Older patients may experience obesity, also exhibit wide mouths with unusual tongue/mouthing behaviors, hypopigmentation, and abnormal sleep cycles noted in over 80% of patients include microcephaly, seizures, and a specific, abnormal EEG pattern (1). Additional features include scoliosis, epilepsy, poor growth, and irregular breathing. There is broad clinical variability in the severity of Rett syndrome, including variants of Rett syndrome which may be associated with atypical features compared to the classic phenotype. Classic Rett syndrome primarily affects females.

Angelman syndrome [OMIM #105830] is characterized by functionally severe developmental delay or intellectual disability, movement or balance disorders of variable severity, behavioral uniqueness exemplified by apparent happy demeanor (frequent laughing/smiling) and easy excitability, and severe speech impairment (2). Other characteristics noted in over 80% of patients include microcephaly, seizures, and a specific, abnormal EEG pattern (2). Patients may also exhibit wide mouths with unusual tongue/mouthing behaviors, hypopigmentation, and abnormal sleep-wake cycles. Older patients may experience obesity (2).

Clinical Features:
Angelman and Rett syndrome are neurodevelopmental disorders with significant phenotypic overlap. Classic Rett syndrome [OMIM#312750] is a progressive disorder characterized by acquired microcephaly, loss of purposeful hand movements, and autistic behaviors, following a period of normal growth and development (1). Additional features include scoliosis, epilepsy, poor growth, and irregular breathing. There is broad clinical variability in the severity of Rett syndrome, including variants of Rett syndrome which may be associated with atypical features compared to the classic phenotype. Classic Rett syndrome primarily affects females.

Angelman syndrome [OMIM #105830] is characterized by functionally severe developmental delay or intellectual disability, movement or balance disorders of variable severity, behavioral uniqueness exemplified by apparent happy demeanor (frequent laughing/smiling) and easy excitability, and severe speech impairment (2). Other characteristics noted in over 80% of patients include microcephaly, seizures, and a specific, abnormal EEG pattern (2). Patients may also exhibit wide mouths with unusual tongue/mouthing behaviors, hypopigmentation, and abnormal sleep-wake cycles. Older patients may experience obesity (2).

Our Rett/Angelman Deletion/Duplication Panel includes deletion/duplication analysis of all 19 genes listed below in bold.

