<td>50</td>
<td>18</td>
<td>e1</td>
</tr>
<tr>
<td>Intellectual disability</td>
<td>26</td>
<td>106</td>
<td>25</td>
<td>e2</td>
</tr>
<tr>
<td>Mitochondrial disorders</td>
<td>22</td>
<td>102</td>
<td>22</td>
<td>e3</td>
</tr>
<tr>
<td><strong>Exome sequencing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ataxia</td>
<td>11</td>
<td>86</td>
<td>13</td>
<td>e4</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>76</td>
<td>21</td>
<td>e5</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>23</td>
<td>39</td>
<td>e6</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>22</td>
<td>41</td>
<td>e7</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>59</td>
<td>44</td>
<td>e8</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>28</td>
<td>46</td>
<td>e9</td>
</tr>
<tr>
<td>Autism spectrum disorder</td>
<td>10</td>
<td>49</td>
<td>20</td>
<td>e8</td>
</tr>
<tr>
<td>Birth defects or multiple congenital anomalies</td>
<td>51</td>
<td>141</td>
<td>36</td>
<td>e8</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>28</td>
<td>54</td>
<td>e10</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>83</td>
<td>298</td>
<td>28</td>
<td>e4</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>43</td>
<td>121</td>
<td>36</td>
<td>e8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>9</td>
<td>78</td>
<td>e11</td>
</tr>
<tr>
<td>Intellectual disability or developmental delay</td>
<td>16</td>
<td>100</td>
<td>16</td>
<td>e12</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>39</td>
<td>33</td>
<td>e13</td>
</tr>
<tr>
<td></td>
<td>105</td>
<td>322</td>
<td>33</td>
<td>e8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>29</td>
<td>34</td>
<td>e10</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>22</td>
<td>74</td>
<td>30</td>
<td>e4</td>
</tr>
<tr>
<td>Neurodevelopmental disorders</td>
<td>425</td>
<td>1,673</td>
<td>25</td>
<td>e14</td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>200</td>
<td>26</td>
<td>e15</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>78</td>
<td>41</td>
<td>e16</td>
</tr>
<tr>
<td>Polyneuropathy</td>
<td>8</td>
<td>27</td>
<td>30</td>
<td>e17</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>15</td>
<td>33</td>
<td>e18</td>
</tr>
</tbody>
</table>

*e-References are available at Neurology.org/cp.*
that does not always immediately lead to improvement in outcome. The second deals with technical issues, predominantly completeness of gene coverage, validation of results, and variant interpretation.21,22

CES is a new and evolving technology. Some payers consider the test to be experimental and investigational.18,23 However, clinicians pursuing a genetic etiology with a broad differential consider exome sequencing as the foremost high-yield and cost-effective evaluation strategy.24–26 Whereas coverage is generally available for noninvasive prenatal genetic testing and genetic evaluation of neoplastic disease, exome sequencing is not yet recognized for coverage. At the center of the coverage debate is the difficulty of establishing an immediate and conventionally measurable index of health benefit from test results. A long-established expectation is that an action, treatment, or prognostication follow closely after the results of traditional tests. Our Code of Federal Regulations (CFR) concerning health care has manualized a definition for all diagnostic tests. It requires that the ordering and treating physician use the results in the management of the beneficiary’s specific medical problem (CFR §411.15(k)(1)). Genetic testing, however, does not result in a proximate actionable intervention or management with such immediacy or short temporal sequence as traditional and familiar tests. The latter tests frequently lead to an action, for example, a pharmacologic treatment, biopsy, stenting, surgery, or conclusive prognostication. In these instances, the standard evidence requirement has been that management decisions follow as a result of test findings. Such a model has served the medical community well and set the standard for any emerging testing modality. The resulting action from these generally reimbursable common tests is often beneficial; however, they may also lead to unnecessary downstream diagnostic therapeutic cascades of interventions.15,16,27,28 For instance, a majority of carotid interventions in the United States are for asymptomatic carotid stenosis. A diagnostic test obviously preceded the detection of the stenosis, although the subsequent occlusion-preventive intervention is seldom a valid indication in asymptomatic patients.29 Because of the above cited considerations, payers are often cautious about utilization of certain tests and their consequent expensive treatments. Thus, circumspection on part of the payers, especially for tests based on novel technologies, would not be an unreasonable expectation. However, the emerging evidence suggests that the benefits of CES favor cost reduction by eliminating the need for subsequent diagnostic procedures and further detailed clinical evaluation.

