Screening for Leukodystrophies: Update on New York State’s Experience

October 29, 2014
ALD screening in New York
ALD Screening

Sequence of Events 1 – Legislated

- Aidan Seeger, a 7 year old from Brooklyn passes 4/29/2012
- Mrs. Seeger called Dr. Caggana in May 2012 to discuss screening
- Family garnered support: NY politicians; website; billboards
- Bill submitted August 2012
- Approved by Health Finance Committee 02/28/2013
- Became law 03/31/2013; start 01/01/2014 (actual 12/30/2013)
Causes damage to the myelin sheath; brain insulator

Accumulation of saturated very long chain fatty acids (VLCFAs)

Lack of a transporter protein that moves VLCFA into peroxisomes for degradation

Affects predominantly males; females can have mild disease; rarely cerebral disease in females

Frequency: 1/17,000 – 1/20,000 births

Expect 12 to 15 cases annually in New York
Three Types of Adrenoleukodystrophy

- Childhood cerebral form (4-8 years/45%): hyperactivity, vision problems, loss of verbal understanding, regression in school, handwriting, seizures, aphagia

- Adrenomyeloneuropathy (males in their 20’s/35%): muscle weakness, difficulty thinking quickly, poor sight memory; uncontrolled urination

- Addison’s disease: lack of steroid hormones (cortisol and aldosterone); decreased appetite, low blood pressure, increased pigmentation, muscle wasting, vomiting, coma
New York State Assay 
(Mod. Krabbe and ALD)

Punch 3-mm specimen, add 200 µL methanol with d4-C26:0 LPC

1 hour extraction

Remove 50 µL of extract and combine with LSD extract

Analyze samples, 1.5 minutes per sample/Marker is C26:LPC

Follow screening algorithm
Interfering compounds – requires second tier HPLC-MS/MS to reduce positives

Adding C26:0-LPC channel to LSD test: – lost GALC-IS signal (corrected)

Adding ALD extract to GALC: Linearity of GALC affected - slope 1.5 (normally 1.1, corrected)

Edge Effects on plates (evaporation, corrected)

C26:0 LPC has low solubility relative to interferent
### Population Statistics (12/30/13 – 10/21/14)

#### C26:0-LPC (uM)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>0.23</td>
</tr>
<tr>
<td>StDev</td>
<td>0.066</td>
</tr>
<tr>
<td>max</td>
<td>2.78</td>
</tr>
</tbody>
</table>

#### ALD N = 2198833 samples

<table>
<thead>
<tr>
<th>C26:0</th>
<th>Count</th>
<th>Yr-Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.35</td>
<td>10803</td>
<td>1228</td>
</tr>
<tr>
<td>&gt;0.4</td>
<td>4339</td>
<td>493</td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>707</td>
<td>80</td>
</tr>
<tr>
<td>&gt;0.6</td>
<td>215</td>
<td>24</td>
</tr>
</tbody>
</table>

Birthrate for NY = ~240,000

First tier cutoff = 0.4 μM
# Positive Controls

## Positive controls, Tier 1 results

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Accession #</th>
<th>Condition</th>
<th>C26:0 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALD_1</td>
<td>20042872073</td>
<td>ALD</td>
<td>1.2</td>
</tr>
<tr>
<td>ALD_2</td>
<td>20131571625</td>
<td>Zellweger</td>
<td>1.75</td>
</tr>
<tr>
<td>ALD_3</td>
<td>20042091488</td>
<td>ALD</td>
<td>1.3</td>
</tr>
<tr>
<td>ALD_4</td>
<td>20070191816</td>
<td>Zellweger</td>
<td>1.53</td>
</tr>
<tr>
<td>ALD_5</td>
<td>20001511848</td>
<td>ALD</td>
<td>0.78</td>
</tr>
<tr>
<td>ALD_6</td>
<td>19991892305</td>
<td>ALD</td>
<td>1.08</td>
</tr>
<tr>
<td>ALD_7</td>
<td>20021191634</td>
<td>ALD</td>
<td>1.09</td>
</tr>
<tr>
<td>ALD_8</td>
<td>20100251314</td>
<td>Carrier</td>
<td>0.78</td>
</tr>
<tr>
<td>ALD_9</td>
<td>20041381090</td>
<td>ALD</td>
<td>1.19</td>
</tr>
<tr>
<td>ALD_10</td>
<td>20023531007</td>
<td>ALD</td>
<td>1.28</td>
</tr>
</tbody>
</table>