Our Rett/Angelman Sequencing Panel includes sequence analysis of all 23 genes listed below.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Clinical Features, Molecular Pathology and Inheritance</th>
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<tbody>
<tr>
<td>ADSL</td>
<td>Mutations in ADSL cause adenylosuccinate lyase deficiency, a metabolic disorder that results in the accumulation of succinylpurines. Affected individuals can exhibit significant overlap with Angelman syndrome, with features including global developmental delays with severe speech deficits, seizures, happy disposition, and hyperactivity (3). Affected individuals have an abnormal ratio of succinyladenosine (S-Ado) and succinylaminimidazole carboxamide riboside (SAICAr) (4).</td>
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<tr>
<td>ARX</td>
<td>Mutations in the ARX gene have been identified in patients with a range of phenotypes, including patients with cryptogenic infantile spasms (5), and patients with X-linked mental retardation (MRX) (6). ARX encodes a transcription factor expressed primarily in fetal and adult brain and skeletal muscle and is important for the maintenance of specific neuronal subtypes in the cerebral cortex. Mutations in ARX are inherited in an X-linked pattern. Carrier females can be asymptomatic.</td>
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<tr>
<td>ATRX</td>
<td>Mutations in ATRX are associated with a wide and clinically heterogeneous spectrum of X-linked mental retardation syndromes (7). Clinical features may include intellectual disability, hypotonia, genital abnormalities, short stature and seizures. Affected individuals may have a microcytic hypochromic anemia characteristic of alpha-thalassemia, however many do not (7). The ATRX gene appears to play a role in chromatin remodeling, and possibly silences expression of certain genes during development (7). Carrier females are typically not affected (7).</td>
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<tr>
<td>CDKL5</td>
<td>CDKL5 mutations have been demonstrated in a broad spectrum of phenotypes including atypical Rett syndrome with infantile spasms (8). Archer et al (2006) identified CDKL5 mutations in 7/42 (17%) of females with severe mental retardation and seizures in the first 6 months of life (9). CDKL5 mutations have been reported in more female than male patients, however, Elia et al (2008) reported CDKL5 mutations in 3/8 boys with severe mental retardation and early-onset seizures (10). CDKL5 contains a serine/threonine kinase domain and is implicated in MeCP2 modification in vitro (11). CDKL5 mutations are typically de novo, however one case of gonadal mosaicism has been reported (11).</td>
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<table>
<thead>
<tr>
<th>Gene</th>
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<tr>
<td>CNTNAP2</td>
<td>Homozygous or compound heterozygous mutations in CNTNAP2 have been described in patients with severe intellectual disability and epilepsy (12). The phenotype is described as overlapping with Pitt-Hopkins syndrome, a condition which also overlaps with Angelman syndrome (12). CNTNAP2 encodes a neuronal cell adhesion molecule, and heterozygous variants in this gene have been associated with susceptibility to a broad spectrum of neuropsychiatric disorders such as epilepsy, schizophrenia and autism spectrum disorder (12).</td>
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<tr>
<td>EHMT1</td>
<td>EHMT1 mutations are associated with Kleefstra syndrome, features of which include severe intellectual disability, hypotonia, brachymicrocephaly, seizures, dysmorphic facial features and congenital heart defects (13). The EHMT protein is one of the components of the E2F6 complex, which represses transcription. Mutations in EHMT1 are typically de novo and autosomal dominant.</td>
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<tr>
<td>PCDH19</td>
<td>The PCDH19 gene encodes a member of the dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family and participates in various cellular processes. It is a highly conserved gene located in the so-called Down Syndrome critical region (DSCR), a part of chromosome 21 that is responsible for the majority of phenotypic features in Down syndrome (14). Both PCDH19 mutations (15) and deletions (14) have been identified in patients with microcephaly and intellectual disability. Mutations in PCDH19 are autosomal dominant and typically de novo.</td>
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<tr>
<td>NAP2</td>
<td>Mutations in NAP2 result in brain-specific folate deficiency early in life, which results in severe developmental regression, movement disturbances, seizures and leukodystrophy (16). NAP2 encodes for folate-binding protein, and treatment with folate can improve symptoms. NAP2 mutations are inherited in an autosomal recessive manner.</td>
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<tr>
<td>MBD5</td>
<td>MBD5 mutations, or disruption due to translocations, are associated with severe intellectual disability, seizures, language impairment, behavioral problems and dysmorphic facial features. Like MECP2, MBD5 belongs to a family of genes involved in DNA methylation or chromatin remodeling. Mutations in MBD5 are inherited in an autosomal dominant manner.</td>
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<tr>
<td>MECP2</td>
<td>MECP2 mutations are present in 70-90% of females with classic Rett syndrome and approximately 20% of males with atypical Rett syndrome (1). Partial deletions of MECP2 are found in approximately 16% of girls with classic or atypical Rett syndrome. In addition, Watson et al. (2001) detected MECP2 mutations in 5 out of 47 patients with a clinical diagnosis of Angelman (19). MECP2 is an X-linked gene that has two functional domains that are involved in gene silencing and transcriptional repression. MECP2 mutations appear to be more common in females than in males, and the majority of cases are de novo. There have been reports of unaffected or mildly affected MECP2 carrier females due to skewed X inactivation.</td>
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<tr>
<td>NRXN1</td>
<td>Zweier et al. (2009) identified compound heterozygous mutations in a patient with severe intellectual disability, breathing abnormalities and dysmorphic facial features. The phenotype was described as similar to Pitt-Hopkins syndrome, which has phenotypic overlap with Angelman syndrome. Heterozygous copy number variants and SNPs in NRXN1 have been associated with susceptibility to a wide spectrum of neuropsychiatric disorders such as developmental language disorders, epilepsy and schizophrenia (21). NRXN1 encodes a presynaptic transmembrane protein which plays an important role in synaptic function (21).</td>
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<tr>
<td>OPHN1</td>
<td>Patients with OPHN1 mutations typically present with moderate to severe mental retardation, cerebellar hypoplasia and dysmorphic facial features (22). Hypotonia and developmental delay are noticed in most patients in early childhood, who then develop moderate to severe mental retardation. About half of all patients experience seizures. The protein encoded for by the OPHN1 gene is postulated to affect cell migration and outgrowth of axons and dendrites (23). Mutations in OPHN1 are inherited in an X-linked pattern and result in clinical features in affected males and females. Males are more severely affected than females (22).</td>
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<tr>
<td>PCDH19</td>
<td>Marini et al. (2010) identified 13 different mutations in the PCDH19 gene in 13 (11%) of 117 female patients with febrile seizures and a wide spectrum of epilepsy phenotypes. The PCDH19 gene encodes for protocadherin-19, which has been found to be expressed in the central nervous system, suggesting a role in cognitive function. PCDH19 mutations are X-linked, with the phenotype being restricted to females. Males with hemizygous mutations are apparently unaffected with normal cognitive functions.</td>
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<tr>
<td>PNKP</td>
<td>Mutations in the PNKP gene are associated with early-onset intractable epilepsy, microcephaly, developmental delay and behavioral abnormalities (25). Both homozygous and compound heterozygous mutations have been reported. The PNKP protein is involved in DNA repair of both double and single-stranded breaks. At this time no features typically associated with DNA repair defects, such as cancer predisposition or immunological abnormalities have been reported in affected individuals (25).</td>
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SLC2A1 [OMIM#606777] | SLC2A1 mutations are associated with drug-resistant infantile-onset seizures, developmental delay, acquired microcephaly, hypotonia, spasticity, ataxia and dystonia (26). Seizures are typically refractory and worsen during periods of fasting. Mutations in the SLC2A1 gene lead to impaired glucose transport in the brain. The majority of reported cases are due to de novo mutations (27).

