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Genetic Testing for Angelman Syndrome

Clinical Features:

Angelman syndrome (AS) [OMIM #105830] is characterized by four essential features, demonstrated by all those affected [1]:

- Functionally severe developmental delay
- Movement or balance disorder, usually manifesting as ataxia, but may be clinically mild
- Behavioral uniqueness, typically exemplified by apparent happy demeanor (frequent laughing/smiling) and easy excitability; often accompanied by unique hand motions/flapping
- Severe speech impairment, resulting in little or no verbal communication; patient may rely predominantly on non-verbal communication

Other characteristics noted in over 80% of patients include microcephaly, seizures, and a specific, abnormal EEG pattern. Patients may also exhibit wide mouths with unusual tongue/mouthing behaviors, hypopigmentation, and abnormal sleep-wake cycles. Older patients may experience obesity [1].

Molecular Genetics:

AS is caused by the absence or dysfunction of the typically active maternal allele at chromosome 15q11-q13, while the clinically distinct Prader-Willi syndrome (PWS) is the result of dysfunction or absence of the paternal allele. The 15q11-q13 region contains several genes that are differentially methylated on maternally and paternally inherited alleles. Within this region, the gene known to be active on the maternal allele is *UBE3A* [OMIM #601623], which encodes the E6AP-3A ubiquitin protein ligase [2]. Ubiquitin molecules typically facilitate protein degradation; although the etiology of AS is still unknown, *UBE3A* has been shown to be imprinted in the brain [3].

To date, AS is known to be caused by four different genetic mechanisms [3]:

- **Deletions of 15q11-q13 on the maternally inherited chromosome (70-75% of cases).** The majority of these cases are the result of large interstitial deletions, though cases involving translocations and smaller deletions have been noted. As a class, patients with AS caused by a deletion exhibit the most severe phenotype with the highest incidence of seizures (90%). Complete absence of speech and severe microcephaly is also typically seen in this group [4].
- **Paternal uniparental disomy (UPD) of chromosome 15 (2-5% of cases).** These patients appear to be more mildly affected than those affected by a deletion. Patients with paternal UPD may have fewer severe seizures and less severe microcephaly; almost 50% can speak a few words [4].
- **Imprinting defects (2-5% of cases).** Defects in the imprinting center (IC) at 15q11-q13 can change the methylation patterns and subsequent transcription activity of the genes within that region. Deletions of the IC region occur in 10-40% of patients with an IC defect and occur more frequently in familial cases. Epigenetic defects of the IC region are thought to comprise the remaining patients in this category and occur in sporadic cases. Patients in this group have a phenotype similar to those in the UPD group [4].
- ***UBE3A* mutations (5-11% of cases).** This mechanism should be considered for patients that fit the classic AS phenotype yet have normal methylation of chromosome 15. Up to 50% of all patients without molecular confirmation of the other mechanisms have a mutation in *UBE3A*, including 75-80% of all familial cases in this category [4,5]. Most reported mutations are unique; the most frequently reported types of mutations are protein-truncating nonsense mutations [4,5]. The phenotype of these patients has been described as intermediate between those of the deletion group and the UPD/IC defect group; seizure frequency, speech impairment, and severity of microcephaly is similar to what is noted in the deletion group, while ability to develop of motor skills and obesity is similar to that in the UPD/IC group [4].

Additionally, about 10-15% of patients expressing the typical features of AS will have no currently discernable molecular defect. This may be the result of *UBE3A* mutations in non-coding regions, *UBE3A* being inactivated by mechanisms other than the ones currently known, mutations in other genes in the ubiquitin pathway, or mutations in other genes in the 15q11-q13 pathway. The phenotype in this group approximates that in the deletion group, with less frequent seizures [4].

Male patients that have clinical features similar to AS that test negative for relevant AS genetic testing, may be considered for mutations in the *SLC9A6* gene. *SLC9A6* mutations have been identified in male patients with an Angelman-like syndrome phenotype who exhibit features such as developmental delay, mental retardation, seizures and a happy demeanor. Patients do not appear to develop obesity with age, as is commonly seen in classic AS [7]. Full gene sequencing of the *SLC9A6* gene is also available in our laboratory (details of this test can be found in our information sheet on *SLC9A6*-related Angelman like syndrome).

