Lysosomal Acid Lipase Deficiency: Biology, Clinical Manifestations, Diagnosis, and Novel Approach to Treatment

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Learning Objectives
At the end of this presentation the participant will be able to:

1. Understand the biology and the clinical manifestations of lysosomal acid lipase deficiency
2. Know how to test for lysosomal acid lipase deficiency
3. Understand treatment options for patients with lysosomal acid lipase deficiency

Lysosomal Acid Lipase (LAL) Deficiency: A single disease, with marked clinical heterogeneity

- Historical terms to describe the disease
  - "Wolman disease" - 1956 by Dr. Wolman
    - Infant who died at the age of ~two months: GI symptoms, hepatosplenomegaly, poor weight gain, and bilateral adrenal calcifications
  - "Cholesteryl Ester Storage Disease (CESD)" - 1963 by Dr. Fredrickson
    - 12y/o with hypercholesterolemia + hepatomegaly (300-500x increase in CE on biopsy)

- Underlying cause is the same
  - Autosomal recessive disease affecting lipid metabolism due to mutations in the LIPA gene encoding lysosomal acid lipase
  - Results in lysosomal accumulation of lipids (cholesteryl esters and triglycerides)

LAL Deficiency Genetics

- Mutations have variable expression of protein
  - Distinction on clinical progression is not based on enzyme activity – variable assay methods/substrate.
- Common mutation (splice mutation) E8SJM-1

Disclosure
Mark Goldberg, M.D. discloses the following relationships with commercial companies:

- Employee and shareholder of Synageva Biopharma

Biology of Lysosomal Acid Lipase (LAL)

Healthy Individuals

- Normal Lysosome
- Normal Lipid homeostasis
- Hydrolysis yielding free cholesterol and free fatty acids

LAL Deficient Patients

- Enlarged Lysosome
- Disruption of lipid homeostasis
- Accumulation of lipid in lysosome

LAL Deficiency Genetics

- Autosomal recessive
- LIPA gene maps to chromosome 10q23.2-q23.3

- Mutations have variable expression of protein
  - Distinction on clinical progression is not based on enzyme activity – variable assay methods/substrate.
- Common mutation (splice mutation) E8SJM-1
**Lysosomal Acid Lipase Deficiency (LAL D) presents across a clinical continuum**

- **Infants**
  - Often fatal within first 6 months of life
  - Marked growth failure in first few months of life
  - Rapidly progressive liver disease

- **Common Aspects**
  - Autosomal recessive disorder of lipid metabolism
  - Increased mortality and early mortality
  - Fatty liver, elevated transaminases fibrosis/cirrhosis

- **Children & Adults**
  - Complications of chronic liver disease (bleeding varices and ascites)
  - Dyslipidemia: Increased LDL and decreased HDL
  - Adrenal calcification frequently present

- **Other**
  - Prominent hepatic and GI manifestations
  - Persistent vomiting, diarrhea
  - Abdominal distension
  - Profound growth failure
  - Hepatomegaly and liver failure
  - Splenomegaly

- **High potential for delayed or misdiagnosis**
  - More frequent presentation of LAL Deficiency
  - Historically known as Cholesteryl Ester Storage Disease
  - 2013 literature review

- **Rapid Disease Progression in LAL D Infants**
  - Rapidly progressive and fatal
  - Prominent hepatic and GI manifestations
  - Persistent vomiting, diarrhea
  - Abdominal distension
  - Profound growth failure
  - Hepatomegaly and liver failure
  - Splenomegaly
  - Adrenal calcification frequently present
  - Incidence: ~1/500,000
  - Treatment options:
    - No safe and effective therapies
    - HSCT (and liver transplant) have limited success and high mortality

- **LALD Presenting in Infants Typical Sign/Symptoms & Differential**

- **Presentation in Children & Adults**

- **LAL Deficiency presenting in childhood or adulthood:**
  - A rare disease with a common phenotype

- **Typical Findings**
  - Metabolic syndrome: combination of fatty liver, elevated serum transaminases, and dyslipidemia
  - Some are diagnosed in childhood, while others remain undiagnosed until adulthood

- **Differential Diagnosis**
  - Prolonged “gastroenteritis” with growth failure
  - Hemophagocytic lymphohistiocytosis (HLH)
  - Glycogen storage disease
  - Cryptogenic liver cirrhosis
  - Niemann-Pick disease Type C
  - Chanarin-Dorfman syndrome
  - Gaucher disease
  - Fruatose intolerance and other amino acid metabolism disorders

- **Lysosomal Acid Lipase Deficiency (LAL D) presents across a clinical continuum**

