Epilepsy

CLINICIAN GUIDE
Ambry’s test offerings are designed to provide flexible, comprehensive options tailored to your patients’ personal and family histories.

Epilepsy is characterized by recurrent, unprovoked seizures. It is a common condition that affects about 1 in every 26 people, with approximately 150,000 new cases diagnosed in the U.S. per year. Epilepsy can develop in any person at any age but is most common in children, particularly in the first year of life.

Did You Know?
Most epilepsies have a genetic component, including emerging evidence of a genetic contribution to focal epilepsies. Genetic testing is increasingly relevant to the clinical management of epilepsy.

**CAUSES OF EPILEPSY**

**STRUCTURAL/ACQUIRED:**
- stroke
- trauma
- congenital lesions
- neoplasms
- other

**GENETIC:**
- single gene
- complex inheritance
- modifiers
- susceptibility alleles


How can genetic testing support clinical practice?
Identifying a genetic diagnosis for an epilepsy syndrome can directly impact management, enabling you to:

- Improve the understanding of prognosis
- Refine genetic counseling, including recurrence risk in future pregnancies
- Select the appropriate anti-convulsant(s) or other interventions
- End the diagnostic odyssey
- Avoid alternative, potentially invasive, testing
- Provide families the opportunity to connect with others in a similar situation
Our Genetic Testing for the Epilepsies

Does this patient fit any of the following clinical categories:
- pmefirst
- pmenext
- epilepsynext
- exomenext
- chromosomal microarray

Ambry participates with the epilepsy genetics initiative (EGI)
This is an option for ongoing data analysis for patients with epilepsy who have undergone ExomeNext testing (diagnostic exome sequencing). Please visit ambrygen.com/epilepsy-genetics-initiative for more information.

PATIENT WITH UNEXPLAINED EPILEPSY

Does this patient have epilepsy in conjunction with one or more of the following:
- developmental delay
- intellectual disability
- an autism spectrum disorder
- congenital anomaly(ies)
- infantile spasms

CHROMOSOMAL MICROARRAY

Does this patient have progressive myoclonus epilepsy (PME)?

- yes
- no

Does this patient have progressive myoclonus epilepsy (PME)?

- yes
- no

PMEFIRST

PMENEXT

Does this patient fit any of the following clinical categories:
- neonatal seizures
- febrile seizures
- infantile spasms
- non-lesional focal epilepsy

EPIFIRST

- EpiFirst-Neonate
- EpiFirst-Fever
- EpiFirst-IS
- EpiFirst-Focal

EPILEPSYNEXT

EXOMENEXT
Did You Know?
Epilepsy genetic testing results can guide therapeutic management decisions.

Points For Your Practice

**SLC2A1** encodes for the glucose transporter 1 (GLUT1), which is responsible for transporting glucose across the blood-brain barrier. Mutations in *SLC2A1* can result in a deficiency of GLUT1 and therefore inadequate levels of glucose in the brain. Affected individuals can have a variety of epilepsy presentations, including infantile epileptic encephalopathy and Doose syndrome.

Mutations in *SLC2A1* are also thought to be responsible for approximately 10% of absence epilepsy with onset prior to four years of age. The ketogenic diet is particularly effective for people with GLUT1 deficiency, and confirmation of this diagnosis can provide an alternative therapy to medication that may result in better seizure control and possibly improved cognitive outcomes.

Benefits of Testing with Ambry

**Flexible testing options:**
- Smaller, targeted panels minimize cost, turnaround time, and potential for variants of uncertain clinical significance
- Larger, comprehensive panels maximize detection rates
- Reflex options allow you to start small and test in a step-wise fashion
- Chromosomal microarray and ExomeNext (diagnostic exome sequencing) can supplement panel testing

**Multiple sample types:** Saliva, blood, and DNA samples are accepted for epilepsy testing and chromosomal microarray. A blood or DNA sample is needed for ExomeNext.

**Up-to-date gene list:** Our epilepsy testing includes multiple, well established and more newly characterized genes. You can be confident that your patients are benefiting from current and relevant information about causative epilepsy genes.

**Ongoing variant classification:** We help clarify clinical implications of variants of uncertain clinical significance (VUS). Follow-up testing of appropriate family members through our Family Studies Program can provide additional, powerful information to aid in variant assessment and classification.

**Insurance support:** We are an in-network provider with 95% of the major U.S. payors, including Medicare and Medicaid, and will perform preauthorization for all testing on your/patient’s behalf. A program is in place to support patients with financial hardship who are having testing.

**Data sharing:** We have always believed in secure data sharing, and are a regular contributor to multiple collaborative repositories and database projects, including ClinVar and the Epilepsy Genetics Initiative.
Neonatal Seizures

Neonatal seizures can occur anytime from birth until 28 days of life (or 4 weeks after expected due date), though the vast majority begin in the first week of life.

Between 1-2% of all neonates in the U.S. will experience seizures. In some neonates, seizures will be self-limiting and occur over only a few days, weeks, or months; others will go on to develop epilepsy.

Did You Know?

One gene can cause multiple epilepsy phenotypes. Distinguishing the underlying molecular mechanism is critical to appropriate treatment, prognosis, and genetic counseling.