The 3 technical issues most commonly debated with regard to exome sequencing include completeness of gene coverage, validation of results, and variant interpretation.21,22 As described above, coverage across the exome is determined on average because, typically due to structural differences, not all portions of the exome are sequenced equally and it is therefore possible that a mutation may exist in a region of reduced coverage. However, coverage and detection of variants can be improved by increasing the number of reads (the depth of exome sequencing)30 or using targeted next-generation strategies to improve capture of troubling regions31 so this concern is not inherent to the method itself and can be resolved through technical performance standards. As of this writing, technological improvements have already significantly reduced this issue to a level of minimal concern. Validation, usually by Sanger-based methodology, which is still considered the gold standard by some, has been shown to not be necessary for most identified variants of sufficient quality.10 Variant interpretation is the process by which the likelihood that a variant is pathogenic or not is determined. For variants that have never been reported in patients, the methods of assessing pathogenicity do not differ between Sanger and next-generation technologies, as they require human application of knowledge (e.g., bioinformatics). The process, however, becomes much more extensive for exome sequencing because of the larger dataset being evaluated and is complicated by technical issues of quality, data processing, genomic alignment, and variant analysis that can vary among laboratories.32 Because most laboratories performing exome sequencing utilize different methods for variant assessment, they may potentially interpret identical variants in different ways as a result (e.g., pathogenic, likely pathogenic, or benign vs uncertain significance, discussed further below). These differences in analytic method, when made transparent, can be assessed by the ordering clinician and
utilized in the final clinical interpretation. Because of this aspect of uncertainty, unique to genomic sequencing methods, exome sequencing results can produce indeterminate yet still clinically relevant results (e.g., variants of uncertain significance). As the testing becomes more widespread, it is also likely that a degree of standardization of testing platforms and analytic methods will occur across laboratories.

**Major considerations in exome sequencing: Variants of uncertain importance and incidental/secondary findings**

The term variant of uncertain significance (VUS) refers to a genetic change in a gene of interest that is novel or rare in the population and has never been reported in connection with disease. Such variants are not unique to exome sequencing and can be observed with any sequencing method. Bioinformatic prediction methods currently are insufficient to solely guide clinical interpretation and the presence of a variant at a low frequency in a population (e.g., <1%) also does not equate to a lack of pathogenicity due to issues of incomplete penetrance, expressivity, improper phenotyping, modes of inheritance, or other considerations. These variants are not insignificant as they can (and in many cases do) represent actual pathogenic mutations that have yet to be verified. In some cases, but not all, this verification can be done

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Indications, contraindications, and Current Procedural Terminology/Healthcare Common Procedure Coding System (CPT/HCPCS) codes for clinical exome sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Indications</strong></td>
<td></td>
</tr>
<tr>
<td>1. Undiagnosed neurologic disorder with nonspecific or clinically heterogeneous phenotype.</td>
<td></td>
</tr>
<tr>
<td>2. Expert evaluation with detailed clinical history, comprehensive neurologic examination, and complete family history.</td>
<td></td>
</tr>
<tr>
<td>3. Complete evaluation for common causes not requiring genetic testing.</td>
<td></td>
</tr>
<tr>
<td>4. Negative initial genetic testing (e.g., high-yield single-gene or multigene testing, chromosomal microarray) based on clinical evaluation, as appropriate.</td>
<td></td>
</tr>
<tr>
<td><strong>B. Contraindications</strong></td>
<td></td>
</tr>
<tr>
<td>1. Exome sequencing is not to be considered as a primary or first-line test for establishing a diagnosis in a patient where a genetic disorder is suspected unless the criteria of part A are met.</td>
<td></td>
</tr>
<tr>
<td>2. Testing is not to be carried out without prior clinical evaluation and confirmation of need by appropriately trained professional health care providers with experience in the diagnostic evaluation of genetic disease.</td>
<td></td>
</tr>
<tr>
<td>3. Testing is not to be carried out without careful consideration, appropriate genetic counseling (including discussion of the possibility of secondary or incidental findings), and the availability of clinical expertise to interpret the findings, render advice, and provide appropriate care and management decisions based on the results of the testing.</td>
<td></td>
</tr>
<tr>
<td><strong>C. CPT/HCPCS codes</strong></td>
<td></td>
</tr>
<tr>
<td>1. 81415 Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis.</td>
<td></td>
</tr>
<tr>
<td>2. +81416 Exome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (e.g., parents, siblings) (list separately in addition to code for primary procedure).</td>
<td></td>
</tr>
<tr>
<td>3. 81417 Exome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (e.g., updated knowledge or unrelated condition/syndrome); do not report 81417 for incidental findings.</td>
<td></td>
</tr>
</tbody>
</table>

