GeneDx
DNA Diagnostic Services

Test Information Sheet

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Whole Genome CGH+SNP Array, Methylation Analysis, *UBE3A* Gene Analysis for Angelman Syndrome

SLC9A6 Gene Analysis for Angelman-Like (Christianson) Syndrome / X-Linked Intellectual Disability

Mendelian Inheritance in Man Numbers: 105830 - Angelman Syndrome (AS); 601623 - *UBE3A* gene; 300243-Mental Retardation, X-Linked, Syndromic, Christianson Type; 300231 - *SLC9A6* gene

***Clinical features*:** Angelman syndrome (AS) is a neurological disorder affecting development and behavior. Individuals with Angelman syndrome exhibit developmental and cognitive delays typically noted in the first year of life, including absent or significantly impaired speech. Neurological features include seizures, ataxia, and characteristic electroencephalogram (EEG) abnormalities. Characteristic behavioral features include sleep disorders and a happy demeanor with recurrent laughter, smiling, and excitability. Individuals with AS are typically noted to have prominent chin, small head circumference and a wide mouth with protruding tongue. The presence and severity of the clinical features can vary among individuals with AS. [1]

Males with Angelman-like (Christianson) syndrome may exhibit many clinical features suggestive of Angelman syndrome such as mental retardation, ataxia, severe speech and language impairment, a happy demeanor with frequent smiling or spontaneous laughter, epilepsy, and microcephaly.[2,3] Mutations in the SLC9A6 gene have also been identified in families with nonsyndromic X-linked intellectual disability.[10,11]

***Inheritance pattern*:** AS is an imprinting disorder caused by one of four known mechanisms that result in absence of expression of the UBE3A gene on the maternally derived chromosome 15 within 15q11.2-q13.1. The majority of cases are de novo with a recurrence risk of <1%; however, the recurrence is 50% for an inherited imprinting center deletion, a maternally inherited *UBE3A* mutation or partial deletion, or for microdeletions inherited from a mother with a balanced chromosome rearrangement.[4]

Angelman-like syndrome is an X-linked disorder that affects males. Most females who are heterozygous carriers of SLC9A6 mutations have normal intelligence, although some carrier females have been reported with learning disabilities and/or mild behavioral abnormalities. [2,12]

Genetics: Angelman syndrome is caused by loss of *UBE3A* gene expression on the maternal copy of chromosome 15q11.2 due to a microdeletion, uniparental disomy (UPD), an imprinting error, or a *UBE3A* gene mutation or deletion. The majority (65-75%) of patients with AS have a large recurrent microdeletion extending from 15q11.2 to 15q13.1 on the maternally inherited chromosome. [4] Paternal UPD accounts for 3-7% of patients with AS.[4] Approximately 3% of patients have an imprinting error that establishes a paternal chromosome-specific methylation pattern despite the presence of both parental alleles, and these imprinting errors can be caused by a microdeletion within the imprinting center in 15q11.2 (0.5% of all AS cases) or by an unknown mechanism that inappropriately silences genes regardless of the parental origin of the chromosome (2.5% of all AS cases). [1,2,4] *UBE3A* mutations detectable by sequencing are responsible for 5-11% of AS cases, while rare patients have been reported to harbor a partial deletion of the *UBE3A* gene.[4] The etiology of the remaining AS cases is unknown; however, the differential diagnosis of AS includes Angelman-like (Christianson) syndrome, a disorder caused by mutations in the *SLC9A6* gene located on chromosome Xq26.3.[4]

Genes/proteins:
The *UBE3A* gene encodes an E3 ligase in the ubiquitin proteasome pathway and functions as a transcriptional coactivator. *UBE3A* is imprinted in many but not all brain tissues, underscoring its importance in neurological development. The SLC9A6 gene consists of 16 exons and encodes the Na^+/H^+ exchanger protein NHE6. This protein plays a role in regulating endosomal membranes and is implicated in long-term potentiation (LTP), the molecular basis of learning and memory.[2]

***Reasons for referral*:**
1. Confirmation of a clinical diagnosis
2. Targeted testing for a known familial mutation
3. Genetic counseling
4. Prenatal diagnosis for known familial mutations in the UBE3A or SLC9A6 genes.
5. Prenatal diagnosis by targeted array CGH for deletions or UPD (methylation analysis not available prenatally)