Points For Your Practice

The \textit{KCNT1} gene encodes a sodium-activated potassium channel subunit widely expressed in the brain. \textit{KCNT1} mutations are associated with autosomal dominant nocturnal frontal lobe epilepsy, related to its expression in neurons of the frontal cortex. \textit{KCNT1} mutations can also cause epilepsy in infancy with migrating focal seizures, a severe epileptic encephalopathy with a poor outcome. Based on functional studies of mutations in \textit{KCNT1} associated with epileptic encephalopathy, treatment with quinidine was proposed and has been successful in some patients.

There are many causes of neonatal seizures, including epilepsy syndromes with onset in the neonatal period. Once initial evaluation has excluded other identifiable causes, diagnostic consideration may include:

<table>
<thead>
<tr>
<th>CLINICAL FEATURES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benign familial neonatal seizures</strong></td>
</tr>
<tr>
<td>• Seizure onset 2-8 days of life, typically tonic-clonic</td>
</tr>
<tr>
<td>• Remission between 1-6 months of age</td>
</tr>
<tr>
<td>• About 15% develop epilepsy later in life</td>
</tr>
<tr>
<td><strong>Early infantile epileptic encephalopathy</strong></td>
</tr>
<tr>
<td>• Seizure onset 2-10 days of life (sometimes \textit{in utero})</td>
</tr>
<tr>
<td>• Epileptic spasms and burst-suppression EEG patterns common</td>
</tr>
<tr>
<td>• Multiple seizure types can occur</td>
</tr>
<tr>
<td><strong>Pyridoxine-dependent epilepsy</strong></td>
</tr>
<tr>
<td>• Seizure onset at birth (sometimes \textit{in utero})</td>
</tr>
<tr>
<td>• Multiple seizure types, often intractable</td>
</tr>
<tr>
<td>• Periods of encephalopathy that precede seizure activity</td>
</tr>
<tr>
<td>• Intellectual disability is common</td>
</tr>
</tbody>
</table>
Our Genetic Testing Options for Neonatal Seizures

Our targeted panel includes genes most commonly associated with neonatal seizures. If a causative mutation is not identified, testing can proceed to a broader epilepsy gene panel.

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>DESCRIPTION</th>
<th>BENEFITS</th>
</tr>
</thead>
</table>
| EpiFirst-Neonate      | Targeted panel of 10 genes (details below) associated with neonatal seizures | • Test for genes most commonly associated with neonatal seizures  
  • Reduce cost and turnaround time  
  • Lower potential for results of uncertain clinical significance |
| EpilepsyNext          | Comprehensive panel of 100 genes associated with various types of epilepsy    | • Increase diagnostic yield by widening the phenotypic spectrum to include many genes known to cause epilepsy                                |

PATIENT WITH UNEXPLAINED NEONATAL SEIZURES

EPIFIRST-NEONATE

ALDH7A1, KCNQ2, KCNQ3
KCNT1, SCN1A, SCN1B, SCN2A
SCN8A, SIK1, STXBP1

EPILEPSYNEXT

Please see the “Comprehensive Epilepsy” section of this brochure for a list of genes included

EXOMENEXT

Please see the “Epilepsy and Genomics” section of this brochure for more information

AMBRY PARTICIPATES WITH THE EPILEPSY GENETICS INITIATIVE

Option for ongoing data analysis. Please visit ambrygen.com/epilepsy-genetics-initiative for more information
Febrile Seizures

Febrile seizures are convulsions associated with a body temperature above 38.0 Celsius (100.4 Fahrenheit), without evidence of any underlying health issue or prior history of seizures. Typical age of onset is six months to five years. Febrile seizures occur in 2-5% of the U.S. population and make up the most common convulsive event in children under the age of five.

Did You Know?

Multiple genes can cause one epilepsy phenotype. In these situations, multi-gene panel testing is often more cost-effective and time-effective than single gene analysis.

Points For Your Practice

Dravet syndrome is an epileptic encephalopathy characterized by infantile onset of prolonged febrile (or afebrile) generalized tonic-clonic or hemiclonic seizures, typically followed by the later development of other seizure types. Although mutations in SCN1A account for the majority of cases, 20-25% of children with Dravet syndrome do not have a SCN1A mutation. In one study, 37% of females without SCN1A mutations were found to have a PCDH19 mutation, establishing an overlapping clinical spectrum for these two genes. Mutations in GABRA1 and STXBP1 have also been identified in a minority of patients with a clinical diagnosis of Dravet syndrome, and are more likely to be found by multi-gene panel testing.

While most children with isolated febrile seizures do not go on to develop epilepsy, febrile seizures can be the presenting symptom of many clinical epilepsy syndromes. The most common epilepsy syndromes that present with febrile seizures include:

### CLINICAL FEATURES

<table>
<thead>
<tr>
<th>Genetic epilepsy with febrile seizures plus (GEFS+)</th>
<th>Childhood absence epilepsy with febrile seizures</th>
<th>Dravet syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Multiple family members with a variety of seizure types, including febrile seizures in early childhood&lt;br&gt;• Occasionally accompanied by various other seizure types, including: afebrile generalized tonic-clonic, atonic, myoclonic, myoclonic-ataxic, absence, focal seizures&lt;br&gt;• Febrile seizures continue past the typical age when febrile seizures are expected to resolve</td>
<td>• Febrile seizures in early childhood, followed by absence seizures from childhood through mid-adolescence that often resolve spontaneously</td>
<td>• Seizure onset in the first year of life&lt;br&gt;• Prolonged febrile and/or afebrile generalized tonic-clonic or hemiclonic seizures&lt;br&gt;• Other seizure types are common, including myoclonic, focal, and atypical absence seizures</td>
</tr>
</tbody>
</table>
Our Genetic Testing Options for Febrile Seizures

Our targeted panel includes genes most commonly associated with febrile seizures. If a causative mutation is not identified, testing can proceed to a broader epilepsy gene panel.

