

Angelman Syndrome: Methylation or *UBE3A* Sequencing

DNA TESTING TO CONFIRM A CLINICAL SUSPICION/ DIAGNOSIS OF ANGELMAN SYNDROME

Disease Overview

- Angelman syndrome (AS) is characterized by severe developmental delay or mental retardation, severe speech impairment, gait ataxia and/or tremulousness of the limbs, and unique behaviors with an inappropriately happy demeanor.
- At birth, newborns with AS have normal head circumference and no major birth defects.
- Infants with AS can present with feeding problems and generalized oral-motor incoordination followed by developmental delays at 6–12 months of age.
- Children with AS develop microcephaly by age 2, and seizures and characteristic EEG pattern by age 3.
- Speech impairment is severe, with little to no development of expressive language.
- Most children with AS learn to walk between 30 months and 6 years of age, but 10 percent remain non-ambulatory.
- Unique behaviors may include frequent laughter, happy demeanor, excitability, hand flapping, short attention span, sleep disturbances, and abnormal food-related behaviors.
- Individuals may have dysmorphic features such as flat occiput, occipital groove, wide mouth and protruding tongue, prognathism, and strabismus. Hypopigmented skin, as well as light hair and eye color relative to the family members, may be present.
- Affected adults require assistance living arrangements. Both men and women have normal fertility and near-normal life span.
- There is currently no effective treatment of AS. Management includes antiepileptic drugs to control seizures; orthotic braces or surgery for scoliosis and other orthopedic problems; safe nighttime confinement; physical, speech, and occupational therapy; behavioral modifications; individual educational plans; and weight control.

Epidemiology

Prevalence is approximately 1:15,000, with males and females equally affected.

Genetics

- Angelman syndrome is caused by lack of functional maternal copy of the *UBE3A* gene on chromosome 15q11.2-q13.
- Ubiquitin protein ligase (*UBE3A*), or E6-associated protein (E6-AP), is an E3 ligase that functions in the E3 complex of the ubiquitin cycle.
- *UBE3A*, which is expressed only from the maternal allele in fetal and adult brain prefrontal cortex neurons, may be regulated through paternally expressed antisense transcript. The *UBE3A* protein controls synaptic function by ubiquitinating and degrading the synaptic protein Arc.

- Disruption of degradation of a number of *UBE3A* substrates is thought to be responsible for the phenotypic effects of AS.
- The etiology of AS is as follows:
 - Maternal deletion involving 15q11.2-q13 (68 percent).
 - Paternal uniparental disomy for chromosome 15 (7 percent).
 - *UBE3A* mutation (11 percent).
 - Imprinting center defect (3 percent).
 - Cytogenetically visible chromosomal translocation (< 1 percent).
 - Presently unidentified genetic mechanism (10 percent).
- Determining the molecular mechanism responsible for AS is important for accurate genetic counseling regarding recurrence risk.
- Inheritance varies depending upon the molecular genetic mechanism. *UBE3A* mutations identified by sequencing may be maternally inherited or de novo.
- Offspring of a female carrier of a *UBE3A* sequence mutation are at 50 percent risk for AS.
- A few individuals with AS have been found to have complete or partial *UBE3A* gene deletions.
- Mosaicism for germline *UBE3A* mutations has been reported. As molecular testing cannot exclude maternal germline mosaicism, prenatal testing for the familial *UBE3A* mutation should be offered in subsequent pregnancies to all females who have a child with AS.

Indications for Ordering

- Angelman syndrome by methylation:
 - To establish a diagnosis of AS in individuals with clinical symptoms.
 - DNA methylation analysis identifies approximately 78 percent of individuals with AS and is the most sensitive diagnostic test.
- Angelman syndrome *UBE3A* sequencing:
 - Individuals with clinical symptoms of AS and normal DNA methylation results should undergo *UBE3A* gene mutation analysis.
 - *UBE3A* sequencing identifies approximately 11 percent of individuals with AS.
 - For optimal test interpretation, provide information regarding patient symptoms/manifestations and family history of AS.

Contraindication

- Testing for individuals with a previously identified familial *UBE3A* mutation.
- To test individuals for a specific sequence mutation, it is more cost-effective to order Familial Mutation, Targeted Sequencing (ARUP test code 2001961). A copy of the laboratory report detailing the familial mutation must be provided for targeted sequencing.

Interpretation

- Angelman syndrome by methylation:
- Unaffected individuals have a methylated, maternally inherited and an unmethylated, paternally inherited allele detectable by methylation-specific PCR. Absence of the methylated maternal allele is indicative of AS.
 - An abnormal methylation result should be followed by FISH or array CGH to determine if a deletion is present. If a deletion is present, chromosome analysis should be performed to exclude a chromosome rearrangement that may alter recurrence risk.
 - If FISH analysis is normal, DNA polymorphism analysis should be performed to distinguish between paternal UPD and an imprinting defect.
 - If there is no UPD, further DNA studies can determine if an imprinting center deletion is present.
 - Parental testing may be indicated to determine if chromosomal deletions, chromosomal rearrangements, or gene mutations are de novo.
- Angelman syndrome *UBE3A* sequencing:
 - Identification of a known pathogenic *UBE3A* mutation in a symptomatic individual confirms a diagnosis of AS.
 - Lack of an identifiable *UBE3A* mutation in a clinically affected individual reduces but does not rule out AS due to the possibility of an undetectable *UBE3A* mutation or another causative genetic mechanism that was not tested.

Methodology and Limitations

- Angelman syndrome by methylation:
 - Bisulfate conversion and PCR amplification to detect methylation using melting-curve analysis.
 - Other molecular mechanisms resulting in AS will not be assessed.

- Angelman syndrome *UBE3A* sequencing:
 - Bidirectional sequencing of the entire *UBE3A* coding region and intron-exon borders.
 - Analytical sensitivity and specificity of sequencing are 99 percent.
 - Clinical sensitivity of *UBE3A* sequencing is 11 percent for AS syndrome.
 - Rare diagnostic errors can occur due to primer-site mutations.
 - *UBE3A* regulatory region mutations, deep intronic mutations, and large deletion/duplications will not be detected.
 - *UBE3A* variants of unknown clinical significance may be detected by sequencing.

Related Tests

- Chromosome FISH, Metaphase (Angelman Syndrome) ([2002299](#))
- Cytogenomic SNP Microarray ([2003414](#))
- Rett Syndrome (*MECP2*), Full Gene Analysis ([0051614](#))

References

1. Chamberlain SJ, Lalande M. Angelman syndrome, a genomic imprinting disorder of the brain. *J Neurosci* 2010;30:9958–63.
2. GeneTests. <http://www.genetests.org/> (accessed on April 4, 2011).
3. Greer PL, et al. The Angelman syndrome protein Ube3A regulates synapse development by ubiquitinating arc. *Cell* 2010;140:704–16.
4. Lossie AC, et al. Distinct phenotype distinguish the molecular classes of Angelman syndrome. *J Med Genet* 2001;38:834–45.
5. Williams CA, Driscoll DJ, Dagli AI. Clinical and genetic aspects of Angelman syndrome. *Genet Med* 2010;12:385–95.

Test Information

2005077 **Angelman Syndrome and Prader-Willi Syndrome by Methylation**
2005564 **Angelman Syndrome (*UBE3A*) Sequencing**

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

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