GeneDx test methods and sensitivity for Angelman syndrome:

Test	Abnormalities Identified	Detection Rate	Comments
Whole genome CGH+SNP array	- common 15q11.2-15q13.1 deletion - UPD - imprinting center deletions - intragenic UBE3A deletion/duplication	~76% AS	can detect other genomic imbalances with features overlapping with AS
Methylation-specific MLPA (MS-MLPA)	- common 15q11.2-15q13.1 deletion - paternal UPD - all types of imprinting abnormalities	~78% AS	can confirm AS diagnosis and determine mechanism
UBE3A Sequencing	- intragenic mutations in UBE3A gene	5-11% AS	for patients with clinical suspicion of AS and normal whole genome CGH+SNP array OR MS-MLPA
UBE3A Del/Dup Testing	- intragenic deletions/duplications of an exon or larger in UBE3A gene	Rare in AS	not necessary if whole genome CGH+SNP array done at GeneDx
SLC9A6 Sequencing	- intragenic mutations in SLC9A6 gene	~6% AS-like	for males with clinical features of AS and no detectable UBE3A abnormalities
Rett/Angelman Syndrome Panel	- intragenic mutations, deletions, and duplications of UBE3A, SLC9A6, and 9 other genes	See website	cannot detect UPD or imprinting abnormalities

Whole-genome CGH+SNP array is available to detect the common 15q11.2-15q13.1 deletion, paternal UPD, imprinting center deletions (but not other types of imprinting errors), and intragenic deletions or duplications of the *UBE3A* gene. Together, these four causes account for an estimated 76% of patients with AS. The array contains 118,000 oligonucleotide probes for detection of copy number variants (CNVs) in the unique sequence of the genome and can therefore identify other genomic imbalances that may produce an AS-like phenotype (e.g., 2q23 or 17q21.31 deletions). In addition, it contains 66,000 SNP probes throughout the genome and can detect stretches of homozygosity extending 5 Mb or longer.

Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) can determine the parent-specific methylation imprint. An abnormal MS-MLPA result can confirm a diagnosis of AS and reveal the mechanism (deletion, UPD, or imprinting error). Methylation analysis is available as reflex testing following normal array results for individuals with a high suspicion of AS to identify UPD or imprinting errors, or as a first-line test for patients with a suspected diagnosis of AS. GeneDx does not offer imprinting center sequence analysis. However, patients with abnormal methylation studies but normal whole-genome CGH+SNP array are assumed to have an imprinting error.

UBE3A and *SLC9A6* gene sequencing are offered as separate tests or are also available as part of a panel of genes causing Rett/Angelman syndrome and other related disorders. Using genomic DNA obtained from the submitted specimens, bi-directional sequence analysis of the coding exons and corresponding intron/exon boundaries of UBE3A (exons 3-13) or SLC9A6 (exons 1-16) is performed. Mutations found in the first person of a family to be tested are confirmed by repeat analysis using sequencing, restriction fragment analysis or another appropriate method. Among patients with normal methylation patterns, FISH results, and UPD studies, UBE3A mutations are detected by DNA sequencing in 50-80% of familial cases and in 10-44% of de novo cases of AS.[1,5,6,7] Mutations in SLC9A6 are rare in patients with an Angelman-like phenotype, accounting for 4 out of 73 (~6%) probands with an AS-like presentation in one study.[2] An SLC9A6 mutation has also been reported in a family with nonsyndromic X-linked intellectual disability.[10]

UBE3A ExonArrayDx deletion/duplication testing is available to evaluate for an intragenic deletion or duplication of the *UBE3A* gene, which is a rare cause of AS.[12,13,14] This test is appropriate for patients with a high suspicion for AS who had normal methylation testing and *UBE3A* gene sequencing. Note that this testing is not necessary for patients having whole genome CGH+SNP array at GeneDx since the array includes exon-level coverage of the UBE3A gene.