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>DESCRIPTION</th>
<th>BENEFITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EpiFirst-Fever</td>
<td>Targeted panel of 13 genes (details below) associated with febrile seizures</td>
<td>• Test for genes most commonly associated with febrile seizures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduce cost and turnaround time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Lower potential for results of uncertain clinical significance</td>
</tr>
<tr>
<td>EpilepsyNext</td>
<td>Comprehensive panel of 100 genes associated with various types of epilepsy</td>
<td>• Increase diagnostic yield by widening the phenotypic spectrum to include many genes known to cause epilepsy</td>
</tr>
</tbody>
</table>

**PATIENT WITH UNEXPLAINED FEBRILE SEIZURES**

**EPINFIRST-FEVER**

CHD2, GABRA1, GABRB3, GABRG2, HCN1, PCDH19, PRRT2, SCN1A, SCN1B, SCN2A, SCN8A, STX1B, STXBP1

**EPILEPSYNEXT**

Please see the “Comprehensive Epilepsy” section of this brochure for a complete list of genes included

**EXOMENEXT**

Please see the “Epilepsy and Genomics” section of this brochure for more information

**AMBRY PARTICIPATES WITH THE EPILEPSY GENETICS INITIATIVE**

Option for ongoing data analysis. Please visit ambrygen.com/epilepsy-genetics-initiative for more information
Infantile Spasms

Infantile spasms (IS) are characterized by epileptic spasms, occasionally associated with hypsarrhythmia on EEG and developmental regression. Peak onset is between four to eight months of age. Spasms typically disappear by age three to four years, but may be replaced by other seizure types. Infantile spasms affect about 1 in every 2,000 infants in the U.S.

Infantile spasms are often seen in the context of West syndrome and Ohtahara syndrome, both early-onset epileptic encephalopathies. Approximately 10-30% of patients with Lennox-Gastaut syndrome evolve from earlier epilepsies that included infantile spasms, including West syndrome and Ohtahara syndrome.

Did You Know?

Genetic testing can determine the cause of infantile spasms in about 40% of cases.

Points For Your Practice

The National Infantile Spasms Consortium conducted a large, prospective multi-center study of 251 infants recently diagnosed with IS and concluded that a cost-effective method of genetic evaluation includes chromosomal microarray (diagnostic yield ~11%) followed by an epilepsy gene panel (diagnostic yield ~31%). Diagnostic exome sequencing is typically reserved for cases in which all other testing has been inconclusive.


Tuberous Sclerosis Complex

Approximately 40% of infants with tuberous sclerosis complex (TSC) will develop infantile spasms. Early diagnosis and rapid treatment can often result in improved developmental outcomes.

TSC is an autosomal dominant multi-system condition. About two-thirds of affected individuals have a de novo mutation in the TSC1 or TSC2 genes, though screening of parents for sub-clinical features of TSC is recommended when a child is diagnosed. Diagnosis can be based on either genetic testing results or clinical criteria.

Genetic diagnostic criteria include the identification of a pathogenic TSC1 or TSC2 mutation in DNA from normal tissue. A negative DNA test does not exclude the diagnosis, and does not impact the use of the clinical diagnostic criteria. However, a test result identifying a gene mutation can confirm a diagnosis of TSC when clinical presentation is not clear, and thus open the door to individualized medical management.
Our Genetic Testing Options for Infantile Spasms (IS)

Our targeted panel includes genes most commonly associated with infantile spasms. If a causative mutation is not identified, testing can proceed to a broader epilepsy gene panel.

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>DESCRIPTION</th>
<th>BENEFITS</th>
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</thead>
<tbody>
<tr>
<td>EpiFirst-IS</td>
<td>Targeted panel of 17 genes (details below) associated with infantile spasms</td>
<td>• Test for genes most commonly associated with infantile spasms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reduce cost and turnaround time</td>
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<tr>
<td></td>
<td></td>
<td>• Lower potential for results of uncertain clinical significance</td>
</tr>
<tr>
<td>EpilepsyNext</td>
<td>Comprehensive panel of 100 genes associated with various types of epilepsy</td>
<td>• Increase diagnostic yield by widening the phenotypic spectrum to include many genes known to cause epilepsy</td>
</tr>
</tbody>
</table>

**EpilepsyNext**

Please see the “Comprehensive Epilepsy” section of this brochure for a complete list of genes included.

