Rett/Angelman Syndromes and Related Disorders Panel Sequence Analysis and Exon-Level Deletion/Duplication Testing Of 20 Genes

Panel Gene List: ATRX, CDKL5, CNTNAP2, CTNNB1, DDX3X, FOXG1*, GABBR2, KCNA2, MBD5, MECP2, MEF2C, NRXN1, SATB2, SLC9A6, STXBP1, TBL1XR1, TCF4, UBE3A, WDR45, ZEB2

*This panel does not include deletion/duplication testing of FOXG1

Clinical Features:

The Rett/Angelman Syndrome and Related Disorders Panel at GeneDx includes genes that cause disorders with overlapping clinical phenotypes including epilepsy, developmental delay and/or regression, movement disorders, and intellectual disability. A brief summary of some of the clinical disorders is included below.

Rett syndrome is a progressive neurodevelopmental disorder that primarily affects females. Classic Rett syndrome is characterized by apparently normal development in the first 6-18 months followed by an arrest in development and subsequent regression in language and motor skills. Frequent symptoms include loss of speech and purposeful hand use, stereotypic hand movements, ataxia, microcephaly, and seizures. Multiple forms of atypical (variant) Rett syndrome have been described, including early-onset Rett syndrome with seizures beginning before six months, forme fruste Rett syndrome with a milder and more slowly progressive course, and the preserved speech variant with a milder clinical phenotype including retained verbal abilities and hand use.[1] Congenital Rett syndrome is a severe congenital encephalopathy that affects males and females and is characterized by hypotonia, irritability, and unresponsiveness in the neonatal period with seizures, progressive microcephaly and developmental delay/regression noted in the first few months of life.[2,3]

Angelman syndrome (AS) is characterized by developmental delay with absent or significantly impaired speech, intellectual disability, ataxia, microcephaly, a characteristic EEG pattern, and a typical behavioral pattern including a happy personality with outbursts of laughter, hyperactivity, excitability, sleep problems, and hand-flapping movements.[4,5] Facial features often observed in individuals with AS include a prominent chin, a wide mouth with a protruding tongue. Angelman-like (Christianson) syndrome is characterized by intellectual disability, ataxia, severe speech and language impairment, epilepsy, and microcephaly in males.[6,7,8]

Pitt-Hopkins syndrome (PHS) is characterized by severe intellectual disability with absent or severely delayed speech that is often associated with breathing abnormalities, stereotypic

movements, seizures, microcephaly and a characteristic facial gestalt consisting of coarse facial features, deep-set eyes, a broad or beaked nasal bridge, a large mouth with a tented upper lip and widely spaced teeth, and cup-shaped ears with a thick helix.[9,10,11] Ataxia, short stature, constipation, gastroesophageal reflux, strabismus, and nonspecific abnormalities on brain MRI have also been described.[9,10,11]

Mowat-Wilson syndrome (MWS) is characterized by severe expressive language delay, seizures, moderate to severe intellectual disability, and a typical facial gestalt consisting of uplifted earlobes with a central depression, broad and medially thick eyebrows, hypertelorism, downslanting palpebral fissures, an open-mouthed expression, and an elongated face with prognathism.[12,13] Hirschsprung disease (HSCR), congenital heart defects, microcephaly, ataxia, urogenital anomalies, short stature, structural eye anomalies, and hypoplasia/agenesis of the corpus callosum have also been described in more than half of patients with MWS.[12]

Inheritance Pattern/Genetics:
The Rett/Angelman Syndrome and Related Disorders Panel includes sequencing and deletion/duplication analysis of 20 genes and methylation-specific multiple ligation probe amplification (MS-MLPA) analysis of the UBE3A gene to also evaluate for paternal UPD (3%-7% of AS) and imprinting errors (~3% of AS) causing Angelman syndrome. The disorders on this panel are inherited in an autosomal dominant, autosomal recessive, or X-linked manner. The genes on this panel are highly expressed in the developing nervous system and encode proteins that are involved in transcriptional activation or repression, are implicated in cell recognition and adhesion, or are involved in regulation of endosomal membranes and long-term potentiation.

