The X (ALD) Factor: Follow-Up and Clinical Perspectives After One Year of Screening in Minnesota

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X-ALD: Background and Testing
<table>
<thead>
<tr>
<th>X-ALD Types/Characteristics</th>
<th>Details</th>
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</thead>
</table>
| **Childhood Cerebral**     | • ~ 35%-50% of males  
• Onset from around 4-10 yrs  
• Adrenal, cognitive, and developmental issues  
• Death within 2-4 yrs after onset of cerebral symptoms |
| **Adrenal Insufficiency**  | • ~ 80% of affected individuals |
| **Adrenomyeloneuropathy (AMN)** | • Seen in most older men with X-ALD  
• Onset around 20s to 40s  
• Progressive weakness |
| **Symptomatic Females**    | • ~10-50% have neurological symptoms  
• Onset around 30s  
• Similar to AMN, but slower progression |
Screening Algorithm

X-ALD Screen (neg ion LC-MS/MS)

**Screen Positive**
C26:0-LPC ≥ 0.30

**Screen Borderline**
C26:0-LPC = 0.16 – 0.29

**Screen Negative**
C26:0-LPC < 0.16

- Repeat heelstick specimen
- Refer to Diagnostic Evaluation

Repeat Screen Borderline
C26:0-LPC = 0.16 – 0.29
Performance and Outcomes: 2/06/2017 to 2/28/2019

137,402 infants screened

68 infants with BORDERLINE results (*1 infant expired, 2 lost to follow-up)

137,321 infants with NORMAL results

16 infants with POSITIVE results

16 infants with NORMAL REPEAT screens

6 infants with BORDERLINE REPEAT screens

22 infants sent to DIAGNOSTIC EVAL

13 males with ↑ VLCFAs and/or ABCD1 variant

5 females with ↑ VLCFAs and ABCD1 variant

137,321 infants with NORMAL results

59 infants with NORMAL REPEAT screens

• 1 female false positive;
• 1 female refused f/u;
• 1 female pending;
• 1 male moved
Performance and Outcomes: 2/06/2017 to 2/28/2019

- **Screen-Borderline Rate** ~ 1 in 2,114 infants
- **Screen-Positive Rate** ~ 1 in 6,245 infants
- **Birth Prevalence*** ~ 1 in 7,633 infants
- **Male Birth Prevalence*** ~ 1 in 5,427 males
- **Female Birth Prevalence*** ~ 1 in 13,416 females

* A case is considered confirmed upon elevated VCFAs and ABCD1 variant identification
X-ALD: Follow-Up and Clinical Considerations
Challenges for the NBS System

- High rate of variants of uncertain significance in *ABCD1* gene
- Inability to predict phenotypic severity
- X-linked inheritance
  - Downstream family member diagnoses
  - Detect females who will not be symptomatic for years (if ever)
  - Heightened need to check unscreened older siblings (especially males)
Variants of Uncertain Significance (*and one unknown Variant*)

- **17** had an *ABCD1* variant detected
  - **11** classified as likely pathogenic/pathogenic
  - **4** variants of uncertain significance
    - **ALL** were eventually reclassified as likely pathogenic
    - **4** variants never seen before