## Mayo positive controls

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Patient information</th>
<th>C26:0 (µM)</th>
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</thead>
<tbody>
<tr>
<td>PLSD 041614-04</td>
<td>XALD #67655 7.7 year old male</td>
<td>1.03</td>
</tr>
<tr>
<td>PLSD 041614-05</td>
<td>XALD #61933 7.8 year old male</td>
<td>0.48</td>
</tr>
<tr>
<td>PLSD 041614-06</td>
<td>XALD #67651 8.8 year old male</td>
<td>0.69</td>
</tr>
</tbody>
</table>
All specimens tested for C26:0 LPC

≥ 0.4 µM

< 0.4 µM

Second Tier HPLC C26:0 LPC

C26:0 LPC ≥ 0.4 µM

DNA testing
For information only

Repeat C26:0 ≥ 0.24 µM

Screen Positive/Referral

C26:0 (0.24-0.39): Presumptive Positive/request repeat

Repeat < 0.24

Screen negative

C26:0 LPC ≤ 0.24
Second Tier: HPLC-MS/MS

Second Tier:
1. Reduce interferents
2. Reduce false positives
C26:0
Internal Standard Peak
Retention Time 4.23 min
Area under the peak is 15,824

C26:0
Screen Negative Infant Peak
Retention Time 4.23 min
Area under the peak is 1,139
C26:0
Internal Standard Peak
Retention Time 4.22 min
Area under the peak is 17,254

C26:0
Screen Positive Infant Peak
Retention Time 4.23 min
Area under the peak is 11,560
Third Tier: DNA Sequencing

1. Full sequencing of ABCD1 gene
2. Not intended to reduce referrals
3. Helps to Determine
   a. if females are ALD carriers
   b. if males have mutation
   c. if no mutation, consider other PGD

4. Genotype does not correlate with phenotype
New York State Newborn Screening for X-ALD

December 30, 2013 to October 21, 2014

212,627 infants screened for C26:0 LPC
First Tier / High-throughput MS/MS

4,339 HPLC/MS-MS C26:0 LPC
PP = 20* (0.24-0.39)
DNA testing = 15 (≥0.4)

15→ referrals:
8 male with mutation
4 no mutation
3 female carrier

8 ALD
4 Zell/
PBD**
## Status of ALD Referrals in New York

### 8 Adrenoleukodystrophy Cases Detected

1. 1.30, 1.14; p.E302K (*de novo* in child, known childhood onset)

2. 1.03, 0.84; p.W601X (known; cerebral adult onset)

3. 0.51, 0.40; p.P534S (phenotype unknown; different aa changes-adult onset)

4. 0.40, 0.26; p.R163H (known; symptomatic carrier -- sibling identified)

5. 1.21, 1.09; p.R189W (known; adult AMN; Addison’s)

6. 0.67, 0.34; p.S572P (novel)

7. 1.24, 0.96; g.E6-10del (known; AMN in ex. 7-10 deletion)

8. 0.61, 0.49; p.G92R (novel) and p.R324C (novel)
Status of ALD Referrals in New York

7 Other Outcomes To Date

0.96, 0.89; (NICU; possible Zellweger; LTFU; BG)

1.29, 1.33; (NICU; peroxisomal biogenesis defect??)

1.79, 1.70; (NICU; peroxisomal biogenesis defect??)

2.56, 3.48; (NICU; Zellweger – two previous ZD siblings)

0.58, 0.40; p.V583M (novel; BG carrier)

0.65, 0.50; p.E272del (reported; BG carrier)

0.62, 0.50; p.Q47Rfs*21 (novel; BG carrier)
ALD by the Numbers (190,368 births)

- Referral rate: 1 in 12,691 or 0.008% of infants screened
- Incidence of ALD*: 1 in 23,796 births
- Incidence of ALD*: 1 in 11,898 males
- Incidence of PGDs: 1 in 47,592 births

Too early for stable incidence rates – prediction is 1 in 17,000 to 1 in 20,000 births

* Assumption that all with Muts will become symptomatic.
Challenges in ALD Screening

- Genetic diversity — *(novel variants?; VOUS)*
- Incomplete genotyping — *(undetected variants?)*
- Later onset condition — *(boys and AMN)*
- Potential for carriers to be symptomatic
- Assay doesn’t identify all carriers
- Potential for Dad to have AMN
- Lack of genotype:phenotype correlation
- Lack of correlation of C26 concentration to severity of disease
Acknowledgements

• Monica Martin: first tier method development
• Mark Morrissey/Cathy Lubowski: second tier method development
• Dieter Matern and Staff: SOP for assay
• Chris Haynes for technical assistance and control samples
• Ann Moser for technical assistance and control samples
• Michele Caggana for many slides