The Rett/Angelman Syndrome Panel includes Next Generation sequencing and deletion/duplication analysis of a panel of genes causing Rett/Angelman syndrome and other related disorders with overlapping clinical phenotypes, including epilepsy, developmental delay and intellectual disability. Additional information about the panel is available on the website http://www.genedx.com/site/neurology

Mutation spectrum and Genotype-Phenotype Correlations:
The UBE3A gene consists of 13 exons with three alternatively spliced transcripts.[8] Most mutations are private and include missense and nonsense changes, small deletions/insertions, splice site alterations, and large deletions.[1,8,9] Mutations have been found throughout the coding region, with a somewhat higher concentration in exon 9 (typically reported as exon 6 using the naming convention that is common in the published literature).[9] Patients with Angelman syndrome who have large deletions have the most severe phenotype, with severe seizures, cognitive delays and an absence of speech.[8] Patients with AS due to paternal UPD and imprinting errors tend to have the least severe presentation, with a lower occurrence of seizures and better cognitive and speech development. Intermediate severity of the phenotype has been reported in patients with AS due to mutations in the UBE3A gene, who have better cognitive and speech development than those with a gene deletion.[8] Multiple mutations that introduce a premature stop codon and a few missense changes in the SLC9A6 gene have been reported in association with an Angelman-like syndrome. No partial or full gene deletions have been reported to date.[2,3,12]

Specimen Requirements and Shipping/Handling:

- *Blood*: A single tube with 1-5 mL whole blood in EDTA (1-2mL for infants). Ship overnight at ambient temperature, using a cool pack in hot weather. Specimens may be refrigerated for one week prior to shipping.
- *Buccal Brushes*: **Buccal brushes are not accepted for methylation analysis, whole-genome CGH+SNP array, UBE3A testing, or the Rett/Angelman syndrome panel.** For adults and children over 6 months, a GeneDx buccal kit (others not accepted) can be used as an alternative to blood for SLC9A6 testing. Submit by mail.
- *Prenatal Diagnosis:* For prenatal testing for a known mutation in the UBE3A or SLC9A6 genes, please refer to the specimen requirements table on our website at: http://www.genedx.com/test-catalog/prenatal/. Ship specimen overnight at ambient temperature, using a cool pack in hot weather.

Required Forms:

Sample Submission (Requisition) Form – complete all pages
Payment Options Form or Institutional Billing Instructions

Prices and Turn-Around Time - *Fees are subject to change without notice:*

Test # 910: Deletion and UPD testing by whole-genome CGH+SNP Array
Test # 566: Methylation Analysis by MS-MLPA
Test # 374: UBE3A sequence analysis in a new patient
Test # 906: UBE3A exon-level deletion/duplication testing
Test # 375: SLC9A6 sequence analysis in a new patient
Test # 337: Targeted testing for a known familial deletion or UPD by CGH+SNP array
Test # 901: Testing of a relative for one specific known mutation in UBE3A or SLC9A6
Test # 902: Prenatal diagnosis for a specific known mutation (including maternal cell contamination studies)

The Rett/Angelman Syndrome Panel is available as a separate test. Please see our website at http://www.genedx.com/neurology for additional information.

References*:* (1) Malzac, P., et al, (1998) Am J Hum Genet 62(6):1353-1360. (2) Gillfillan G et al., Am. J. Hum. Genet. 82(4): 1003-1010, 2008. (3) Christianson A et al. Am J. Hum Genet. 36:759-766, 1999. (4) Williams, C.A. and Dagli A.I. (Updated June 16, 2011). Angelman Syndrome. In: GeneReviews at GeneTests: Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997-2009. Available at http://www.genetests.org. Accessed January 8, 2012. (5) Hitchins, M.P., et al, (2004) Am J Med Genet 125A(2):167-172. (6) Lossie, A.C., et al, (2001) J Med Genet 38(12):834-845. (7) Fang, P., et al, (1991) Hum Molec Genet 8(1):129-135. (8) Clayton-Smith, J., et al (2003) J Med Genet 40(2):87-95. (9) Russo, S., et al, (2000) Hum Mutan 15(4):387-393. (10) Hu et al., (2009) HUGO J 3:41-49. (11) Whibley et al., (2010) Am J Hum Genet 87:173-188. (12) Schroer R., et al. Am J Med Genet. (2009) 152A:2775-2783. (13) Boyes L., et al. Eur J Med Genet. (2006) 49(6):472-80. (14) Beleza-Meireles et al., (2011) Eur J Med Genet 54:348-350. (15) Piard et al., (2011) Am J Med Genet 155:3170-3173.