Rett/ Atypical Rett/ Angelman/ Angelman-like Syndromes
Indication-Specific Gene Panel

including: sequencing and high resolution deletion/duplication analysis

PANEL DESCRIPTION:
Rett syndrome, Angelman syndrome, and several similar conditions cause neurodevelopmental concerns and often include mobility concerns and a risk of seizures. Rett syndrome is found in approximately 1 in 8,500 females.\(^1\) Angelman syndrome is found in as many as 1 in 12,000 males and females.\(^2\) It is unknown how often the atypical presentations of these syndromes occur. This indication-specific panel is designed to detect both sequence-based mutations and small-scale deletions or duplications within \textbf{16 genes} associated with Rett syndrome, atypical Rett syndrome, Angelman syndrome, and Angelman-like syndrome.

PANEL OVERLAP:
Due to the phenotypic overlap between various syndromes, the Autism/Intellectual Disability/Multiple Anomalies Panel, which covers all the genes in the Rett/Atypical Rett/Angelman/Angelman-like Syndromes panel, as well as additional genes of clinical significance, may be appropriate for some patients.

PANEL DETAILS:
- This panel includes both sequencing and high resolution deletion/duplication analysis of the genes specified.
  - Sequencing is performed using a customized next generation sequencing library. Analysis includes the coding exons of all genes in the panel plus ten bases into the introns and untranslated regions (5' and 3'). Sanger sequencing is performed to confirm variants suspected or confirmed to be pathogenic.
  - Deletion/duplication analysis is performed using a high resolution, custom microarray platform designed to target the genes of interest at the exon level.
- Detection rates are limited to the genes specified; this test does not provide whole genome analysis.
- Gene panels are a more cost-effective approach than single gene testing to confirm or establish a diagnosis. However, if single gene testing is desired for the patient or family members of an individual with a known mutation, that must be ordered separately.

ADDITIONAL TESTING DETAILS:
- If microarray analysis is performed, it will be done using a high resolution, single nucleotide polymorphism (SNP) platform designed to interrogate the whole genome at a resolution much higher than is possible using traditional karyotyping or fluorescence \textit{in situ} hybridization (FISH) methodologies. Our High Density SNP array contains a total of 2.6 million markers distributed throughout the genome for the detection of both genomic dosage anomalies (deletions and duplications) and regions of homozygosity (ROH; regions lacking typical amounts of genetic variation). This marker density provides a global resolution of 10 Kb to 20 Kb for copy number changes and 5 Mb resolution for ROH.
- If methylation analysis is performed, it will be done using a methylation-specific PCR assay to identify imprinting abnormalities of chromosome 15. Methylation analysis is used to detect and identify the parental origin of deletions, uniparental disomy (UPD), and methylation defects occurring in the imprinted promoter regions of chromosomes 14 and 15.

RECOMMENDED TESTING STRATEGY:
Tests below can be ordered individually, however our laboratory’s recommended Comprehensive Testing for Rett, Atypical Rett, Angelman, and Angelman-like syndromes includes the following three tests:

\textbf{High Density SNP Microarray Analysis}
- Approximately 8% of individuals with classic Rett syndrome and \sim 3\% of those with atypical Rett syndrome are found to have partial or whole \textit{MECP2} gene deletions which are NOT identifiable by sequencing\(^3\). Phenotypic features of Rett...
syndrome, atypical Rett, Angelman and Angelman-like syndromes overlap with the features of multiple microdeletion and microduplication syndromes. High Density SNP Microarray assesses for these copy number changes.

- NOTE: High Density SNP Microarray is NOT a substitution for Deletion/Duplication Analysis of MECP2.

**Next Generation Sequencing**

- This gene test panel covers genes associated with Rett syndrome, atypical Rett syndrome, Angelman syndrome, and Angelman-like syndrome. Next generation sequencing (NGS) analyzes multiple genes at once, making this a cost-effective method of testing genes known to be as associated with these indications.

**Targeted Deletion/Duplication Analysis**

- If SNP Microarray Analysis and gene sequencing are normal, Deletion/Duplication Analysis is performed to identify partial or whole gene deletions and duplications, another potential cause of developmental disabilities, autism, or congenital anomalies.

**IF INDICATED, add Methylation 15 studies**

- Methylation abnormalities of Chromosome 15 are found in ~78% of individuals with Angelman syndrome. Methylation Analysis will identify methylation errors as a result of mechanisms NOT detected by SNP Microarray Analysis and is recommended for patients with a phenotype suggestive of Angelman syndrome.

**DISORDERS INCLUDED IN THIS PANEL:**

- Atypical Rett
- Angelman [GeneReviews®](http://www.ncbi.nlm.nih.gov/books/NBK1144/)
- Angelman-like
- Pitt-Hopkins [GeneReviews®](http://www.ncbi.nlm.nih.gov/books/NBK100240/)
- X-linked intellectual disability, Christianson type

**INDICATIONS FOR TESTING:**

- Developmental delay, with or without regression
- Mild to severe intellectual disability
- Seizures
- Lack of speech/apraxia
- Low muscle tone
- Ataxic gait
- Dysmorphic or normal facial features
- Concerning stereotypic behaviors such as hand-wringing, hand-flapping, inappropriate laughter

**SPECIMEN COLLECTION & TRANSPORT:**

Complimentary test kits are available upon request, but are not required.