AMA CPT® Copyright Statement: CPT codes, descriptions, and other data are copyright 2013 American Medical Association. All Rights Reserved. Applicable FARS/DFARS clauses apply. *For certain broad clinically heterogeneous phenotypes, specific high-yield genetic testing may justifiably not be identifiable. In those circumstances, exome sequencing could be considered as initial testing if all other ordering conditions are met.
through additional testing of other family members or through detection of associated biomarkers. Furthermore, as DNA sequencing does not change over time, exome sequencing results can be reinterpreted as new clinical data is reported, increasing the chances for an ultimately positive result even if initial testing is inconclusive.

Exome sequencing can also identify incidental or secondary findings (e.g., unexpected but medically significant results such as a predisposition to cancer). Such findings are not uncommon and have been seen in approximately 4%–5% of patients. The American College of Medical Genetics has established recommendations for specific genes that should be screened in all clinical exomes. Patients must be counseled regarding this possibility prior to testing and provided the opportunity to decide whether they would wish to know such results if any are found. It is important to note that although this technology is capable of finding unexpected genetic mutations, due to multiple issues such as the variability associated with gene expressivity and penetrance, as well as ethical considerations, there is currently no clinical indication to perform exome sequencing as an elective screening test for assessing the risk of future disease in asymptomatic healthy individuals in the absence of other concerns.

**Indications, contraindications, and billing of CES**

The current and generally accepted indications for exome sequencing testing and the relative contraindications of such testing are indicated in table 3. Accumulating evidence in this relatively new field may alter these indications and limitations in the future.

Not every payer accepts all submitted procedure codes for payment and not all providers submit the same set of codes. A new section in 2015 on genomic sequencing procedures describes DNA sequence analysis methods that simultaneously assay multiple genes or genetic regions and includes CES (table 3). These commonly used codes for exome sequencing should be used regardless of the technique employed, unless specifically noted in the code descriptor. Not all new molecular pathology diagnostic codes are recognized by common payers such as Medicare or Medicaid and coverage environment is variable (table 4), due in part to the lack of a comprehensive payer policy justifying the use of this testing clinically in neurologic disease.

<table>
<thead>
<tr>
<th>Insurer/payer</th>
<th>Coverage status</th>
<th>Basis</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicare</td>
<td>No NCD; some contractor LCDs cover specific cancer diagnostic tests, but not noncancer exome sequencing tests</td>
<td>One contractor evaluates each test, by application and review, based on its specific merit; laboratories may ask for a denial of noncovered tests to facilitate secondary payer submissions</td>
<td>Exome sequencing tests are grouped as molecular diagnostic tests; coverage is based on each test rather than as CPT codes</td>
</tr>
<tr>
<td>Medicaid</td>
<td>Nonstandardized; varies among states; coverage usually not sufficient to cover costs of tests, so hospital must contract with the laboratory to guarantee difference</td>
<td></td>
<td>Some Medicaid plans may cover by prior authorization</td>
</tr>
<tr>
<td>Private payers (e.g., BCBS, Anthem, Aetna, UHC)*</td>
<td>Generally insurer medical policies do not cover but coverage varies by policy and insurer; in most instances, physician must supply documentation for need and explanation as to how results would change management</td>
<td>Payers justify denials by considering as either investigational or experimental or not medically necessary</td>
<td>If covered after prior authorization, may still require copayment by patient or family</td>
</tr>
</tbody>
</table>

Abbreviations: BCBS = Blue Cross/Blue Shield; CPT = Current Procedural Terminology; LCD = local coverage determination; NCD = national coverage determination; UHC = United Healthcare.


