PEROXISOMAL DISORDERS AND NEWBORN SCREENING

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Peroxisomal Disorders

Disorders of peroxisomal biogenesis

- Zellweger syndrome
- Neonatal adrenoleukodystrophy
- Infantile Refsum disease
- Rhizomelia chondrodysplasia punctata
- Hyperpipecolic acidemia

Single enzyme defects

- X-linked adrenoleukodystrophy (X-ALD)
- Acyl-CoA oxidase deficiency
- Bifunctional protein deficiency
- Thiolase deficiency
- Acatalasaemia
- Refsum disease
- Glutaryl-CoA oxidase deficiency
- Mevalonate kinase deficiency
- Hyperoxaluria type I
Disorders of Peroxisome Biogenesis

- The peroxisome fails to be formed normally, and defects involve multiple peroxisomal proteins
- Incidence 1:50,000
- Twelve PBD complementation groups have been identified
- Two clinical spectra:
  - the Zellweger spectrum
  - rhizomelic chondrodysplasia punctata
Disorders of Peroxisomal Biogenesis

Clinical Phenotypes

Zellweger syndrome spectrum (ZSS) disorders

Rhizomelic chondrodysplasia punctata

Zellweger syndrome
Neonatal adrenoleukodystrophy
Infantile Refsum disease

Rhizomelic chondrodysplasia punctata type 1

Differential diagnosis with Down Syndrome

CMLS 59 (2002)
## Disorders of Peroxisomal Biogenesis

### Clinical Phenotypes

<table>
<thead>
<tr>
<th>Abnormal features</th>
<th>Zellweger spectrum</th>
<th>Rhizomelic chondrodysplasia punctata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>severe form</td>
<td>mild forms: pseudo-Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease</td>
</tr>
</tbody>
</table>

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Age of survival</td>
<td>&lt; 1 year</td>
<td>≥ 1 year</td>
</tr>
<tr>
<td>Dyssmorphic features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical face</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypotonia</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Epileptic seizures</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Psychomotor retardation</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Gavage feeding</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cataract</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatorenal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver fibrosis or cirrhosis</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Renal cysts</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skeletal system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcific stippling</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Rhizomelia</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+ mild; ++ moderate; +++ severe; – not present.
## Disorders of Peroxisomal Biogenesis
### Biochemical Phenotypes

<table>
<thead>
<tr>
<th>Screening Tests</th>
<th>PBD: Zellweger spectrum</th>
<th>β-oxidation single enzyme defects</th>
<th>Rhizomelic Chondrodysplasia Punctata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severe</td>
<td>Mild</td>
<td>DBP</td>
</tr>
<tr>
<td>Very long chain fatty acids (VLCFA)</td>
<td>↑↑</td>
<td>↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Phytanic acid</td>
<td>Normal*</td>
<td>↑</td>
<td>Normal*</td>
</tr>
<tr>
<td>Pristanic acid</td>
<td>Normal*</td>
<td>↑</td>
<td>Normal*</td>
</tr>
<tr>
<td>Plasmalogens</td>
<td>↓↓</td>
<td>↓— Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Palmitoleic acid, plasma</td>
<td>↑</td>
<td>↑↑</td>
<td>Normal</td>
</tr>
<tr>
<td>Palmitoleic acid, urine</td>
<td>↑↑</td>
<td>↑</td>
<td>Normal</td>
</tr>
<tr>
<td>Bile acid metabolites</td>
<td>↑↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

### Follow up Tests (in fibroblasts):

<table>
<thead>
<tr>
<th>VLCFA</th>
<th>Plasmalogen synthesis</th>
<th>Phytanic acid oxidation</th>
<th>Pristanic acid oxidation</th>
<th>Catalase solubility</th>
<th>Fibroblast peroxisome morphology</th>
<th>Fibroblast PTS1 import</th>
<th>Fibroblast PTS2 import</th>
<th>Molecular Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑↑</td>
<td>↓↓</td>
<td>↓↑</td>
<td>Normal</td>
<td>Normal</td>
<td>Enlarged, reduced number or Absent</td>
<td>Enlarged, reduced number</td>
<td>Enlarged, reduced number</td>
<td>PEX1, 2, 3, 5, 6, 10, 12, 13, 14, 16, 19, or 26, DBP gene</td>
</tr>
<tr>
<td>Normal</td>
<td>↓— Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