**Ambry participates with the Epilepsy Genetics Initiative**

Option for ongoing data analysis. Please visit ambrygen.com/epilepsy-genetics-initiative for more information.
Non-Lesional Focal Epilepsy

Focal seizures are the most common type of seizure experienced by people with epilepsy. Most focal epilepsies are caused by structural brain abnormalities, head injury, or brain tumor. However many occur in the setting of a normal brain MRI or head CT and can be referred to as non-lesional.

Originally thought to have no genetic basis, there is now increasing evidence indicating that genetics plays a role in the development of focal epilepsy. Many focal epilepsy syndromes are now known to be caused by mutations in single genes.

Did You Know?
Understanding the underlying molecular basis of an epilepsy syndrome can assist you in tailoring treatment for your patient.

Points For Your Practice

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a non-lesional focal epilepsy syndrome that can be caused by a mutation in one of six different genes: CRH, CHRNA2, CHRNA4, CHRNA2, DEPDC5, or KCNT1. Testing of these genes reveals causative mutations in approximately 20% of affected patients with a family history of this condition. Carbamazepine is the most frequently utilized anti-seizure medication for ADNFLE, providing remission in about 70% of individuals. However, patients who undergo genetic testing and are found to have the CHRNA4 pathogenic mutation p.Ser284Leu are more responsive to zonisamide than carbamazepine.

Several of the more common non-lesional focal epilepsy syndromes with known causative gene associations are below:

<table>
<thead>
<tr>
<th>CLINICAL FEATURES</th>
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</thead>
<tbody>
<tr>
<td><strong>Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)</strong></td>
</tr>
<tr>
<td>• Clusters of brief, nocturnal seizures</td>
</tr>
<tr>
<td>• Cognition and other neurological functions are most often unaffected</td>
</tr>
<tr>
<td>• Onset in childhood or adolescence</td>
</tr>
<tr>
<td><strong>Autosomal dominant partial epilepsy with auditory features (ADPEAF); also called familial lateral temporal lobe epilepsy</strong></td>
</tr>
<tr>
<td>• Focal seizures with auditory hallucinations</td>
</tr>
<tr>
<td>• Can shift into generalized convulsive seizures</td>
</tr>
<tr>
<td><strong>Familial partial epilepsy with variable foci (FPEVF)</strong></td>
</tr>
<tr>
<td>• Multiple family members with focal epilepsies with different localizations (e.g. some may have temporal lobe epilepsy, whereas others may have frontal lobe epilepsy)</td>
</tr>
</tbody>
</table>
Our Genetic Testing Options for Non-Lesional Focal Epilepsy

Our targeted panel includes genes most commonly associated with non-lesional focal epilepsy. If a causative mutation is not identified, testing can proceed to a broader epilepsy gene panel.

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>DESCRIPTION</th>
<th>BENEFITS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EpiFirst-Focal</td>
<td>Targeted panel of 11 genes (details below) associated with non-lesional focal epilepsy</td>
<td>• Test for genes most commonly associated with focal epilepsies&lt;br&gt;• Reduce cost and turnaround time&lt;br&gt;• Lower potential for results of uncertain clinical significance</td>
</tr>
<tr>
<td>EpilepsyNext</td>
<td>Comprehensive panel of 100 genes associated with various types of epilepsy</td>
<td>• Increase diagnostic yield by widening the phenotypic spectrum to include many genes known to cause epilepsy</td>
</tr>
</tbody>
</table>

**PATIENT WITH UNEXPLAINED NON-LESIONAL FOCAL EPILEPSY**

**EPIFIRST-FOCAL**

CRH, CHRNA2, CHRNA4, CHRN2B, DEPDCS, GRIN2A, KCNT1, LGI1, PRRT2, SCN1A, SCN1B

**EPILEPSYNEXT**

Please see the “Comprehensive Epilepsy” section of this brochure for a complete list of genes included

**EXOMENEXT**

Please see the “Epilepsy and Genomics” section of this brochure for more information

**AMBRY PARTICIPATES WITH THE EPILEPSY GENETICS INITIATIVE**

Option for ongoing data analysis. Please visit ambrygen.com/epilepsy-genetics-initiative for more information
**Progressive Myoclonus Epilepsy**

The progressive myoclonus epilepsies (PME) are a group of disorders that affect the central nervous system, causing progressive myoclonus, other generalized epilepsy, cognitive impairment, ataxia, and other neurologic deficits. People with PME frequently have a combination of seizure types, most often including myoclonic and tonic-clonic seizures.

The presence of the following findings are indicative of PME:

- Progressive neurologic disability
- Failure to respond to antiepileptic drug therapy
- Background slowing on EEG

Distinction from more common forms of genetic generalized epilepsies can be challenging early on in the disease course. Additionally, inappropriate therapy in the genetic generalized epilepsies can result in symptoms that mimic PME, including ataxia, impaired cognition, and uncontrolled seizures. Genetic testing can assist with establishing the correct diagnosis.