Inheritance:

AS has an estimated incidence of approximately 1 in 12,000-20,000. Most cases of AS are *de novo*, with a <1% recurrence rate, yet some cases may be familial, caused by inherited imprinting center or *UBE3A* mutations, or unbalanced translocations involving 15q11-q13. Mutations inherited maternally will result in AS; daughters inheriting AS-causing mutations from their fathers are at risk to have children with AS. Germline mosaicism of a *UBE3A* mutation has been reported [6].

Additional Resources:

Angelman Syndrome Foundation

4255 Westbrook Drive, Ste. 216, Aurora, IL 60504

Phone: 630-978-4245; 800-432-6435

Email: info@angelman.org

www.angelman.org

Test Methods:

M-PCR is a rapid, inexpensive test to identify individuals with AS due to deletions of 15q11-q13, UPD15, or imprinting abnormalities.

We recommend methylation-specific PCR (M-PCR), along with chromosome analysis to rule out chromosomal abnormalities, as the initial test for AS. For patients identified as having AS by M-PCR, additional testing is required to determine if the individual has a deletion, paternal UPD15, or an imprinting center deletion in order to provide accurate genetic counseling. A normal M-PCR result does NOT rule out AS as patients with *UBE3A* mutations will exhibit a normal M-PCR result.

In addition to M-PCR and chromosome analysis, our laboratories offer FISH analysis for deletions of 15q11-q13, polymorphic microsatellite analysis for UPD15, real-time quantitative PCR (RT-PCR) for imprinting center deletions, and *UBE3A* sequencing.

Chromosome analysis

Sample specifications:	3-10 cc of blood in a green top/sodium heparin tube
Cost:	\$700
CPT codes:	88230, 88262, 88291
Turn-around time:	12 days

Methylation-specific PCR (M-PCR)

Sample specifications:	3-10 cc of blood in a purple top (EDTA) tube
Cost:	\$315
CPT codes:	83891, 83898, 83894, 83912
Turn-around time:	2 – 4 weeks

FISH for deletions of 15q11-q13

Sample specifications:	3-10 cc of blood in a green top/sodium heparin tube
Cost:	\$325
CPT codes:	88230, 88271, 88291, 88273
Turn-around time:	10 – 12 days

Microsatellite analysis for UPD15 testing

Sample specifications:	3-10 cc of blood from patient and BOTH parents in purple top (EDTA) tubes
Cost:	\$540 (total for patient's and both parents' blood samples)
CPT codes:	83891, 83898 x 4, 83894, 83912
Turn-around time:	2 – 4 weeks

Real-time quantitative PCR (RT-QPCR) for imprinting center (IC) deletions

Sample specifications:	3-10 cc of blood in a purple top (EDTA) tube
Cost:	\$450
CPT codes:	83891, 83900, 83901, 83912
Turn-around time:	2 – 4 weeks

UBE3A sequence analysis

Sample specifications:	3-10 cc of blood in a purple top (EDTA) tube
Cost:	\$2025
CPT codes:	83891, 83898 x 4, 83904 x 9, 83912
Turn-around time:	4 – 6 weeks

Testing for a known mutation in additional family members

Sample specifications:	3 - 10 cc of blood in a purple top (EDTA) tube
Cost:	\$390
CPT codes:	83891, 83898 x 2, 83894, 83912
Turn-around time:	3 - 4 weeks

Prenatal testing for a known mutation

Sample specifications:	2 T25 flasks of cultured cells from amniocentesis or CVS or 10 mL of amniotic fluid
Cost:	\$590
CPT codes:	83891, 83898 x 2, 83894, 83912, 99051
Turn-around time:	1-2 weeks

Results:

You will be informed of the results of your case as soon as it has been completed. Results, along with an interpretive report, will be faxed and mailed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

Laboratory Faculty and Staff:

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References:

1. Williams C, et al. Angelman syndrome 2005: Updated consensus for diagnostic criteria. (2006). Am J Med Genet 140A:413-418.
2. Huibregtse J, et al. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. (1993). Mol Cell Biol 13(2):775-84.
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4. Lossie A, et al. Distinct phenotypes distinguish the molecular classes of Angelman syndrome. (2001). J Med Genet 38:834-845.
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7. Gilfillan G, et al. *SLC9A6* mutations cause X-linked mental retardation, microcephaly, epilepsy and ataxia, a phenotype mimicking Angelman syndrome (2008). Am. J. Hum. Genet. 82:1003-1010.

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