THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

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. An absence/defect/lack of expression of the *UBE3A* gene is thought to be the basis of the large majority of AS (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).

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 (X-linked Angelman-like syndrome) (6).

Other disorders that have several overlapping features with AS include Pitt-Hopkins syndrome (caused by mutations of the *TCF4* gene) and atypical Rett syndrome (caused by mutations in the *MECP2* gene).

- De Pontual *et al* [2009] detected *TCF4* mutations in 13 of 36 patients with severe psychomotor delay and facial features consistent with PHS, some of whom had previously been investigated for AS, Mowat-Wilson or Rett syndrome (7).
- Watson *et al* [2001] detected *MECP2* mutations in 5 out of 47 patients with a clinical diagnosis of AS (8).

Testing for these disorders are available in our laboratory as individual tests, or as part of the Angelman Syndrome Tier 2 Panel or the Rett/Angelman Syndrome Panel. More details can be found on our website.

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 unidentified defects of the *UBE3A* gene, mutations in other genes in the ubiquitin pathway, or mutations in other genes. The phenotype in this group resembles the deletion group, with less frequent seizures (4).

Inheritance:

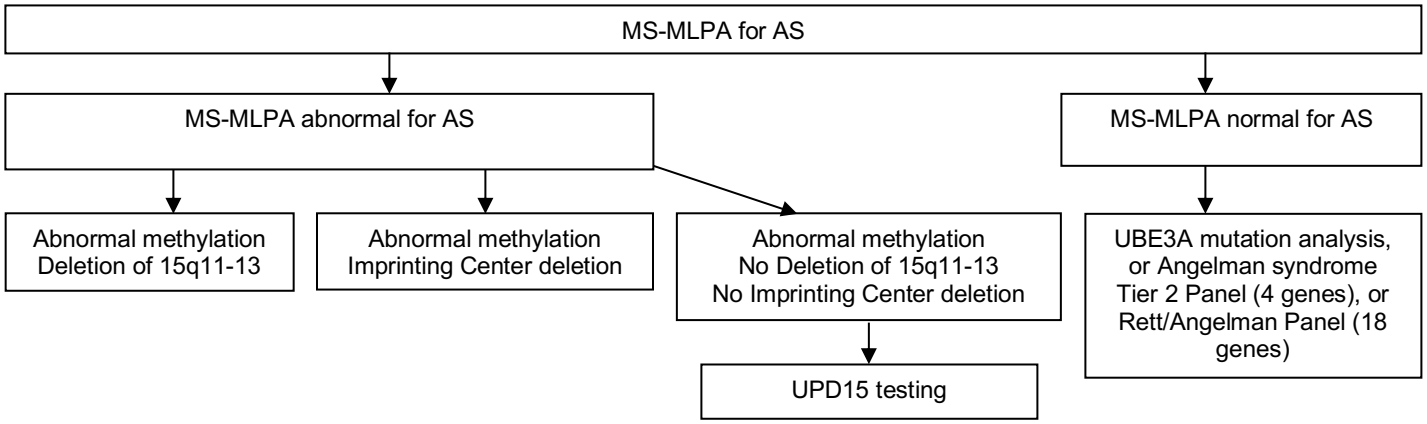
AS has an estimated incidence of approximately 1 in 12,000-20,000. Most cases of AS are *de novo*, with a <1% recurrence rate, yet some cases may be familial, caused by inherited imprinting center or *UBE3A* mutations, or unbalanced translocations involving 15q11-q13. Mutations inherited maternally will 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 (9). *MECP2* and *SLC9A6* are X-linked. *MECP2* mutations appear to be more common in females than in males. The majority of cases are *de novo*. There have been reports of unaffected or mildly affected *MECP2* carrier females due to skewed X inactivation. Mutations in *SLC9A6* result in clinical features in affected males and occasionally some mild features in carrier females. All reported cases of *TCF4* mutations are due to *de novo* mutations, with the exception of one case of maternal mosaicism (7).

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:

We recommend methylation-specific (MS)-MLPA as the initial test for AS. This testing will identify patients with abnormal methylation, large deletions and imprinting center deletions. Those patients with abnormal methylation, but no deletion, should pursue UPD testing to distinguish between UPD15 and non-deletion IC defect. Patients with normal methylation should consider the Angelman Syndrome Tier 2 Panel (*UBE3A, SLC9A6*, *MECP2* and *TCF4* sequencing and deletion/duplication analysis) or the Rett/Angelman Panel (18 genes).



Methylation-specific MLPA (MS-MLPA)

Sample specifications:	3-10 cc of blood in a purple top (EDTA) tube
Cost:	$525
CPT codes:	81331
Turn-around time:	4 weeks

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:	81402
Turn-around time:	4 weeks

Imprinting center deletion analysis

Sample specifications:	3-10 cc of blood in a purple top (EDTA) tube
Cost:	$450
CPT codes:	81403
Turn-around time:	4 weeks

Angelman Syndrome Tier 2 Panel (*UBE3A* and *SLC9A6*, *MECP2* and *TCF4* mutation analysis)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2500
CPT codes:	81406 81407
Turn-around time:	4 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

Rett/Angelman Syndrome Panel (18 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2500
CPT codes:	81406 81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the Rett/Angelman Panel. The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

1. Williams CA, Beaudet AL, Clayton-Smith J et al. Angelman syndrome 2005: updated consensus for diagnostic criteria. Am J Med Genet A 2006: 140: 413-418.
2. Huibregtse JM, Scheffner M, Howley PM. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Mol Cell Biol 1993: 13: 775-784.
3. Williams C. Angelman Syndrome. In: Cassidy S, Allanson J, eds. Management of Genetic Syndromes. Hoboken, NJ: John Wiley & Sons, 2005.
4. Lossie AC, Whitney MM, Amidon D et al. Distinct phenotypes distinguish the molecular classes of Angelman syndrome. J Med Genet 2001: 38: 834-845.
5. Fang P, Lev-Lehman E, Tsai TF et al. The spectrum of mutations in UBE3A causing Angelman syndrome. Hum Mol Genet 1999: 8: 129-135.
6. Gilfillan GD, Selmer KK, Roxrud I et al. SLC9A6 mutations cause X-linked mental retardation, microcephaly, epilepsy, and ataxia, a phenotype mimicking Angelman syndrome. Am J Hum Genet 2008: 82: 1003-1010.
7. de Pontual L, Mathieu Y, Golzio C et al. Mutational, functional, and expression studies of the TCF4 gene in Pitt-Hopkins syndrome. Hum Mutat 2009: 30: 669-676.
8. Watson P, Black G, Ramsden S et al. Angelman syndrome phenotype associated with mutations in MECP2, a gene encoding a methyl CpG binding protein. J Med Genet 2001: 38: 224-228.
9. Hosoki K, Takano K, Sudo A et al. Germline mosaicism of a novel UBE3A mutation in Angelman syndrome. Am J Med Genet A 2005: 138A: 187-189.