Many different syndromes cause PME, some of the most common are:

<table>
<thead>
<tr>
<th>CLINICAL FEATURES</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epilepsy, progressive myoclonus 1 (EPM1); Unverricht-Lundborg disease</strong></td>
<td>Seizure onset between 6-13 years of age, with myoclonus onset 1-5 years later</td>
</tr>
<tr>
<td><strong>Epilepsy, progressive myoclonus 2A (EPM2A); Lafora disease</strong></td>
<td>Seizure and myoclonus onset in teens, Presence of Lafora bodies in nervous system, muscles, and/or skin</td>
</tr>
<tr>
<td><strong>Neuronal ceroid lipofuscinosis (NCL); Batten disease</strong></td>
<td>Progressive neurological impairment, including seizures, visual problems, dementia, loss of motor skills, gait disturbance, Onset can vary depending on type, anywhere from infancy to adulthood</td>
</tr>
</tbody>
</table>
Our Genetic Testing Options for Progressive Myoclonus Epilepsy

Our targeted panel, PMEFirst, includes three genes that account for approximately 50% of all PME. If a causative mutation is not identified, testing can proceed to a broader panel, PMENext. To ensure that testing automatically proceeds to the larger panel, you can choose PMEReflex.

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>DESCRIPTION</th>
<th>BENEFITS</th>
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</thead>
<tbody>
<tr>
<td>PMEFirst</td>
<td>Small, targeted panel of 3 genes (details below) that account for approximately 50% of all PME</td>
<td>- Test for the genes most commonly associated with PME</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Reduce cost and turnaround time</td>
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<tr>
<td></td>
<td></td>
<td>- Lower potential for results of uncertain clinical significance</td>
</tr>
<tr>
<td>PMENext</td>
<td>Larger, panel of 21 genes (details below) associated with PME</td>
<td>- Increase diagnostic yield by including additional genes known to cause PME</td>
</tr>
<tr>
<td>PMEReflex*</td>
<td>Begin with PMEFirst; if negative, automatically continue to PMENext</td>
<td>- Maximize efficiency by prioritizing high-potential genes, while ensuring eventual testing of all relevant genes (as appropriate)</td>
</tr>
</tbody>
</table>

*If PMEReflex is ordered, your patient is billed only for the most comprehensive test performed.

PATIENT WITH PME

PMEFIRST

CSTB, EPM2A, NHLRC1

PMENext

ATP13A2, CLN3, CLN5, CLN6, CLN8, CSTB, CTSD, CTSF, DNAJC5, EPM2A, FOLR1, GOSR2, GRN, KCNC1, KCTD7, MFSD8, NHLRC1, PPT1, PRICKLE1, SCARB2, TPP1

EXOMENext

Please see the “Epilepsy and Genomics” section of this brochure for more information

AMBRY PARTICIPATES WITH THE EPILEPSY GENETICS INITIATIVE

Option for ongoing data analysis. Please visit ambrygen.com/epilepsy-genetics-initiative for more information
Comprehensive Epilepsy Testing

Recent discoveries regarding the genetics of the epilepsies have highlighted two important patterns:

One gene can be associated with multiple epilepsy syndromes.
One epilepsy syndrome can be associated with multiple genes.

Taken together, these findings support the use of a broad multi-gene panel for genetic testing in the epilepsies. Our comprehensive epilepsy panel, EpilepsyNext, is constructed to maximize diagnostic yield by including relevant genes covering a wide phenotypic spectrum, including newly characterized genes with sufficient causative evidence.

Who Could Benefit From EpilepsyNext?

Anyone with an inconclusive result on an EpiFirst panel.
Anyone with epilepsy of unknown etiology after physical exam, neuroimaging, and biochemical testing.

EpilepsyNext includes genes associated with the following epilepsy syndromes:

- Benign familial neonatal seizures
- Dravet syndrome
- Genetic epilepsy with febrile seizures plus (GEFS+)
- Childhood absence epilepsy with febrile seizures
- Familial hemiplegic migraine
- Early infantile epileptic encephalopathy
- Infantile spasms
- West syndrome
- Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)
- Autosomal dominant partial epilepsy with auditory features (ADPEAF); also called familial lateral temporal lobe epilepsy
- Familial partial epilepsy with variable foci (FPEVF)
- Progressive myoclonus epilepsy
- Myoclonic epilepsy myopathy sensory ataxia (MEMSA)
- Pyridoxine-dependent epilepsy
- Tuberous sclerosis complex
- Neuronal ceroid lipofuscinosis (Batten disease)
Our Comprehensive Epilepsy Genetic Testing

EpilepsyNext is our broad, comprehensive epilepsy panel that includes 100 genes known to cause a variety of epilepsies.

EpilepsyNext is an appropriate reflex option following any EpiFirst test, allowing for targeted testing of the most relevant genes prior to a broader testing approach. In cases with an unclear history or atypical presentation, EpilepsyNext could be an appropriate first-line test.

Did You Know?
Tiered testing with automatic reflex from targeted to broader panels is cost- and time-effective for you and your patients, and also maintains comprehensiveness.

