Genetics 101

Stormy J. Chamberlain
Department of Genetics and Genome Sciences,
University of Connecticut Health Center

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Cell

Nucleus

Chromosome

http://www.gencodys.eu
Neurons (brain cells)

Nucleus

Chromosome 15

http://www.gencodys.eu
Humans have 22 pairs of chromosomes plus 2 sex chromosomes

46, XX

46, XY
We get one copy of each chromosome from mom and one copy from dad.

46, XY
Unlike most chromosomes, mom’s and dad’s copies of chromosome 15 are different from one another.

The chromosome 15q11-q13 region is where the *UBE3A* gene is located.
In neurons, UBE3A is produced from mom’s copy, but not dad’s.

In nearly every other cell type, UBE3A is produced from both mom’s and dad’s copies.
Genes in the chromosome 15q11-q13 region

* Methylation imprint ultimately determines the difference between mom’s and dad’s chromosomes 15.
Molecular classes of AS

[Diagram showing different molecular classes of Angelman Syndrome (AS) with labels for typical/normal, deletion, UBE3A mutation, paternal UPD, and imprinting defect]
Different Molecular Classes of AS

<table>
<thead>
<tr>
<th>Chromosomal/Genetic Abnormality</th>
<th>% in AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion of maternal 15q11–q1</td>
<td>70%</td>
</tr>
<tr>
<td>Mutation in $UBE3A$ gene</td>
<td>10</td>
</tr>
<tr>
<td>Paternal Uniparental Disomy (UPD)</td>
<td>5</td>
</tr>
<tr>
<td>Imprinting Defect (ID)</td>
<td>5</td>
</tr>
<tr>
<td>Unknown</td>
<td>10</td>
</tr>
</tbody>
</table>

Adapted from: Molecular epigenetics of Angelman syndrome (2007). M. Lalande and M. A. Calciano
Molecular Classes of AS

Typical/NORMAL

DELETION

UBE3A mutation

UPD

Imprinting defect

UBE3A

70%

10%

5%

5%

AS
Why do we want to know what type of AS a child has?

Credit: Dr. Charlie Williams
Risk of Recurrence—
What are the chances of having another child with AS?

- **Typical/Normal**: 70%
- **Deletion**: 10%
- **UBE3A mutation**: 5%
- **UPD**: 5%
- **Imprinting defect**: 5%

**Prevalence in AS**:
- Typical/Normal: 70%
- Deletion: 10%
- UBE3A mutation: 5%
- UPD: 5%

**Risk of recurrence**:
- **Typical/Normal**: <<1%* if inherited
- **Deletion**: <<1%* for most
- **UBE3A mutation**: <1%* if spontaneous
- **UPD**: <<1%* for most
- **Imprinting defect**: 50% if deletion

* exceptions—translocation, germline mosaicism
Example of Imprinting Inheritance in Familial AS:
Inherited UBE3A Mutation

- = UBE3A mutation carrier
○ = Angelman syndrome

Diagnostic Testing for AS

- DNA methylation analysis
  - Normal
  - Diagnostic of AS
    - UBE3A gene sequencing
      - Normal
      - AS unlikely
      - UBE3A mutation
    - FISH or Array/CGH/CMA
      - Deletion
      - IC mutation analysis
        - Normal
        - Imprinting defect
          - Normal
          - Paternal UPD
            - Two paternal copies of chromosome 15
        - Abnormal
          - Epimutation
          - IC deletion
DNA methylation testing

* methylation-specific PCR
Diagnostic Testing for AS

1. DNA methylation analysis
   - Normal
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         - AS unlikely
         - UBE3A mutation
       - Deletion
         - IC mutation analysis
           - Normal
             - Epimutation
           - Abnormal
             - IC deletion
     - FISH or Array/CGH/CMA
       - No deletion
         - DNA marker analysis
           - Normal
             - Two paternal copies of chromosome 15
           - Imprinting defect
     - Paternal UPD
UBE3A gene sequencing

A

B

Patient (VSM)

mutated allele

non-mutated allele

What do we learn from UBE3A gene sequencing

ACC GUG CUU ACG GGG UAC AUA
TVLTGYI

The UBE3A gene encodes a protein called a ubiquitin ligase.

1. A polymorphism is a change that doesn’t change the meaning of the sequence.

ACC GUG CUU ACC GGG UAC AUA
TVLTYI

The UBE3A gene encodes a protein known as a ubiquitin ligase.

2. Some mutations may change the meaning of the sequence impacting the protein.

ACC GUG CCU ACC GGG UAC AUA
TVPTGYI

The UBE3A gene explodes a protein called a ubiquitin ligase.