Test Methods:
Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions of the genes on this panel are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be > 99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. For the FOXG1 gene(s), sequencing but not deletion/duplication analysis, is performed. Additionally, MS-MLPA is performed to evaluate for abnormal methylation of the UBE3A gene. If indicated, multiplex ligation-dependent probe amplification (MLPA) of the FOXG1 gene is available as a separate test (test code 904).

If clinically appropriate and the Rett/Angelman and Related Disorders Panel is negative, sequencing and deletion/duplication analysis of the remaining genes on the Comprehensive Epilepsy Panel is available as a separate test.

Test Sensitivity:
The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in the Rett/Angelman Syndrome and Related Disorders depends in part on the patient's clinical phenotype. Specific information about the diagnostic yield for each gene in selected populations is summarized in the table below.

Disorder	Gene	Protein	Inh	Diagnostic Yield in Selected Population(s)
Rett/atypical Rett syndrome	*MECP2*	Methyl CpG binding protein 2	XL	88% females with Rett syndrome[13]
	CDKL5	Cyclin-dependent kinase-like 5	XL	2-8% females with atypical Rett syndrome[14,15]
	CTNNB1	Catenin beta 1	AD	Rare in Rett-like syndromes[29]
	DDX3X	DEAD-box helicase 3, X-linked	XL	Rare in Rett-like syndromes[30]
	*FOXG1**	Forkhead box protein G1	AD	~1% Rett syndrome overall[16;] 25% congenital variant of Rett[17]
	GABBR2	Gamma-aminobutyric acid type B receptor subunit 2	AD	Rare in Rett-like syndromes[31]
	KCNA2	Potassium voltage-gated channel subfamily A member 2	AD	Rare in Rett-like syndromes[32]
	MBD5	Methyl-CpG-binding domain protein 5	AD	Rare in Rett-like syndromes[26]
	MEF2C	Myocyte-specific enhancer factor 2C	AD	Rare in Rett-like syndromes[23]
	SATB2	SATB homeobox 2	AD	Rare in Rett-like syndromes[33]

Disorder	Gene	Protein	Inh	Diagnostic Yield in Selected Population(s)
	STXBP1	Syntaxin binding protein 1	AD	Rare in Rett-like syndromes[34]
	TBL1XR1	Transducin (beta)-like 1 X-linked receptor 1	AD	Rare in Rett-like syndromes[35]
	WDR45	WD repeat domain 45	XL	Rare in Rett-like syndromes[27,28]
Angelman/ Angelman-like syndromes	*UBE3A*	Ubiquitin protein ligase E3A	AD	78% Angelman syndrome by methylation[18]; 11% Angelman syndrome by sequencing of UBE3A[18]
	SLC9A6	Sodium/hydrogen exchanger 6	XL	6% Angelman-like syndrome[7]
	TCF4	Transcription factor 4	AD	2% Angelman syndrome[19]
	MBD5	Methyl-CpG-binding domain protein 5	AD	Rare in Angelman-like syndrome[25]
Pitt-Hopkins syndrome (PHS)	*TCF4*	Transcription factor 4	AD	36% PHS[19]
	NRXN1	Neurexin-1	AR	Rare in PHS[20]
	CNTNAP2	Contactin-associated protein-like 2	AR	Rare in PHS[20]
Mowat-Wilson syndrome	*ZEB2*	Zinc finger E-box-binding homeobox 2	AD	95% Mowat Wilson syndrome[21,22]
Alpha-thalassemia X-linked intellectual disability	*ATRX*	Transcriptional regulator ATRX	XL	95% males with alpha-thalassemia X-linked intellectual disability[36]

** Does not include deletion/duplication testing of FOXG1

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