THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Rett/Angelman Syndrome Panel

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 Sequencing Panel and Rett/Angelman Deletion/Duplication Panel include analysis of the 23 genes listed below.

Rett/Angelman Syndrome Panel			
ADSL	EHMT1	NRXN1	TCF4
ARX	FOLR1	OPHN1	TRAPPC9
ATRX	FOXG1	PCDH19	UBE3A
CDKL5	MBD5	PNKP	WDR45
CNTNAP2	MECP2	SLC2A1	ZEB2
DYRK1A	MEF2C	SLC9A6	

Gene	Clinical Features, Molecular Pathology and Inheritance
ADSL [OMIM#608222]	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 succinylaminoimidazole carboxamide riboside (SAICAr) (4).
ARX [OMIM#300382]	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.
ATRX [OMIM#300032]	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).
CDKL5 [OMIM#300203]	*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).

CNTNAP2 [OMIM#604569]	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).
EHMT1 [OMIM#607001]	*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.
DYRK1A [OMIM#60085]	The *DYRK1A* 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 *DYRK1A* mutations (15) and deletions (14) have been identified in patients with microcephaly and intellectual disability. Mutations in *DYRK1A* are autosomal dominant and typically *de novo*.
FOLR1 [OMIM#136430]	Mutations in *FOLR1* result in brain-specific folate deficiency early in life, which results in severe developmental regression, movement disturbances, seizures and leukodystrophy (16). *FOLR1* encodes for folate-binding protein, and treatment with folate can improve symptoms. *FOLR1* mutations are inherited in an autosomal recessive manner.
FOXG1 [OMIM#164874]	Abnormalities of the *FOXG1* gene have been identified in patients with the congenital variant of Rett syndrome (17). Patients with the congenital variant of Rett syndrome have features similar to classic Rett syndrome, however hypotonia and severe developmental delay starts in the first months of life (17). The *FOXG1* gene encodes a brain-specific transcriptional repressor. *FOXG1* mutations are inherited in an autosomal dominant pattern and are typically *de novo*.
MBD5 [OMIM#164874]	*MBD5* mutations, or disruption due to translocations, are associated with severe intellectual disability, seizures, language impairment, behavioral problems and dysmorphic facial features (18). Like *MECP2, MBD5* belongs to a family of genes involved in DNA methylation or chromatin remodeling (18). Mutations in *MBD5* are inherited in an autosomal dominant manner.
MECP2 [OMIM#300005]	*MECP2* mutations are present in 70-90% of females with classic Rett syndrome and approximately 20% of females with atypical Rett syndrome (1). Partial deletions of *MECP2* are found in approximately 16% of girls with classic or atypical Rett syndrome (1). 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.
MEF2C [OMIM#600662]	Mutations of the *MEF2C* gene have been identified in patients with severe mental retardation, stereotypic movements, hypotonia, and epilepsy [14]. Phenotypic overlap exists between patients with *MEF2C* mutations and atypical Rett syndrome (20). The *MEF2C* gene plays an important role in the development and maintenance of multiple organ systems, and phenotype overlap of *MEF2C* and atypical Rett syndrome is probably due to the involvement of a common pathway (20). *MEF2C* mutations are inherited in an autosomal dominant pattern and are typically *de novo*.
NRXN1 [OMIM#600565]	Zweier *et al.* (2009) identified compound heterozygous mutations in a patient with severe intellectual disability, breathing abnormalities and dysmorphic facial features (21). 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).
OPHN1 [OMIM#300127]	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).
PCDH19 [OMIM#300088]	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 (24). 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.
PNKP [OMIM#613402]	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).

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 [OMOM#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 episodes, 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 (NBIA), 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 pathogenic and likely 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:	$2,500
CPT codes:	81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for this panel.

Note: For patients with a classical Angelman syndrome phenotype, methylation studies of the 15q11-q13 region are recommended as the initial test. Please see our website for more details.

Rett/Angelman Syndrome Deletion/Duplication Panel (deletion/duplication analysis of 23 genes)

Sample specifications:	3 to10 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.

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