Epilepsy Advanced Sequencing Evaluation

**Clinical Use**
- Determine cause of epilepsy (multiple unprovoked seizures) in patients with nonspecific epilepsy phenotypes
- Guide treatment selection
- Counsel patient and relatives regarding recurrence risk

**Clinical Background**
Epilepsy is a common neurologic condition marked by recurrent unprovoked seizures, affecting roughly 2.3 million adults and 470,000 children in the United States.\(^1\) Approximately 150,000 new cases are diagnosed annually,\(^1\) with incidence being highest in young children and the elderly. The type, frequency, and severity of seizures are highly heterogeneous, as are the potential causes. Determining the cause of epilepsy can help avoid diagnostic uncertainty and aid in selecting appropriate therapy.\(^2\)

Known causes of epilepsy include stroke, head trauma, infections, and genetic disorders. Once physical causes of epilepsy have been ruled out, a genetic cause or predisposition can reasonably be suspected.\(^3\) Evidence such as specific phenotypic features and family history can help guide the search for genetics causes. However, patients often present with relatively nonspecific findings and have no known family history. In a large population-based study from Minneapolis, for example, only 4.7% of relatives of probands had developed epilepsy by age 40.\(^4\)

Genetic testing has been reported to be a useful tool for diagnosis of certain phenotypic subgroups of epilepsy; such subgrouping might provide information helpful for clinical care.\(^5\) For example, detection of an SCN1A mutation suggests that sodium channel blockers should be avoided as they could aggravate seizure activity. Detection of an ALDH7A1 mutation, on the other hand, might suggest responsiveness to pyridoxine in some patients.\(^6\) Results may also be useful in assessing the prognosis and in counseling of family members.

Chromosomal microarray analysis (CMA), which detects chromosomal rearrangements and subtle microdeletions/duplications, is often the first-tier genetic test for individuals with unexplained epilepsy.\(^7,8\) It is appropriate when clinical findings do not point to a specific cause of cause of certain epilepsy-related disorders, such as global developmental delay, intellectual disability, multiple congenital anomalies, and autism spectrum disorders. CMA has been reported to detect abnormalities in up to 30% of patients with epilepsy.\(^8\) If CMA does not identify an abnormality, then targeted gene sequencing may be appropriate to establish a specific genetic diagnosis.

When phenotypic features suggest a syndrome associated with a specific gene, sequence analysis of individual genes associated with the syndrome may be most appropriate.\(^7\) However, the large number of genes putatively associated with epilepsy, each with relatively low rates of mutation detection, complicates testing for underlying genetic aberrations with traditional methods such as Sanger sequencing of individual mutations.\(^3\) Next-generation sequencing (NGS), on the other hand, allows sequencing of many genes simultaneously. Using a 265-gene NGS assay, Lemke and colleagues identified causative mutations in 16 of 33 (47%) patients with a wide variety of epilepsy phenotypes.\(^3\) Using a 67-gene NGS assay, Mina and colleagues subsequently detected causative mutations in 9 of 19 (47%) epilepsy patients with diverse phenotypes.\(^9\) In both studies, detection rates were much higher for patients with phenotypes suggestive of a genetic...
cause than in patients with nonspecific presentations.\textsuperscript{3,9} The diagnostic yield of NGS panels for epilepsy will vary depending on characteristics of the population tested and the specific genes included in the assay.

The Epilepsy Advanced Sequencing Evaluation test is based on the 265-gene panel described by Lemke and colleagues, but excludes genes reflecting phenotypes that could be readily diagnosed with MRI or biochemical methods. The resulting 141-gene panel covers genes associated with multiple epilepsy phenotypes (Appendix). Smaller, more specific panels are available for patients whose phenotypes suggest a specific syndrome.

**Individuals Suitable for Testing**
- Patients with a nonspecific epilepsy phenotype in whom physical causes have been ruled out and family history is uninformative

**Method**
- Sequence analysis of 141 genes (Appendix)
  - NGS performed on sheared genomic DNA libraries enriched for target regions by in-solution hybrid capture; resulting sequences analyzed using bioinformatics tools
  - Target regions include complete exonic coding regions and at least 10 noncoding nucleotides adjacent to each exon
  - Sanger sequencing performed for regions that are difficult to sequence using NGS
- Patient report provides a list of mutations detected, their associated phenotype, and their predicted clinical relevance
- Analytical sensitivity for sequence variation: >99%
- Analytical specificity for sequence variation: >99%

**Interpretive Information**
Detection of a pathogenic mutation associated with a specific phenotype is consistent with a diagnosis of, or a predisposition to develop, the given disorder. A finding of a variant(s) of unknown clinical significance (VUS) indicates that the variant detected has not been definitively associated with genetic epilepsy. Thus, a VUS result does not confirm the diagnosis of a genetic form of epilepsy.