- **Disease Spectrum**

- **Kaplan-Meier Estimate: Survival in LAL D Infants with Growth Failure**

- **Typical Findings**
  - Age at Symptom Onset: 1.0 Month
  - Age at Diagnosis: 2.6 Months
  - Age at Death: 3.7 Months

- **Laboratory**
  - Elevated transaminases
  - Elevated ferritin
  - Elevated triglycerides
  - Elevated cholesterol

- **Imaging**
  - Hepatomegaly and/or splenomegaly
  - Adrenal calcifications (may not be present)

- **Presentation in Children & Adults**

- **Prevalence:** 1:40,000 – 1/300,000

- **High potential for delayed or misdiagnosis**
  - Metabolic syndrome: combination of fatty liver, elevated serum transaminases, and dyslipidemia
  - Focusing on non-obesity may increase the suspicion for LAL D

- **More frequent presentation of LAL Deficiency**

- **Historically known as Cholesteryl Ester Storage Disease**

- **2013 literature review**

- **Disease presentation is variable**

- **High potential for delayed or misdiagnosis**

- **Population shown are subjects who did not undergo HSCT or liver transplant

- **Data on File, Synageva BioPharma Corp.**

- **Typical Findings**
  - Fatty liver, elevated transaminases fibrosis/cirrhosis

- **Adrenal calcifications (may not be present)**

- **Prevalence:** 1:40,000 – 1/300,000
ALT Elevation Is Common, Persistent And Present From A Young Age

- ALT values were persistently elevated
- The majority (458 of 499 values; 92%) ALT values were above 43 U/L, with only a small proportion (41 of 499 values; 8%) of values being ≤ 43 U/L at any time.
- ALT values were generally comparable in subjects with and without biopsy-proven fibrosis and/or cirrhosis.

Quinn et al. WORLD 2014

LDL Elevation is Common

- Most LDL values (88%; 270 of 306) were > 100 mg/dL, with many values in a range indicative of substantial dyslipidemia in the study population.
- LDL values > 100 mg/dL were common even if LLM had been initiated, with 27 subjects having at least 1 LDL value > 100 mg/dL while receiving LLM.

Quinn et al. WORLD 2014

LAL Deficiency Not Widely Recognized As a Cause of Low HDL

- Highest recorded Total Cholesterol
- Lowest Recorded HDL

Tripuraneni et al. NLA 2013

Lipid Abnormalities In LALD are Broader than Classically Described Type II Hyperlipidemia

- Highest recorded Total Cholesterol
- Highest recorded Triglyceride

NLA 2013

Clinical Summary from Bernstein et al.

- Distinctive histopathological features that support a diagnosis of cholesterol ester storage disease in liver biopsy specimens

Hepatomegaly
- Presented in 134 (99%) of patients

Splenomegaly
- Presented in 100 (74%) of patients

Transaminase Levels
- Elevated AST and/or ALT activities in all cases

Bernstein et al. Cholesterol Ester Storage Disease: Review of the Findings in 135 Reported Patients with an Under Diagnosed Disease.

<table>
<thead>
<tr>
<th>Clinical Summary from Bernstein et al.</th>
<th>Review of the 135 cases/publications describing LALD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age of Onset</td>
<td>5 years of age</td>
</tr>
<tr>
<td>Male (birth – 44)</td>
<td>Female (1 month-68)</td>
</tr>
<tr>
<td>Distribution of Age of Onset (131 pts)</td>
<td>116 (89%) presented between age 3 and 12 years,</td>
</tr>
<tr>
<td></td>
<td>15 (11%) had onset or diagnosis during adolescence or</td>
</tr>
<tr>
<td></td>
<td>as adults</td>
</tr>
<tr>
<td></td>
<td>5 patients whose diagnoses were made at autopsy</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>Presented in 134 (99%) of patients</td>
</tr>
<tr>
<td>Splenomegaly</td>
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</tr>
<tr>
<td>Transaminase Levels</td>
<td>Elevated AST and/or ALT activities in all cases</td>
</tr>
</tbody>
</table>
Clinical Summary from Bernstein et al. (cont)

Liver Injury and/or Liver Failure (135 pts)
- Occurred in all patients
- Death due to liver disease progression: 7-66 years old
- 50% of deaths were in patients under 21 years of age.