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>GENES</th>
</tr>
</thead>
<tbody>
<tr>
<td>EpilepsyNext</td>
<td>ALDH7A1, ARHGEF9, ARX, ATP1A2, ATP13A2, CACNA1A, CASK, CDKL5,</td>
</tr>
<tr>
<td></td>
<td>CHD2, CHRNA2, CHRNA4, CHRNb2, CLN3, CLN5, CLN6, CLN8,</td>
</tr>
<tr>
<td></td>
<td>CNTNAP2, CSTB, CTSD, DEPDC5, DNAJC5, DNMB1, DYNCH1H1, EPMZ2A,</td>
</tr>
<tr>
<td></td>
<td>FOLR1, FOXG1, GABRA1, GABRB3, GABRG2, GAMA1, GATM, GNAO1,</td>
</tr>
<tr>
<td></td>
<td>GOSR2, GRIN1, GRIN2A, GRIN2B, HCN1, HNRPNL, KCNCl, KCNJ10,</td>
</tr>
<tr>
<td></td>
<td>KCNQ2, KCNQ3, KCNT1, KCTD7, LG11, MECP2, MEF2C, MFSD8, NLRCl,</td>
</tr>
<tr>
<td></td>
<td>NRXN1, PCDH19, PIGA, PNKl, PNPO, POLG, PPT1, PRICKLE1, PRRT2,</td>
</tr>
<tr>
<td></td>
<td>SCARB2, SCNA1A, SCN1B, SCN2A, SCN8A, SLC13A5, SLC25A22, SLC2A1,</td>
</tr>
<tr>
<td></td>
<td>SLC35A2, SLC9A6, SNAP25, SPTANI, ST3GAL3, STXBPl, SYNl,</td>
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<tr>
<td></td>
<td>SYNGAPI, SZT2, TBC1D24, TCF4, TAPP1, TSC1, TSC2, UBE3A, ZEB2, PLCBl,</td>
</tr>
<tr>
<td></td>
<td>CRH, STX1B, CTSF, IQSEC2, GRN, EEF1A2, KCNA2, SIK1, SLC6A1, TBL1XR1,</td>
</tr>
<tr>
<td></td>
<td>DCX, DYRK1A, FLNA, KIAA2022, PURA, SMC1A, WDR45</td>
</tr>
</tbody>
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PATIENT WITH UNEXPLAINED EPILEPSY
PATIENT WITH INCONCLUSIVE EPIFIRST PANEL

EPILEPSYNEXT

EXOMENEXT

Please see the “Epilepsy and Genomics” section of this brochure for more information

AMBRY PARTICIPATES WITH THE EPILEPSY GENETICS INITIATIVE
Option for ongoing data analysis. Please visit ambrygen.com/epilepsy-genetics-initiative for more information.
Epilepsy and Clinical Genomics

The genetic architecture of the epilepsies is proving to be complex. In addition to interrogating specific genes associated with epilepsy via targeted multi-gene panels, searching more broadly across your patient’s DNA (called genomic testing) can be useful in the diagnostic process.

Chromosomal Microarray (CMA)

CMA is a technique that allows for high resolution genome-wide detection of unbalanced structural and numerical chromosomal abnormalities, and is a powerful tool for identifying molecular causes of disease. CMA is most frequently used as a first-tier screening test, followed by targeted gene testing if no informative answer is found.

Did You Know?

CMA is the best first-tier test for a patient with epilepsy who presents with comorbid developmental delay, intellectual disability, and/or an autism spectrum disorder.

Points For Your Practice

CMA has been found to be particularly useful in identifying a genetic cause for developmental delay, intellectual disabilities, and autism spectrum disorders. The diagnostic yield for CMA for these indications is approximately 15-20%. Current recommendations from the American Academy of Neurology, the American Academy of Pediatrics, and the American College of Medical Genetics and Genomics indicate that CMA is a first-tier test for anyone with these clinical findings.

The use of CMA in the epilepsies is an area of current investigation, with some studies reporting a diagnostic rate of up to 11% for this patient population. Some of the more frequent alterations detected by CMA for patients with epilepsy include deletions of 15q13.3, 15q11.2, and 16p13.11.

ExomeNext (Diagnostic Exome Sequencing)

Despite testing using CMA and multi-gene epilepsy panels, many patients do not receive an answer as to the cause of their epilepsy. In these instances, diagnostic exome sequencing can help. Our ExomeNext test targets approximately 20,000 genes in the nuclear and mitochondrial genomes for analysis, often uncovering unexpected causes of clinical symptoms.

Did You Know?

In our first 1,100 exomes analyzed, the detection rate in the epilepsies cohort was 38.2%.
Our Clinical Genomics Testing Options

<table>
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<th>TEST</th>
<th>DESCRIPTION</th>
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| Chromosomal microarray (SNP Array) | • Array CGH supplemented with SNP probes that cover the entire genome at a median probe spacing of 1.1 kb  
   • Captures copy number changes and copy neutral aberrations, such as regions of homozygosity (ROH) and uniparental disomy (UPD)  
   • Includes probe coverage for genes located in the pericentromeric and subtelomeric regions |
| Chromosomal microarray (180K oligo array) | • Covers the entire genome at a median probe spacing of 13 kb and 5 kb on the X chromosome  
   • Detects deletions/duplications of ~680 disease causing genes with exon level resolution  
   • Detects all known microdeletion/duplication syndromes and most disorders detected by chromosomal analysis and fluorescence in situ hybridization (FISH) tests |
| ExomeNext | • Exons of ~20,000 nuclear genes and mitochondrial (mtDNA) genome are sequenced  
   • Genetic alterations filtered through in-house bioinformatics pipeline and analyzed by our medical team  
   • Analysis of characterized, as well as novel genetic etiologies, performed |

Did You Know?
Between 60-75% of patients with epilepsy who undergo diagnostic exome sequencing at a clinical lab receive an inconclusive result.