↑ = higher than normal; ↓ = lower than normal; * since a severe case would present in the new born period and branched chain fatty acids accumulate with dietary exposure, these fatty acids are usually normal at that time; DBP = D-bifunctional protein; ACOX1 = straight chain acyl-CoA oxidase; DHAPAT = dihydroxyacetonephosphate acyltransferase; ADHAPS = alkyl-dihydroxyacetonephosphate synthase.
Disorders of Peroxisomal Biogenesis

Therapy

• Symptomatic and supportive therapy
  – Treating seizures and liver dysfunction
  – Providing hearing aids
  – Ophthalmologic interventions

• Experimental treatments
  – Steroid-replacement therapy
  – Supplementation with fat-soluble vitamins (A, D, E, and K), and docosahexaenoic acid (DHA)
  – Bile acids and ether lipids
X-linked Adrenoleukodystrophy

- Most common peroxisomal disorder
- Incidence: 1:21,000 males
- XALD gene mapped on chromosome Xq28 (protein ALDP)
- Abnormal accumulation of VLCFAs in brain white matter and adrenal cortex
- Several different phenotypes
- More than 50% of the heterozygotes could be symptomatic
### XALD Phenotypes in Males

**Table 1** X-linked adrenoleukodystrophy phenotypes in males.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Description</th>
<th>Estimated relative frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCER</td>
<td>Onset at 3–10 years of age. Progressive behavioral, cognitive and neurologic deficit, often leading to total disability within 3 years. Inflammatory brain demyelination</td>
<td>31–35%</td>
</tr>
<tr>
<td>Adolescent</td>
<td>Similar to CCER, but somewhat slower progression. Onset age 11–21 years</td>
<td>4–7%</td>
</tr>
<tr>
<td>AMN</td>
<td>Onset 28±9 years, progressive over decades. Involves spinal cord mainly, distal axonopathy inflammatory response mild or absent. Approximately 40% of patients have or develop cerebral involvement, with varying degrees of inflammatory response and more-rapid progression</td>
<td>40–46%</td>
</tr>
<tr>
<td>Adult cerebral</td>
<td>Dementia, behavioral disturbances. Sometimes focal deficits, without preceding AMN. White matter inflammatory response present. Progression parallels that of CCER</td>
<td>2–5%</td>
</tr>
<tr>
<td>Olivo-ponto-</td>
<td>Mainly cerebellar and brainstem involvement in adolescence or adulthood</td>
<td>1–2%</td>
</tr>
<tr>
<td>cerebellar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Addison-only</td>
<td>Primary adrenal insufficiency without apparent neurologic involvement. Onset common before 7.5 years. Most patients eventually develop AMN</td>
<td>Varies with age. Up to 50% in childhood</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>Biochemical and gene abnormality without demonstrable adrenal or neurologic deficit. Detailed studies often show adrenal hypofunction or subtle signs of AMN</td>
<td>Diminishes with age. Common &lt;4 years. Very rare &gt;40 years</td>
</tr>
</tbody>
</table>

Abbreviations: AMN, adrenomyeloneuropathy; CCER, childhood cerebral adrenoleukodystrophy.

*Nat Clin Pract Neurol, 3 (2007)*
## Phenotypes in Females

### Table 2 Phenotypes in females who are heterozygous for X-linked adrenoleukodystrophy.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Description</th>
<th>Estimated relative frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>No evidence of adrenal or neurologic involvement</td>
<td>Diminishes with age. Most women &lt;30 years do not show neurologic involvement</td>
</tr>
<tr>
<td>Mild myelopathy</td>
<td>Increased deep tendon reflexes and distal sensory changes in lower extremities, with absent or mild disability</td>
<td>Increases with age. ~50% at &gt;40 years</td>
</tr>
<tr>
<td>Moderate to severe myeloneuropathy</td>
<td>Symptoms and pathology resemble adrenomyeloneuropathy, but milder and with later onset</td>
<td>Increases with age. ~15% at &gt;40 years</td>
</tr>
<tr>
<td>Cerebral involvement</td>
<td>Rarely seen in childhood and slightly more common in middle age and later</td>
<td>~2%</td>
</tr>
<tr>
<td>Clinically evident adrenal insufficiency</td>
<td>Rare at any age</td>
<td>~1%</td>
</tr>
</tbody>
</table>

*Nat Clin Pract Neurol, 3 (2007)*
X-linked Adrenoleukodystrophy

**Therapy**

- **Hormone replacement therapy**
  - Adrenal insufficiency is a major cause of morbidity and mortality in XALD patients, if left untreated
  - 70% of XALD patients have adrenocortical insufficiency
  - X-ALD is estimated to be the cause of adrenal insufficiency in approximately 35% of patients with idiopathic Addison disease
  - Adrenal function can be impaired as early as 6 months of age

- **Dietary therapy with “Lorenzo’s oil”**
  - Reduction of VLCFAs levels in plasma and fibroblasts of XALD patients, more significant in asymptomatic patients
  - No effect on clinical progression once the inflammatory demyelination starts

- **Hematopoietic stem cell transplantation (HSCT)**
  - Narrow window of opportunity
  - Effective only in early stages of the childhood cerebral form
2. Gather expert opinion to delineate the best evidence for screening for specified conditions and develop recommendations focused on newborn screening, including but not limited to the development of a uniform condition panel.
ACMG Panel: Final Score

Fig. 1. Scoring by test availability (separates out those conditions that have an acceptable, validated, population-based screening test from those that do not).
ACMG Panel: Fact Sheet

Newborn screening panel and system

ACMG Newborn Screening Expert Group

CONDITION
X-Linked adrenoleukodystrophy

TYPE OF DISORDER
Inborn error of peroxisomal fatty acid oxidation

ETHNICITY
Panethnic

SCREENING METHOD(S)
No test available at the present time

NBS STATUS in the US
Screened for in 6 of 51 states, 0% of annual births (August 2004)

CIVILIAN INCIDENCE
1:50,000

Phenotype at birth
Almost never

Burden if undetected
Profound

Survey scores

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>1/50,000</td>
</tr>
<tr>
<td>Phenotype at birth</td>
<td>Almost never</td>
</tr>
<tr>
<td>Burden if undetected</td>
<td>Profound</td>
</tr>
</tbody>
</table>

The test

Screening test
No

Cotesting in tile or by physical method
No

High throughput
No

Overall case <$1
No ($1 test)

Secondary targets
No

Multiplex platform
No

Treatment

Availability of treatment
Not available

Efficacy of treatment
Potential to prevent 80% of cases is not considered effective

Benefits of early intervention
Some evidence that early intervention optimizes outcomes

Benefits of early identification
Some evidence that early intervention optimizes outcomes

Prevention of mental retardation
Not available

Confirmation of diagnosis
Limited availability

Acute management
Limited availability

Simplicity of therapy
Regular involvement of specialist

REFERENCES AND WEB SITES

ASSESSMENT

Not included in uniform panel (no test)

COMMENT

Childhood form accounts for 35% of cases.

Adrenomyeloneuropathy (AMN) form accounts for 40-45% of cases; "Addisons only" form accounts for 10% of cases; 5-10% of cases have a variable phenotype. The therapeutic efficacy of Lorenzo's oil continues to be evaluated and debated. There are limited reports of potential efficacy, but a randomized placebo controlled clinical trial for childhood ALD has not been done to date.

Lovastatin and 4-phenylbutyrate have been proposed as therapeutic agents, but their clinical efficacy has not been tested.
Basic Requirements for NBS

• Condition  Yes
  – Incidence 1:21,000 males
  – Clinically normal at birth
  – Profound burden if untreated

• Treatment  Yes
  – Adrenal hormonal replacement
  – Lorenzo’s oil
  – Hematopoietic Cell Transplantation

• Test  NO
Brief Communication

Combined liquid chromatography–Tandem mass spectrometry as an analytical method for high throughput screening for X-linked adrenoleukodystrophy and other peroxisomal disorders: Preliminary findings

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b Kennedy-Krieger Institute, 700 North Broadway, Baltimore, MD 21205, USA
c Mayo Clinic, 3050 Superior Drive, NW, Rochester, MN 55901, USA

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Available online 7 July 2006
<table>
<thead>
<tr>
<th>Selected LysoPC species&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Controls&lt;sup&gt;n = 19&lt;/sup&gt;</th>
<th>X-ALD and AMN&lt;sup&gt;n = 25&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C22:0</td>
<td>0.021 ± 0.007 0.007–0.033</td>
<td>0.036 ± 0.016 0.009–0.069</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.037 ± 0.019 0.007–0.084</td>
<td>0.163 ± 0.073 0.039–0.308</td>
</tr>
<tr>
<td>C26:0</td>
<td>0.017 ± 0.008 0.005–0.036</td>
<td>0.170 ± 0.119 0.047–0.620</td>
</tr>
</tbody>
</table>

<sup>a</sup>Lysophosphatidylcholine species expressed as nanograms per blood spot area. Data based on blood spot area = 0.0314 cm<sup>2</sup>, or approximately 2.5 μl of blood. Permission obtained from © Hubbard WC et al. (2006) Mol Genet Metab 89: 185–187. Abbreviations: X-ALD, X-linked adrenoleukodystrophy; AMN, adrenomyeloneuropathy; LysoPC, lysophosphatidylcholine.
Alternative Approaches

• LC MS/MS method
  – High selectivity
  – Minimal matrix interference
  – Precision
  – Multiplexing (run time 7 min)

• Flow Injection Analysis
  – Rapid analysis (run time 1.5 min)
  – Software data processing
  – Easier implementation (screening labs)
Clinical Validation

**Internal standard**

<table>
<thead>
<tr>
<th>Masses, amu</th>
<th>Intensity, cps</th>
</tr>
</thead>
<tbody>
<tr>
<td>496.4/104.1</td>
<td>2000</td>
</tr>
<tr>
<td>524.5/104.1</td>
<td></td>
</tr>
<tr>
<td>552.5/104.1</td>
<td></td>
</tr>
<tr>
<td>580.5/104.1</td>
<td></td>
</tr>
<tr>
<td>608.5/104.1</td>
<td></td>
</tr>
<tr>
<td>636.5/104.1</td>
<td></td>
</tr>
<tr>
<td>640.5/104.1</td>
<td></td>
</tr>
</tbody>
</table>

**Q1/Q3 Masses, amu**

**Controls vs. NBS**

**X-ALD vs. NBS**

**MRM experiment**

*NBS: newborn blood spots*
Required Instrumentation

- Instrumentation (API 5000 vs API 3200)

- Sample size

- 3/16” punch
- 1/8” punch
LPC-C26 Species Values
API 5000 vs API 3200 (1/8”)

- 3200 Controls
- 5000 Controls

- 3200 AMN
- 5000 AMN

- 3200 PBD
- 5000 PBD

C26 (μg/mL)
samples
Control N=181  XALD (NB) N=16  XALD (A) N=14
LC-MS/MS Analysis
C26 Lyso-PC

Control N=181  XALD (NB) N=16  XALD (A) N=14
NBS for XALD

Impact on Medical Practice

- Confirmation of diagnosis
- Treatment (how to monitor? when to start?)
- Counseling
- Affected family members:
  - (older) siblings?
  - (symptomatic) female carriers?
  - maternal uncles?
  - etc.

+NBS
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