

Angelman Syndrome: *UBE3A* Gene Sequencing

Test Code: SUBE3

Turnaround time: 6 weeks

CPT Codes: 81406 x1

Condition Description

Angelman syndrome (AS) is characterized by severe developmental delay or mental retardation, severe speech impairment, gait ataxia and/or tremulousness of the limbs, and a unique behavior with an inappropriate happy demeanor that includes frequent laughing, smiling, and excitability. Consensus criteria for the clinical diagnosis of AS have been developed in conjunction with the Scientific Advisory Committee of the US Angelman Syndrome Foundation. Newborns typically have a normal phenotype. Developmental delays are first noted at around six months of age. However, the unique clinical features of AS do not become manifest until after one year of age, and it can take several years before the correct clinical diagnosis is obvious.

Findings typically present in affected individuals:

- Normal prenatal and birth history, normal head circumference at birth, no major birth defects
- Normal metabolic, hematologic, and chemical laboratory profiles
- Structurally normal brain by MRI or CT, although mild cortical atrophy or dysmyelination may be observed
- Delayed attainment of developmental milestones without loss of skills
- Evidence of developmental delay by six to twelve months of age, eventually classified as severe
- Speech impairment, with minimal to no use of words; receptive language skills and nonverbal communication skills higher than expressive language skills
- Movement or balance disorder, usually ataxia of gait and/or tremulous movement of the limbs
- Behavioral uniqueness, including any combination of frequent laughter/smiling; apparent happy demeanor; excitability, often with hand-flapping movements; hypermotoric behavior; short attention span
- Delayed or disproportionately slow growth in head circumference, usually resulting in absolute or relative microcephaly by two years of age

- Seizures, usually starting before three years of age

- Abnormal EEG, with a characteristic pattern of large-amplitude slow-spike waves

The diagnosis of AS rests on a combination of clinical features and molecular genetic testing and/or cytogenetic analysis. Consensus clinical diagnostic criteria for AS have been developed. Analysis of parent-specific DNA methylation imprints in the 15q11.2-q13 chromosome region detects approximately 78% of individuals with AS, including those with a deletion, uniparental disomy (UPD), or an imprinting defect (ID); fewer than 1% of individuals have a cytogenetically visible chromosome rearrangement (i.e., translocation or inversion). *UBE3A* (15q11-q13) sequence analysis detects mutations in an additional approximately 11% of individuals. Accordingly, molecular genetic testing (methylation analysis and *UBE3A* sequence analysis) identifies alterations in approximately 90% of individuals. The remaining 10% of individuals with classic phenotypic features of AS have a presently unidentified genetic mechanism and thus are not amenable to diagnostic testing.

For diagnosis of a proband

- DNA methylation analysis identifies approximately 80% of individuals with AS and should be the first test ordered.
- If DNA methylation analysis is normal, *UBE3A* sequence analysis is the next appropriate diagnostic test.

AS is caused by the loss of the maternally imprinted contribution in the 15q11.2-q13 Angelman syndrome/Prader-Willi syndrome (AS/PWS) region that can occur by one of at least five different known genetic mechanisms. The risk to sibs of a proband depends on the genetic mechanism of the loss of the maternally contributed AS/PWS region: typically less than 1% for probands with a deletion or UPD; as high as 50% for probands with an ID or a mutation of *UBE3A*. Members of the mother's extended family are also at increased risk when an ID or a *UBE3A* mutation is present. Cytogenetically visible chromosome rearrangements may be inherited or de novo. Prenatal testing for pregnancies at increased risk is possible when the underlying genetic mechanism is a deletion, UPD, an ID, a *UBE3A* mutation, or a chromosome rearrangement.

The prevalence of AS is one in 12,000-20,000.

For patients with suspected Angelman syndrome, methylation analysis of the 15q11.2-q13 chromosome region is recommended as the first step in analysis. For patients in whom a methylation defect is not identified, full gene sequence analysis is appropriate. If sequence analysis is negative, deletion/duplication analysis is appropriate.

[Click here](#) for the GeneTests summary on this condition.

Indications

This test is indicated for:

- Confirmation of a clinical diagnosis of Angelman syndrome after methylation analysis of the 15q11.2-q13 chromosome region is negative

Methodology

PCR amplification of 10 exons contained in the *UBE3A* gene is performed on the patient's genomic DNA. Direct sequencing of amplification products is performed in both forward and reverse directions, using automated fluorescence dideoxy sequencing methods. The patient's gene sequences are then compared to a normal reference sequence. Sequence variations are classified as mutations, benign variants unrelated to disease, or variations of unknown clinical significance. Variants of unknown clinical significance may require further studies of the patient and/or family members. This assay does not interrogate the promoter region, deep intronic regions, or other regulatory elements, and does not detect large deletions.

Detection

Clinical Sensitivity: *UBE3A* sequence analysis detects mutations in approximately 11% of clinically diagnosed individuals. Mutations in the promoter region, some mutations in the introns and other regulatory element mutations cannot be detected by this analysis. Large deletions will not be detected by this analysis. Results of molecular analysis should be interpreted in the context of the patient's biochemical phenotype.

Analytical Sensitivity: ~99%

Specimen Requirements

Submit only 1 of the following specimen types

Type: Whole Blood (EDTA)

Specimen Requirements:

EDTA (Purple Top)

Infants and Young Children (2 years of age to 10 years old): 3-5 ml

Older Children & Adults: 5-10 ml

Autopsy: 2-3 ml unclotted cord or cardiac blood

Specimen Collection and Shipping:

Ship sample at room temperature for receipt at EGL within 72 hours of collection. Do not freeze.

Type: DNA, Isolated

Specimen Requirements:

Microtainer

8µg

Isolation using the Perkin Elmer™ Chemagen™ Chemagen™ Automated Extraction method or Qiagen™ Puregene kit for DNA extraction is recommended.

Specimen Collection and Shipping:

Refrigerate until time of shipment in 100 ng/µL in TE buffer. Ship sample at room temperature with overnight delivery.

Type: Saliva

Specimen Requirements:

Oragene™ Saliva Collection Kit

Oragene™ Saliva Collection Kit used according to manufacturer instructions. Please contact EGL for a Saliva Collection Kit for patients that cannot provide a blood sample.

Specimen Collection and Shipping:

Please do not refrigerate or freeze saliva sample. Please store and ship at room temperature.

Special Instructions

Submit copies of diagnostic biochemical test results with the sample, if appropriate. Contact the laboratory if further information is needed.

Methylation analysis of the 15q11.2-q13 chromosome region is required before *UBE3A* sequence analysis. If methylation analysis is performed outside of EGL Genetics, please submit a copy of the methylation report with the test requisition.

Sequence analysis is required before deletion/duplication analysis by targeted CGH array. If sequencing is performed outside of EGL Genetics, please submit a copy of the sequencing report with the test requisition.

Related Tests

- Methylation analysis of the 15q11.2-q13 chromosome region (test code PW listed under Prader-Willi/Angelman) detects approximately 78% of individuals with AS and is recommended before *UBE3A* analysis.
- Deletion/duplication analysis of the *UB3EA* gene by CGH array is available for those individuals in whom sequence analysis is negative.
- [Custom diagnostic mutation analysis \(KM\)](#) is available to family members if mutations are identified by targeted mutation testing or sequencing analysis.
- Prenatal testing is available to adults who are confirmed carriers of mutations. Please contact the laboratory genetic counselor to discuss appropriate testing prior to collecting a prenatal specimen.