- **One male case did not have a detectable *ABCD1* variant**
  - Family history consistent with X-linked inheritance
  - Functional ALDP expression study on fibroblasts pending in Amsterdam
Clinical Findings: Male X-ALD Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>NBS C26:0-LPC (umol/L)</th>
<th>VLCFA C26:0 (umol/L)</th>
<th>ABCD1 Variant</th>
<th>Classification (as reported by specialists)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>0.26</td>
<td>3.34</td>
<td>None Found</td>
<td>N/A</td>
</tr>
<tr>
<td>M2</td>
<td>0.72</td>
<td>10.6</td>
<td>c.293C&gt;T</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>M3</td>
<td>1.06</td>
<td>7.38</td>
<td>c.900+1G&gt;A</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>M4</td>
<td>0.74</td>
<td>6.43</td>
<td>c.1028G&gt;T</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>M5</td>
<td>1.12</td>
<td>8.07</td>
<td>c.1973C&gt;T</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>M6</td>
<td>0.76</td>
<td>6.9</td>
<td>c.487C&gt;T</td>
<td>Likely Pathogenic</td>
</tr>
<tr>
<td>M7</td>
<td>0.4</td>
<td>2.17</td>
<td>c.1597A&gt;G</td>
<td>Likely Pathogenic</td>
</tr>
<tr>
<td>M8</td>
<td>0.43</td>
<td>1.92</td>
<td>c.593C&gt;T</td>
<td>Likely Pathogenic</td>
</tr>
<tr>
<td>M9</td>
<td>0.31</td>
<td>2.37</td>
<td>c.593C&gt;T</td>
<td>Likely Pathogenic</td>
</tr>
<tr>
<td>M10</td>
<td>0.16</td>
<td>1.53</td>
<td>c.80A&gt;C</td>
<td>VUS*</td>
</tr>
<tr>
<td>M11</td>
<td>0.35</td>
<td>3.18</td>
<td>c.823C&gt;T</td>
<td>VUS*</td>
</tr>
<tr>
<td>M12</td>
<td>0.47</td>
<td>N/A</td>
<td>c.823C&gt;T</td>
<td>VUS*</td>
</tr>
<tr>
<td>M13</td>
<td>0.49</td>
<td>3.73</td>
<td>c.1747G&gt;A</td>
<td>VUS*</td>
</tr>
</tbody>
</table>

* = novel variants

* reclassified as likely pathogenic

0.9 7.72
0.415 2.27
0.41
Case Example I

Shared with permission from UMN and family
No known history of cerebral disease or adrenal insufficiency in family; AMN symptoms only
Variant Re-classified from VUS to Likely Pathogenic

The c.823C>T variant in ABCD1 results in the p.Arg275Trp substitution. This variant has been reported as likely pathogenic by a single submitting laboratory without supporting evidence. This variant is present in one hemizygous individual in the gnomAD database of 140,000 controls. In silico prediction models estimate this variant to be pathogenic; however, the accuracy of such models is limited. Due to insufficient data to clearly classify this variant as pathogenic or benign, this variant is categorized as a variant of uncertain significance.

- Co-segregation of variant with VLCFA elevations
- Variant identified in other families following X-linked inheritance

This addendum is issued to reflect additional evidence regarding the c.823C>T (p.Arg275Trp) variant in the ABCD1 gene. Since this patient's report was originally issued, this variant has been identified in additional probands with X-linked adrenoleukodystrophy by other laboratories and the variant has also been shown to segregate with an X-linked ALD phenotype in another family tested in this laboratory. Based upon this additional evidence, this variant is now classified as likely pathogenic. Genetic counseling regarding these results is recommended.
Downstream Diagnoses (*first year cases only*)

- Approximately 4 family members are being detected for every newborn
  - Age Range = 1 to 67 years
  - Median = 10 years

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Percentage of Identified Family Members</th>
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<tr>
<td>Males with cerebral disease</td>
<td>~6%</td>
</tr>
<tr>
<td>Males with adrenal insufficiency</td>
<td>~6%</td>
</tr>
<tr>
<td>Males with AMN symptoms</td>
<td>~12% (~67% of males &gt; 18 years)</td>
</tr>
<tr>
<td>Females with AMN symptoms</td>
<td>~30%</td>
</tr>
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### Clinical Care Process

#### Screen Positive Result

- Primary Care Provider Notified
- XALD Specialists Alerted
- Family notified of result and need for further evaluation. Referral placed to specialist.
- Upon referral, family contacted by Genetic Counselor

#### Initial Appointment with Genetic Counselor
- Confirmatory Testing
- Identification of At-Risk Male Relatives

#### Full Assessment in Comprehensive ALD Clinic
- Pediatric Neurology
- Pediatric Endocrinology
- HSCT Counseling
- Service Available

#### Clinical Nurse Coordinator
- Full Assessment in Comprehensive ALD Clinic
Conclusions

• X-ALD incidence may be more common than previously observed

• Can expect numerous subsequent family member diagnoses
  • Phenotypic spectrum may be broader/more mild than expected

• Challenges include:
  • Delayed understanding of true clinical picture of detected cases
  • Numerous VUS’ and reclassifications based on biochemical/molecular findings only
  • Inability to predict phenotypic severity

• Given the above, thoughtful planning and coordination by dedicated specialists is imperative for successful implementation of population-based screening for X-ALD
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