SLC9A6 [OMIM#300231] | Mutations of the SLC9A6 gene have been identified in patients with X-linked mental retardation with features similar to Angelman syndrome (28). Males with SLC9A6 mutations typically have developmental delay, ataxia, flexed arms, excessive drooling, happy demeanor with spontaneous smiling and laughter, and progressive microcephaly by two years of age (28). It is thought that the SLC9A6 protein product is important for sodium/hydrogen exchange as well as normal mitochondrial function (29). Mutations in SLC9A6 result in clinical features in affected males and occasionally some mild features in carrier females.

TCF4 [OMIM#602272] | Mutations in TCF4 are associated with Pitt-Hopkins syndrome (PHS), which has phenotypic overlap with Angelman syndrome. PHS is characterized by severe mental retardation and dysmorphic facial features, which tend to coarsen with age (30). Other common features include hyperventilation phenotypes, epilepsy, acquired microcephaly, short stature, stereotypic hand movements, and absent speech. De Pontual et al [2009] detected TCF4 mutations in 13 of 36 patients with PHS, some of whom had previously been investigated for Angelman, Mowat-Wilson or Rett syndrome (30). TCF4 is thought to be specifically required for brain development, and has a role in pontine neuron differentiation (30). All reported cases of TCF4 mutations are due to de novo mutations, with the exception of one case of maternal mosaicism (30).

TRAPPC9 [OMIM#611966] | Marangi et al. (2013) concluded that the phenotype associated with loss-of-function mutations in the TRAPPC9 gene is recognizable and can be characterized by dysmorphic facial appearance, obesity, hypotonia, moderate to severe intellectual disability, and consistent brain abnormalities (31). Trappc9 is highly expressed in the mouse brain (32). TRAPPC9 is inherited in an autosomal recessive manner, and all mutations reported to date have been homozygous and protein truncating.

UBE3A [OMIM#601623] | The majority of cases of Angelman syndrome are associated with abnormal methylation patterns in the 15q11-q13 region, which can be caused by multiple mechanisms. Up to 50% of all patients with a classic Angelman syndrome phenotype and a normal methylation pattern on chromosome 15 have a mutation in UBE3A, which is located at 15q11.2 (33). The UBE3A gene is active on the maternal allele, and an absence of UBE3A expression is thought to be the basis of the large majority of Angelman syndrome cases, including those associated with abnormal methylation patterns. Most mutations in UBE3A are de novo, with a <1% recurrence rate, yet some cases may be familial. Mutations inherited maternally will result in Angelman syndrome; daughters inheriting Angelman syndrome-associated mutations from their fathers are at risk to have children with Angelman syndrome. Germline mosaicism of a UBE3A mutation has been reported (34).

WDR45 [OMIM#30056] | Mutations in WDR45 are associated with X-linked neurodegeneration with brain iron accumulation (NBA), also known as beta-propeller protein-associated neurodegeneration (BPAN). Some of the features of this condition overlap with Rett syndrome, and include global developmental delay, progressive dystonia, and parkinsonism with iron accumulation in the brain detected on MRI (OMIM#300894). In a study by Hoffjan et al 2016, a de novo splice site mutation in WDR45 was identified in a female child with Rett-like features. The patient presented with febrile seizures, language delays, stereotypical hand movements including wringing and washing, and had no brain iron accumulation noted on MRI. A review of the literature from the same group identified several other cases where patients with Rett-like features were found to have a mutation in WDR45 via whole exome sequencing (35).

ZEB2 [OMIM#605802] | Mutations in ZEB2 are associated with Mowat-Wilson syndrome (MWS), the features of which overlap with Angelman syndrome. MWS is characterized by distinctive facial features, moderate-to-severe mental retardation, seizures and microcephaly (36). ZEB2 encodes a transcriptional corepressor which likely to have a crucial role in embryonic development (36). ZEB2 mutations are inherited in an autosomal dominant pattern and most cases are de novo. Germline mosaicism has been reported; recurrence risk for unaffected parents of an isolated case is approximately 2% (37).

**Test methods:**
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All novel and/or potentially pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Deletion/duplication analysis of the panel genes is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

**Rett/Angelman Syndrome Sequencing Panel (sequence analysis of 23 genes)**

| Sample specifications: | 3 to10 cc of blood in a purple top (EDTA) tube |
| Cost: | $4,400 |

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Rett/Angelman Syndrome Deletion/Duplication Panel (deletion/duplication analysis of 19 genes)

Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
Cost: $2,500
CPT codes: 81407
Turn-around time: 4-6 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

Results:
Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References: