X-ALD Newborn Screening and Follow-up testing in USA

Ann B Moser, the Hugo W. Moser Research Institute, Kennedy Krieger Institute and Johns Hopkins Univ.

ALD Life Meeting, London, May 5, 2018

14:00 Dr Ann Moser- Update on New Born Screening (NBS) in USA
Purpose of Newborn Screening

Presymptomatic Diagnosis of Males with X-ALD to:

1. Prevent overt adrenal insufficiency
2. Reduce the risk for childhood phenotype with Lorenzo’s Oil diet
3. Improve prognosis of cerebral ALD by facilitating early hematopoietic stem cell and gene transplants
4. Improve success of future therapies

Identification of 85% of X-ALD Heterozygotes to:

Provide family screening for male X-ALD relatives.

Diagnosis of Peroxisome Biogenesis Disorders & SEDs:

Prevent diagnostic odyssey, provide genetic counseling and early supportive therapy.

In 2015, ALD was added to the Recommended Uniform Screening Panel in the USA. Some states are now screening for it, with others hoping to begin screening shortly.
MINIMUM FREQUENCY OF X-ALD IN THE UNITED STATES

<table>
<thead>
<tr>
<th></th>
<th>Male Population</th>
<th>Female Population</th>
<th>Total Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemizygotes (a)</td>
<td>1:21,000</td>
<td></td>
<td>1:42,000</td>
</tr>
<tr>
<td>Heterozygotes (b)</td>
<td></td>
<td>1:14,000</td>
<td>1:28,000</td>
</tr>
<tr>
<td>(calculated)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Hemizygotes + Heterozygotes</strong></td>
<td></td>
<td></td>
<td><strong>1:16,800</strong></td>
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(a) Similar results in France and Canada  
(b) 60% of heterozygotes develop symptoms in middle age or later


**History of Development Newborn Screening Test for X-ALD**

- **2004** Newborn Screening Meeting at NIH, X-ALD added to list of disorders, but no test available on newborn blood spots
- **2006** LC-MS/MS Assay for X-ALD newborn screening, Hubbard et al. Found 5 to 10-fold increase of C26:0 lyso phosphatidyl choline, C26:0 LPC in X-ALD dried blood spots
- **2008** D4-C26:0 LPC synthesized by Avanti
- **2009** Validation of LC-MS/MS assay for X-ALD newborn screening, Hubbard et al.
- **2013** X-ALD newborn screening pilot 5000 screened in MD
- **December 2013, New York using the marker C26:0 LPC adds newborn screening for X-ALD; as of July 2017 and 820,000 screened, birth incidence of X-ALD is 1/15,472
- **August 2015**-Secretary’s Advisory Committee voted to add X-ALD to the Recommended Uniform Screening Panel (RUSP)
Authentic Standards for X-ALD newborn screening

26:0-Lyso-PC, MW 635.5
Elevated in blood spots X-ALD

\( ^{2}H_{4}-26:0\)-Lyso-PC, MW 639.5
Internal Standard
C26:0 LPC measured in 1/8" DBS by LC-MSMS by Ann Moser (NBS cutoff 0.27uM)

C26:0 LPC uM

<table>
<thead>
<tr>
<th></th>
<th>N</th>
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<tbody>
<tr>
<td>normals</td>
<td>190</td>
</tr>
<tr>
<td>NBS</td>
<td>416</td>
</tr>
<tr>
<td>ALD NBS</td>
<td>18</td>
</tr>
<tr>
<td>ALD</td>
<td>90</td>
</tr>
<tr>
<td>ALDH</td>
<td>117</td>
</tr>
<tr>
<td>PBD</td>
<td>72</td>
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Newborn Screening for ALD and Krabbe Disease, a combined high-throughput assay:

The New York State Newborn Screening Lab under the directorship of Michelle Caggana implemented screening for ALD in December 2013 in response to Aidan’s Law and successful lobbying by Elisa Seeger, Aidan’s mother. The first tier test is the combined high-throughput assay. The second tier is LC-MSMS. Those with positive 2nd tier test are confirmed by analysis of ABCD1 gene and those with negative ABCD1, PBDs by clinical, genetic and biochemical tests. In NY State there is an established referral network of geneticists, pediatricians, endocrinologists, and pediatric neurologists.
# Newborn Screening for ALD: Methods in Use for Analysis of C26:0 LysoPC on Newborn Bloodspot

## 3 tier testing: 1st tier high throughput MSMS, 2nd tier LCMSMS, and 3rd tier ABCD1 mutation analysis

<table>
<thead>
<tr>
<th>State</th>
<th>Testing Tier</th>
<th>2nd Tier Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>New York</td>
<td>3 tier testing</td>
<td>2nd tier; C26:0LPC cutoff 0.23uM, ABCD1</td>
</tr>
<tr>
<td>California</td>
<td>3 tier testing</td>
<td>2nd tier; C26:0LPC cutoff 0.20uM, ABCD1</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>3 tier testing</td>
<td>2nd tier; C26:0LPC cutoff 0.23uM, ABCD1</td>
</tr>
<tr>
<td>Connecticut</td>
<td>2 tier testing</td>
<td>Negative ion LCMSMS and ABCD1 mutation</td>
</tr>
<tr>
<td>Minnesota</td>
<td>2 tier testing</td>
<td>Positive ion LCMSMS and plasma VLCFA</td>
</tr>
<tr>
<td>Washington</td>
<td>3 tier testing</td>
<td>2nd tier; C26:0LPC cutoff 0.23uM, ABCD1</td>
</tr>
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Bigger states have introduced 3 tier testing, while smaller states have a two-tier system due to the lower number of births and availability of resources.
## NY State NBS Program – “3-Tier” Screening for X-ALD

<table>
<thead>
<tr>
<th>Tier - Screening Activity</th>
<th>Rate Definition</th>
<th>Rates</th>
</tr>
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<tbody>
<tr>
<td>TIER 1</td>
<td>MS/MS for C26:0 LPC</td>
<td>Re-test rate (same specimen)</td>
</tr>
<tr>
<td>TIER 2</td>
<td>HPLC &amp; MS/MS for C26:0 LPC</td>
<td>Repeat rate (independent specimen)</td>
</tr>
<tr>
<td>🎖 Mutation analysis – ABCD1 gene</td>
<td>Referral rate</td>
<td>= 80 of 880,000 newborns = 0.009%</td>
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**Confirmed Status:**
- 30 male with ABCD1 mutations (1/29,333)
- 27 female carriers with ABCD1 mutations (1/32,593)
- All X-ALD (1/15,439 births)
- 10 Other PBD/SED confirmed (1/88,000 births)
- 1 Aicardi-Goutieres syndrome confirmed
- 12 no mutation found

Total 880,000 newborns screened (Dec 30 2013 – September 30, 2017)

In New York, for example:
- Tier 1 testing: 1.84% retest rate
- Tier 2 testing: 0.009% repeat rate
- Tier 3 testing: 0.0009% referral rate
### X-ALD Newborn Screening in CT using negative ion LCMSMS with C26:0-LPC as marker

<table>
<thead>
<tr>
<th>Description</th>
<th>Count/Details</th>
</tr>
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<tbody>
<tr>
<td>Number of infants analyzed as of 06/01/17 (10/1/2015-06/01/2017)</td>
<td>61341</td>
</tr>
<tr>
<td>Total Screen Positive</td>
<td>17</td>
</tr>
<tr>
<td>Samples reported with 2nd request</td>
<td>7</td>
</tr>
<tr>
<td>Samples normal on second sample analysis</td>
<td>5</td>
</tr>
<tr>
<td>False Positive 2016</td>
<td>1</td>
</tr>
<tr>
<td>Pending 2017</td>
<td>3</td>
</tr>
<tr>
<td>Confirmed X- ALD diagnosis newborn infant results</td>
<td>9 (4 male, 5 female)</td>
</tr>
<tr>
<td>Siblings Identified (and confirmed at Treatment Center) with X-ALD</td>
<td>2 (1 male, 1 female)</td>
</tr>
</tbody>
</table>
| Other                                                                       | 1
| **Zellweger 2017**                                                          |                                                   |
| **X-ALD Incidence Overall**                                                 | **~1:6815**                                       |