Points For Your Practice
Ongoing reinterpretation of the data generated by exome sequencing has eventually led to an answer for some patients, as new gene discoveries are made and more is learned about the genetic causes of epilepsy. We are partnered with the Epilepsy Genetics Initiative (EGI), a large secure repository of genetic data from patients with epilepsy that utilizes exome sequencing data to search for novel genetic causes of epilepsy.

Should your patient choose to enroll in this project, we can quickly facilitate the transfer of data to the EGI repository. Patient data that is entered into this secure database will be reanalyzed repeatedly and any new information relevant to the patient that becomes available will be reported back to their clinician. Visit ambrygen.com to learn more.
Variants of Uncertain Clinical Significance and Secure Data Sharing

Variants of uncertain clinical significance (VUS) are changes in the DNA sequence for which there is currently insufficient evidence to determine whether the DNA change is a mutation that causes disease. VUS are common in genetic testing panels because multiple genes are analyzed simultaneously.

Did You Know?

Genetic testing using smaller, targeted panels can greatly reduce the likelihood of a VUS result.

We are committed to careful analysis and timely reclassification of VUS. If a VUS is identified, complimentary testing of informative relatives may be offered through our Family Studies Program. Familial tracking can assist in clarifying the nature of a VUS. When enough evidence has been accumulated to reclassify a VUS as either disease-causing or benign, you will be automatically notified.

Did You Know?

Clinical laboratories often house a vast amount of data on variants. Sharing this data can advance the collective understanding about the relationship between a DNA change and a disease, which is vital to patient care advancement.

We have long believed in secure data sharing and regularly contribute variant data to the following projects:

ClinVar

ClinVar is the premier public database that aggregates information about genomic variants and their relationship to human health. [ncbi.nlm.nih.gov/clinvar](ncbi.nlm.nih.gov/clinvar)

Epilepsy Genetics Initiative (EGI)

EGI is a repository that contains confidential genetic information about people with epilepsy. It is used for research purposes, with the goal of understanding genetic causes of epilepsy. [cureepilepsy.org/egi](cureepilepsy.org/egi)

Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA)

ENIGMA is a research-based consortium of investigators focused on determining the involvement of all variants of unknown significance in BRCA1 and BRCA2. [enigmaconsortium.org](enigmaconsortium.org)
Ambry and Advocacy

We are more than just a laboratory—we have a deep commitment to patients and provide continual support to numerous community foundations. Giving back is a large part of our company spirit, and we are proud to support the following epilepsy advocacy organizations:

CITIZENS UNITED FOR RESEARCH IN EPILEPSY (CURE)

CURE is the leading non-governmental agency providing funding for research in epilepsy. In 2015, CURE launched a new signature program, the Epilepsy Genetics Initiative (EGI), a research project providing ongoing analysis of diagnostic exome data for individuals with epilepsy. Ambry is proud to be an inaugural industry partner for EGI. cureepilepsy.org

DANNY DID FOUNDATION

Danny Did Foundation is dedicated to advancing the public awareness of epilepsy and Sudden Unexpected Death in Epilepsy (SUDEP). Danny Did works to gain mainstream awareness of SUDEP and to promote the use of seizure detection and prediction devices that may assist in preventing seizure-related deaths. dannydid.org

EPILEPSY AWARENESS DAY DISNEYLAND (EADDL)

EADDL is dedicated to uniting the epilepsy community, advancing the public awareness of epilepsy, and removing the stigma that can be associated with this condition. EADDL hosts an annual event at Disneyland to provide education, inclusion, and joy for individuals and families living with epilepsy. epilepsyawarenessday.org

GLUT1 DEFICIENCY FOUNDATION

The Glut1 Deficiency Foundation is dedicated to providing education about Glut1 deficiency, promoting awareness and advocacy, and supporting researchers working toward a cure for this condition. g1dfoundation.org

PCDH19 ALLIANCE

The PCDH19 Alliance is committed to improving the lives of children and families who are affected by PCDH19 Epilepsy. The Alliance provides information and support to affected families, and provides support for medical research targeting more effective treatments. pcdh19info.org

BATTEN DISEASE SUPPORT AND RESEARCH ASSOCIATION (BDSRA)

BDSRA is dedicated to improving the lives of those affected by Batten disease by supporting medical science, research, and families working together to discover treatments and cures. bdsra.org
Genetic Test Results Explained

A patient undergoing genetic testing will receive one of three possible results: positive, negative, or inconclusive (i.e. variant of uncertain clinical significance or VUS).

<table>
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<tr>
<th>RESULTS</th>
<th>EXPLANATION</th>
</tr>
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| Positive | • A mutation was found in one of the genes tested  
• Management recommendations specific to the gene  
• Testing at-risk relatives for specific alteration may be recommended |
| Negative | • No clinically significant genetic changes identified in any of the genes tested  
• Management recommendations based on personal and family history  
• Genetic testing not typically indicated for family members |
| Inconclusive | • A genetic change was identified, but current knowledge cannot predict if the change is disease-causing or benign  
• Management recommendations based on personal and family history  
• Family studies may be indicated |

Specimen Requirements

**Blood:** Collect 6-10cc (adult) or at least 5cc (children) in purple top EDTA tube (preferred) or yellow top citric acetate tube. If CSTB dodecamer repeat expansion analysis is requested as part of EpilepsyNext, a minimum of 10-15cc whole blood in EDTA (purple top, preferred) or Citric Acetate (yellow top, acceptable) tubes is required for this testing. Storage: 2-8°C and do not freeze. Shipment: Room temperature for two-day delivery.