*One payer (Cigna) has initiated criteria-based coverage as of November 2015.
One Medicare contractor has established a Molecular Diagnostic Services Program (MolDx; Palmetto; http://www.palmettogba.com/palmetto/MolDX.nsf/DocsCatHome/MolDx). It acts as a repository and a registry primarily for coding help and it also details its expectations for coverage. It is advisable to consult with individual payers, diagnostic laboratories, and responsible billing agencies before selecting and submitting codes. The name of the specific test being performed may need to be provided either during submission of claim forms or subsequently. Similarly, prior clearance of the specific test by the payer contractor may also be necessary.

One payer has provided the helpful definition for a molecular diagnostic test (MDT) as “any test that involves the detection or identification of nucleic acid(s) (DNA/RNA), proteins, chromosomes, enzymes, cancer chemotherapy sensitivity and/or other metabolite(s). The test may or may not include multiple components. A MDT may consist of a single mutation analysis/identification, and/or may or may not rely upon an algorithm or other form of data evaluation/derivation” (MolDx; Palmetto; http://www.palmettogba.com/palmetto/MolDX.nsf/DocsCatHome/MolDx).

**Regulatory status**

No US Food and Drug Administration–approved genotyping tests are identifiable as of this writing. Therefore, genotyping by CES is offered as a laboratory-developed test. Clinical laboratories may develop and validate such tests in-house (e.g., home brew). These tests must comply with the regulatory standards of the Clinical Laboratory Improvement Act (CLIA) and the laboratory offering the service must be licensed by CLIA for high-complexity testing. At the time of this writing, only a select number of laboratories (<10) are currently performing CLIA-certified exome sequencing. Because of the sophisticated nature of this testing and analysis, diagnostic laboratories must invest major resources into this testing methodology (e.g., equipment, computers and software, and trained personnel). Consequently, the number of laboratories performing this testing is expected to remain limited and of high performance and quality.

**Future directions**

As illustrated above, CES is rapidly establishing itself as the standard for efficient and cost-effective diagnosis of complicated neurogenetic cases featuring broad differentials and clinically heterogeneous phenotypes. The field of human genetics and genomics is rapidly changing and this is likely a prelude to further advances including better methods of bioinformatic analysis, development of more extensive databases of normal human genetic variation, improvements in next-generation sequencing methodologies and equipment, and more widespread clinical use of genome sequencing. Establishing the appropriate clinical role for exome sequencing and integrating payer policies at this stage will ease the incorporation of these new advances as they become available and transition into the clinic to further improve diagnosis as well as patient management and care.

**REFERENCES**


**Take-home points**

- Next-generation sequencing methodology allows for the rapid and relatively inexpensive analysis of large amounts of DNA, up to entire genomes.
- Exome sequencing utilizes next-generation technology to analyze all protein-coding sequences in the human genome, comprising approximately 21,000 genes.
- Exome sequencing can broadly improve the diagnosis rate for suspected neurogenetic disorders with clinically heterogeneous phenotypes in both children and adults, leading to benefits in patient care.
- Clinical exome sequencing should be utilized to complement, not supersede, a systematic comprehensive patient evaluation. Physicians should receive proper training on its appropriate use and interpretation of results.
- We urge payers to utilize these recommendations in the development of comprehensive coverage policies for clinical exome sequencing.


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speakers’ bureaus for Transgenomic Labs, Courtagen Labs, United Mitochondrial Disease Foundation, and Courtagen Labs; is a consultant to Health and Human Services for the Division of Vaccine Injury; receives research support from Edison Pharmaceuticals, Raptor Pharmaceuticals, Stealth Biotherapeutics, and Reata Pharma; serves on the Board of Trustees for the United Mitochondrial Disease Foundation; and has participated in medico-legal cases. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/cp.

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CORRECTION
Clinical exome sequencing in neurologic disease: AAN model coverage policy

The article “Clinical exome sequencing in neurologic disease” by B.L. Fogel et al.¹ was endorsed as official AAN policy on April 7, 2016, by the AAN Board of Directors, who suggested a modification to the title to include the subtitle “AAN model coverage policy.” As this endorsement was subsequent to publication, the subtitle was not included in the original published article.

REFERENCE