3. A “stop” mutation may alter the sequence and truncate the protein so it isn’t functional.

ACC GUG CUU ACC GGG UAA AUA
TVLTV

The UBE3A gene encodes a protein called.

4. Mutations that add or delete sequence will change how the sequence is read impacting the protein.

ACC GUG CUU AAC GGG GUA CAU A
TVLNGVH

The UBE3A gene encodes a protein called.

http://www.cureangelman.org/what-testing101.html
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DNA methylation testing

* methylation-specific PCR
Diagnostic Testing for AS

DNA methylation analysis

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UBE3A gene sequencing

- Normal
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FISH or Array/CGH/CMA

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DNA marker analysis

- No deletion
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- Two paternal copies of chromosome 15

IC mutation analysis

- Normal
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- Epimutation
- IC deletion

Imprinting defect

Paternal UPD
FISH—fluorescent in situ hybridization

Green = the tip of chromosome 15, which should be present in everyone
Red = UBE3A gene. If missing, then the individual is deletion-positive.

http://www.cureangelman.org/what-testing101.html
Microarray/Chromosomal Microarray/aCGH

DNA extraction and digestion

Test = patient DNA labeled with red dye
Reference DNA labeled with green dye

combine equal amounts of DNA

Probes

Hybridisation

Scanning

Spotting

Image analysis software

Image

Less AS patient DNA = deletion
Less reference DNA = duplication
Microarray/Chromosomal Microarray/aCGH
There are additional breakpoints (BPs) downstream of BP3 that are used less frequently.

The BPs are nearly identical DNA sequences that are repeated. Repeats = possible deletions.
Diagnostic Testing for AS

DNA methylation analysis

- Normal
- Diagnostic of AS

UBE3A gene sequencing

- Normal
- AS unlikely
- UBE3A mutation

FISH or Array/CGH/CMA

- Deletion

DNA marker analysis

- No deletion
- Normal
- Two paternal copies of chromosome 15

IC mutation analysis

- Normal
- Abnormal

- Epimutation
- IC deletion

Imprinting defect

Paternal UPD
DNA marker analysis

Maternal (upper), proband (middle) and paternal (lower) samples analysed together using Devyser UPD-15. The highlighted allele is inherited from the father. No allele is inherited from the mother.

AS caused by paternal UPD

Diagnostic Testing for AS

1. DNA methylation analysis
   - Normal
     - UBE3A gene sequencing
       - Normal
         - AS unlikely
         - UBE3A mutation
       - Deletion
         - Imprinting defect
         - DNA marker analysis
           - Normal
             - Normal
             - Two paternal copies of chromosome 15
           - Imprinting defect
             - Paternal UPD
           - IC deletion
             - IC mutation analysis
               - Normal
                 - Epimutation
               - Abnormal
                 - IC deletion
In females, both mom and dad chr15 become mom chr15 in oocytes or eggs.
If AS-IC is missing or doesn’t work, one of mom’s chr15 gets labeled as a dad chr15.
Diagnostic Testing for AS

1. DNA methylation analysis
   - Normal
   - Diagnostic of AS

2. DNA marker analysis
   - No deletion
   - Two paternal copies of chromosome 15
   - Normal
   - Paternal UPD

3. IC mutation analysis
   - Normal
   - Abnormal
      - Epimutation
      - IC deletion

4. FISH or Array/CGH/CMA
   - Deletion
   - Imprinting defect

5. UBE3A gene sequencing
   - Normal
   - AS unlikely
   - UBE3A mutation

6. Diagnostic of AS
   - UBE3A mutation
   - Deletion
   - Imprinting defect
   - Paternal UPD
   - Epimutation
   - IC deletion
Advanced Genetics
DNA usually encodes RNA, which then encodes a protein.
Gene expression across the chromosome 15q11-q13 region

Non-neurons

Neurons

UBE3A-ATS
UBE3A-ATS silences paternal UBE3A
Topotecan reduces UBE3A-ATS

AS Neurons

AS Neurons + topotecan
Topotecan reduces UBE3A-ATS
Antisense oligonucleotides (ASOs) reduce UBE3A-ATS

AS Neurons

AS Neurons + ASO
Antisense oligonucleotides (ASOs) reduce UBE3A-ATS
Acknowledgments

Families who have donated samples

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DPH Keeping Connecticut Healthy

Fighting Angels Foundation Fighting to talk...Fighting to walk...Fighting for a cure.

UCONN HEALTH