The incidence rate so far is slightly higher in Connecticut, with no reason for this yet apparent.
X-ALD Newborn Screening in MN using negative ion LCMSMS, C26:0-LPC as marker
Oct 2017

- 2/6/17 to 9/30/17 Total screened = 45,095
- 5 X-ALD males
- 4 X-ALD females
- Incidence of ABCD1 proven X-ALD = 1/5010

- Impact of X-linked disorder on families
- 24 additional family members carry ABCD1 mutation
  - Holly Winslow, Research Scientist and Amy Gaviglia, Laboratory Supervisor, MN Dept. of Health
X-ALD gene (ABCD1)

- >2642 mutations of which 799 are non-recurrent and 227 VUS as of 2/2018
- www.x-ald.nl
- No correlation with phenotype
- Mutation Analysis is essential for accurate heterozygote detection

Current Follow-up Recommendations For X-ALD Babies

1. For all X-ALD babies: Genetic counseling for family.

2. Males only: Assess adrenal function in newborn period and every 6 months; Adrenal hormone replacement if found to be deficient.

3. Males only: Starting at 1 year of age, MRI every year to age 3 years, then every 6 months to 12 years, then annually; if abnormal and progressive, refer for bone marrow transplantation if there is a matched sibling donor, or umbilical cell transplant if matched, or if no match, refer for ABCD1 gene therapy.

The current follow-up recommendations following discovery of ALD through newborn screening:
Genetic counselling for families
Assessing adrenal function in boys, including hormone replacement if necessary
MRI every year until the age of 3, then every 6 months until the age of 12
Acknowledgements

Richard O. Jones, Paul Watkins, S. Ali Fatemi, Carol Tiffany– Kennedy Krieger Institute
Gerald Raymond- Minnesota Children’s Hospital
Walter Hubbard– Dept. of Medical Pharmacology, Johns Hopkins
Susan R. Panny, Fizza Gulamali– Majid –Newborn Screening Program Maryland
Dept. Mental Health/Hygiene
Michele Caggana, Joseph Orsini, Michael Morrissey, Beth Vogel- NY State
Newborn Screening
Rasoul Koupaei, Partha Neogi, Fred Lorey, Lisa Feuchtbaum – Genetic Disease
Screening Program, California
Adrienne Manning, Connecticut Newborn Screening Lab
Amy Gaviglia, Holly Winslow, Minnesota Newborn Screening Lab
Silvia Tortorelli, Coleman Turgeon– Mayo Biomedical Genetics Laboratory
Can(John) Ficicioglu, Children’s Hospital of Philadelphia
Christopher Haines – CDC, Newborn Screening Branch
Michael Gelb, University of Washington, Seattle

Funding:
United Leukodystrophy Foundation, European Leukodystrophy Association, Myelin
Project, Run 4 ALD, Brian’s Hope, Stop ALD Foundation, Aidan Jack Seeger
Foundation
NINDS: Drug discovery for X-linked adrenoleukodystrophy: 1R21NS091988-01
Watkins
NIH: Newborn Screening Grant: HD057136 Raymond
NIH: Instrumentation API4000 1-S10 RR16798 (Hubbard)
X-linked Adrenoleukodystrophy (X-ALD)
Newborn Screening and Emerging Therapies

- Most Common Leukodystrophy with overall incidence of 1:14,700 due to mutations in *ABCD1*
- Loss of function of the ALD protein to transport very long chain fatty acids (VLCFA) ≥C24:0 to the peroxisome for oxidation resulting in accumulation of VLCFA
- Affecting the nervous system, adrenal glands and testicles
- Several phenotypes in X-ALD males: adrenal disease, childhood cerebral, and an adult spastic paraparesis, AMN, affecting both men and X-ALD females.

**X-ALD newborn screening**
will save the lives of X-ALD boys by providing early detection of adrenal dysfunction and monitoring for first signs of childhood cerebral disease when they will receive treatments with proven and emerging therapies.