This method does not detect large deletions, insertions, or structural variations, and may not detect mutations in patients with low-level mosaicism. It also does not detect mutations in regions of the gene not analyzed. Thus, although negative results indicate that mutations were not detected in the epilepsy-associated genes tested, they do not rule out a genetic cause of epilepsy.

Additional testing is available to identify large rearrangements; see the Athena Diagnostics Web site (AthenaDiagnostics.com) for additional testing options.

Additional assistance in interpretation of results is available from our Genetic Counselors and Laboratory Directors by calling the Athena Diagnostics Client Services Department (800-394-4493).

**References**
**Appendix**

Table. Genes Tested in the Epilepsy Advanced Sequencing Evaluation, by Associated Phenotype

<table>
<thead>
<tr>
<th>Epilepsy Phenotype</th>
<th>Genes Tested</th>
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<tr>
<td><strong>Generalized, absence, focal, and myoclonic epilepsies (5001)</strong></td>
<td>ALDH7A1, CACNA1A, CASR, CHRNA2, CHRNA4, CHRNB2, CSTB, DEPDC5, EFHC1, EPM2A, GABRA1, GABRB3, GABRD, GABRG2, GRIN2A, KCNMA1, KCNQ2, KCNQ3, KCN1, KCTD7, LGI1, MBDS3, ME2, NHLRC1, PCDH19, PRICKLE1, PRICKLE2, PRRT2, SCARB2, SCN1A, SCN1B, SCN2A, SCN9A, SLC2A1, SLC4A10, TBC1D24</td>
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<tr>
<td><strong>Epileptic encephalopathies (5002)</strong></td>
<td>ARHGEF9, ARX, CDKL5, CNTNAP2, FOXL1, GABRG2, GRIN2A, KCN1, MECP2, NRXN1, PCDH19, PANK, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, SCN1A, SCN1B, SCN2A, SCN8A, SCN9A, SLC2A22, SLC2A1, SLC9A6, SPTAN1, STXBP1, SYNGAP1, TCF4, TREX1, UBE3A, ZEB2</td>
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<tr>
<td><strong>Neuronal migration disorders (5003)</strong></td>
<td>ARFGEF2, ARX, COL18A1, COL4A1, CPT2, DCX, EMX2, FGFR3, FKRP, FKTN, FLNA, GPR56, LAMA2, LARGE, PAFAH1B1, PAX6, PEX7, POMMT1, POMT1, POMT2, PQBP1, RAB3GAP1, RELN, SNAP29, SRPX2, TUBA1A, TUBA8, TUBB2B, WDR62</td>
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<tr>
<td><strong>Epilepsy in X-linked intellectual disability (5004)</strong></td>
<td>ARHGEF9, ARX, ATP6AP2, ATRX, CASK, CDKL5, CUL4B, DCX, FGD1, GPC3, GRIA3, HSD17B10, KDM5C, MECP2, OFD1, OPN1, PAX3, PCDH19, PHF6, PLP1, PQBP1, RAB39B, SLC9A6, SMC1A, SMS, SRPX2, SYN</td>
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<tr>
<td><strong>Neuronal ceroid lipofuscinosis (5005)</strong></td>
<td>CLN3, CLN5, CLN6, CLN8, CTSD, DNAJC5, KCTD7, MFSD8, PPT1, TPP1</td>
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<td><strong>Epilepsy with migraine (5006)</strong></td>
<td>ATP1A2, CACNA1A, NOTCH3, POLG, PRRT2, SCN1A, SLC2A1</td>
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<tr>
<td>** Syndromic disorders (5007)**</td>
<td>ATP2A2, ATP6V0A2, CCDC88C, CLCNKA, CLCNKB, VPS138, KCNA1, KCNJ1, KCNJ10, KIAA1279, LBR, LGI1, ML2, NIPBL, PANK2, SERPINS1, PIGV, PLA2G6, RAII, SETBP1, SMC3, SYNGAP1, TBX1, TSC1, TSC2, VPS13A</td>
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<tr>
<td><strong>Infantile spasms (5008)</strong></td>
<td>ARX, CDKL5, FOXL1, GABRB3, GRIN2A, MEF2C, SCN2A, SLC2A22, SPTAN1, STXBP1</td>
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</table>

*Some genes in this panel are associated with more than 1 epilepsy phenotype.

*Testing for the groups of genes associated with each phenotype may be ordered separately, using the test codes listed in parentheses, for patients whose phenotypes suggest specific syndromes.

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*The CPT codes provided are based on AMA guidelines and are for informational purposes only. CPT coding is the sole responsibility of the billing party. Please direct any questions regarding coding to the payer being billed.

This test was developed and its performance characteristics have been determined by Athena Diagnostics. Performance characteristics refer to the analytical performance of the test.

Polymerase chain reaction (PCR) is performed pursuant to a license agreement with Roche Molecular Systems, Inc.