**Blood Spot:** Blood spots are not accepted.

**Saliva:** Fill 1 tube (2 tubes for pediatric patients) with saliva up to black line (1cc of saliva) in Oragene Self Collection container. After tube is closed, 1cc of buffer will mix with saliva for a total volume of 2cc. Store at room temperature in sterile bag. Shipment: Room temperature for two-day delivery. **Saliva cannot be accepted for PMEFirst, PMENext, ExomeNext, or for EpilepsyNext if CSTB dodecamer repeat expansion analysis is requested.**

**DNA:** 20 μg of DNA in TE (10mM Tris-Cl pH 8.0, 1mM EDTA); preferred 200 μl at -100 ng/μl. If CSTB dodecamer repeat expansion analysis is requested, at least 350-400μl at -250 ng/μl concentration of extracted DNA is needed. Please provide DNA OD 260-280 ratio (preferred 1.7-1.9) and send agarose picture with high mw genomic DNA, if available. Store at -20°C. Ship frozen on dry ice (preferred) or ice. It is recommended if DNA has undergone multiple freeze/thaw cycles that it not be submitted. DNA isolated from fixed tissue is not recommended.

**Cultured Cells:** We accept cultured cells from fibroblasts. Prefer two T25 cell flasks (one flask is acceptable) or suitable alternative at 80% confluence. Store in tissue culture incubator. Do not freeze. Please call ahead when sending prenatal samples.
Ambry Expertise

SUPPORT
Board-certified genetic counselors, laboratory directors, and medical directors are readily available to assist with test selection, case reviews, and result interpretation.

INSURANCE
We are contracted with the majority of commercial insurances and Medicare. All out-of-network patients are treated as in-network to minimize out-of-pocket costs. Insurance, Medicare, and Medicaid coverage vary. Preverification is recommended.

PATIENT PROTECTION PLAN
Ambry’s billing policy is to preverify insurance coverage (with or without patient sample) for genetic testing. We will contact the patient after their sample is received, if their out-of-pocket cost is estimated to exceed $100. We are committed to working with you and your patients to make the genetic testing process as simple and cost-effective as possible, and our Billing Department is available to answer any questions your patient may have. Our Billing Department can be reached by phone at +1-949-900-5795 or billing@ambrygen.com.

PROGENY’S FAMILY HISTORY QUESTIONNAIRE (FHQ)
With Progeny’s FHQ, patients can complete their family health history at their own pace. Pedigrees are automatically generated and managed along with all submitted data. With no re-entry of data, referral and clinical decisions can be made immediately. Template FHQs are available for use in your clinic at no cost, or custom questionnaires can be designed for you. Visit progenygenetics.com/clinical/trial for more.

ABOUT AMBRY GENETICS
Ambry Genetics is a privately-held healthcare company with the most comprehensive suite of genetic testing solutions for inherited and non-inherited diseases. Since 1999, Ambry has tested approximately half a million patient samples benefiting 90% of all U.S. patients covered by public and private insurers. Ambry is dedicated to scientific collaboration by offering its rapidly growing database of anonymized genomic data (variant frequencies) free to the global medical research community to fulfill the promise of the human genome to cure or manage all human disease. Ambry is dedicated to the belief that human health should not be patented or owned, and genomic data should be freely shared so we can try to understand all human disease.

To order your complimentary sample submission kits, please contact:
Ambry Genetics
15 Argonaut
Aliso Viejo, CA 92656 USA
+1 (866) 262-7943
info@ambrygen.com

For more details about these tests, visit ambrygen.com

References used to develop clinical content are available at ambrygen.com
AmbryPort2.0

AmbryPort2.0 is an online customer interface, which includes features such as:

- Insurance preverification and order submission
- Ability to get status updates and track samples
- Ability to print and/or download patient reports
- Patient-specific auto-generation of letters of medical necessity
- Patient signature form to easily obtain patient signature during clinic
- Ability to upload insurance paperwork, medical records and other patient-specific documents

CURRENT AMBRY CUSTOMERS

If you currently use Ambry Genetics and have an email on file with us, please attempt to login to your account by going to ambrygen.com and clicking the AmbryPort 2.0 login link on the upper right corner of any screen. Click reset password and insert your email address. If successful, a password reset will be sent to your email account. You can then change your password.

If you have any questions or concerns, please contact our Client Services department at 949-900-5500.

USER GUIDE

A user guide is available and can be found here at ambrygen.com/ap2

SYSTEM REQUIREMENTS

- Compatible with: desktop, tablet and mobile devices
- AmbryPort 2.0 works with: Safari, Chrome, Firefox, and Internet Explorer Version 8 or higher
- We recommend using the most current browser version to insure full functionality of the AmbryPort 2.0 interface

Now available: new enhancements to Patient List Screen and secure document downloading through AP2 -- visit ambrygen.com/ap2 for more details