**SAMPLE TYPE and REQUIREMENTS:**

- blood: 3 - 5 ml whole blood in an EDTA tube (purple top)
  - newborn minimum requirement: 1 - 3 ml
- buccal swab: 5 swabs
- extracted DNA: 70 ng/µg with a total yield of 7-10 µg (in TE) in a DNA microcentrifuge tube

* When submitting extracted DNA for genetic testing, nucleic isolation must have occurred in a CLIA-certified laboratory or a laboratory meeting equivalent requirements as determined by the CAP and/or CMS.

**SHIPPING:**

- Maintain and ship samples at room temperature.
- Coordinate transport for sample to be received in our laboratory within 24-48 hours of collection.
  - LOCAL: Call 402-559-5070 (option 1)
  - OUT OF AREA: Prior to shipment, please fax the completed test request form to 402-559-7248, including the FedEx® airbill tracking number.
    - Saturday delivery MUST be checked when sending FedEx® on Friday.
    - Please include Internal Billing Reference # 3155070600 on the FedEx® airbill.
    - Ship To: Human Genetics Laboratory – Zip 5440
    - UNMC Shipping & Receiving Dock
    - 601 S. Saddle Creek Road
    - Omaha, NE 68106
FORMS FOR TESTING: The following forms can be downloaded via our website.

- **Required:** Postnatal Test Request Form
- **Optional:** Informed Consent for Genetic Testing

POTENTIAL TEST RESULTS:
Once a variant is confirmed, our laboratory team interprets this information in conjunction with the patient’s clinical findings and the scientific literature in order to classify a finding. There are three possible results:

- **A normal** result indicates that sequencing or deletion/duplication analysis of the genes analyzed did not find any pathogenic mutations or variants of uncertain clinical significance (or no clinically-significant chromosome anomalies were identified by microarray analysis).

- **An abnormal (or pathogenic)** result indicates that a pathogenic mutation was identified (or microarray analysis identified a genomic dosage anomaly [deletion or duplication] or ROH that likely provides an explanation for the individual’s clinical findings). Any available information regarding the phenotype associated with that mutation will accompany the technical details on the report.

- In some cases, the clinical significance of an identified sequence variant (or chromosomal anomaly detected by microarray) may not be well understood. These variants (anomalies) will be reported as **variants of uncertain clinical significance (UCS)**. Any available information about the molecular characteristics of the genetic change and the relationship of the genetic change to phenotype will be included on the report. Over time, as more patients are reported, a variant of uncertain clinical significance may be revised to an informative result, and a revised report will be generated.

- Parental testing may be recommended in order to classify the result as **de novo** or familial for the purpose of recurrence risk calculation.

TURNAROUND TIMES: For all sample types, results are typically available in 4 - 5 weeks.

BILLING: Our laboratory offers patient/self-pay, insurance (including Medicare/Medicaid), and client/institution billing options. Verifying coverage requirements or obtaining preauthorization PRIOR TO OR AT THE TIME OF SPECIMEN COLLECTION is often necessary. We provide preauthorization services upon request by calling 402-559-5070 (option 3); the following form is helpful for obtaining the information required by insurance providers and can be downloaded via our website.

- Insurance Preauthorization Request

In some circumstances, a test may be warranted even though insurance coverage is denied or not guaranteed. For these situations, we request the following form be signed by the patient and submitted with the sample. This helps inform patients of their potential financial responsibility, should the costs of genetic testing not be paid by their insurance provider.

- Advanced Beneficiary Notice of Noncoverage (ABN) – required when billing Medicare

CPT CODES:

- **High Density SNP Microarray Analysis:** 81229, 88230
- **Next Generation Sequencing:** 81407
- **Targeted Deletion/Duplication Analysis:** 81405
- **Methylation Analysis (of chromosome 15):** 81331

PRICING: For current costs contact the laboratory billing staff at 402-559-5070 (option 3).
GENE LIST: (version 2_16)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARX</td>
<td>EHMT1</td>
<td>MEF2C</td>
<td>SLC9A6</td>
</tr>
<tr>
<td>ATRX</td>
<td>FOXG1</td>
<td>NRXN1</td>
<td>TCF4</td>
</tr>
<tr>
<td>CDKL5</td>
<td>MBD5</td>
<td>PCDH19</td>
<td>UBE3A</td>
</tr>
<tr>
<td>CNTNAP2</td>
<td>MECP2</td>
<td>PNKP</td>
<td>ZEB2</td>
</tr>
</tbody>
</table>

REFERENCES:


updated 1-1-2019