Liver Biopsy (112 (83%) pts)
- A striking orange-yellow in color
- Diffuse, uniform microvesicular steatosis
- 72 (64%) had fibrosis and/or cirrhosis
- Findings were consistent among patients, and appeared independent of age, genotype, or other factors

Management Options & Clinical Trials
- Infants
  - Electrolyte replacement, parenteral nutrition, formula modifications, etc.
- Children & Adults
  - Statins/lipid lowering agents – but liver disease progression can still occur
- Hematopoietic stem cell or liver transplantation
  - Has been associated with serious complications (e.g., death, graft-versus-host disease)
- Enzyme replacement therapy (ERT)
  - Sebelipase alfa is an investigational ERT
  - Reported encouraging phase ½ results in LAL deficient adults
  - Ongoing trials for LAL deficient infants (LAL-CL03) and children/adults (ARISE)

Sebelipase Alfa: Pre-Clinical and Clinical Development

Sebelipase alfa Preclinical Targeting and Activity

Sebelipase alfa: Targeting and Activity

Terminal mannose/GlcNac and mannose-6-phosphate (M6P) for targeted delivery

In Vivo Activity

LAL Deficiency Rat Model Reproduces Key Aspects of Human Disease

Accumulation Lipid Substrate in Liver, Spleen and Gut

Growth failure

Increased Mortality

Maximal life span in LAL-/- rats is approximately 14 weeks

Rat model 1st described Japan 1980

In Vivo Activity

*sebelipase alfa 5mg/kg* once weekly for 4 weeks
Sebelipase alfa
Preclinical Targeting and Activity

In Vivo Activity

* sebelipase alfa 5mg/kg once weekly for 4 weeks

Sebelipase alfa (SBC-102) Restores Normal Growth And Increases Survival in Preclinical Disease Model

Improvements In Growth And Organ Size Is Associated With Correction Of Underlying Pathology

Liver

Placebo

Sebelipase alfa

SBC-102

Placebo

Liver

Sebelipase alfa

LAL-CL03 (Phase 2/3 Trial)

Trial Design

Endpoints

- LAL D infants with growth failure first 6 months of life
- Open label
- Intra-patient dose escalation of sebelipase alfa
  - 0.35 mg/kg to 3 mg/kg
- Once weekly dosing
- Multicenter

Primary Endpoint:
- Survival at 12 months of age

Key Secondary Endpoints:
- Safety and tolerability
- Survival beyond 12 months of age
- Growth
- Liver parameters (AST, ALT, GGT, Alk Phos, Bilirubin)
- Lipids
- Development (Denver II)
- Pharmacokinetics

Key Inclusion & Exclusion Criteria

Inclusion:
- Confirmed diagnosis of LAL D
- Growth failure with onset before 6 months of age
  - Weight decreasing across at least 2 of the 11 centile lines on WHO weight for age chart OR
  - Body weight in kg below the 10th centile AND no weight gain for the 2 weeks prior to screening OR
  - Loss of ≥ 5% of birth weight in a child older than 2 weeks
- Infants with rapidly progressive course if no growth failure*

Exclusion:
- Myeloablative preparation, or other systemic pre-transplant conditioning
- Previous hematopoietic stem cell or liver transplant
*Exceptional circumstances and require review with the safety committee prior to enrollment

Patient Disposition & Baseline Characteristics

- Data as of June 2014
- 9 patients enrolled
- Median age at time of first infusion: 3.0 months
  - Range 1.1 – 5.8 months
- Range of time in the trial: 0.6 to 31.8 months

Signs and Symptoms:
- Diarrhea or Vomiting (6 of 9)
- Hepatomegaly (7 of 9)
- Spleenomegaly (6 of 9)
- Adrenal calcification (4 of 9)

Laboratory Values:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>96 U/L</td>
<td>16-297 U/L</td>
</tr>
<tr>
<td>AST</td>
<td>125 U/L</td>
<td>71-716 U/L</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>7 umol/L</td>
<td>2.4-446 umol/L</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>9.3 g/dL</td>
<td>7.2-10.2 g/dL</td>
</tr>
<tr>
<td>Platelets</td>
<td>173 x 10^12/L</td>
<td>39-583 x 10^12/L</td>
</tr>
</tbody>
</table>
Kaplan-Meier Estimate of Survival in LAL D Infants with Growth Failure*: Results of Natural History Study

*Population shown are subjects who did not undergo HSCT or liver transplant

Source: Jones et al, LDN 2014 poster #113

Dosing Status

6 Subjects continue on sebelipase alfa
3 Deceased Subjects
  - Not related to sebelipase alfa

<table>
<thead>
<tr>
<th>Infusion History</th>
<th>Age at Death</th>
<th>Relationship to sebelipase alfa</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 infusion (0.35 mg/kg/wk)</td>
<td>3 months</td>
<td>Not related</td>
<td>Complications of disease</td>
</tr>
<tr>
<td>1 infusion (0.35 mg/kg/wk)</td>
<td>2 months</td>
<td>Not related</td>
<td>Complications of disease</td>
</tr>
<tr>
<td>4 infusions • 2 infusions (0.35 mg/kg/wk) • 2 infusions (1.0 mg/kg/wk)</td>
<td>4 months</td>
<td>Not related</td>
<td>Complications of disease</td>
</tr>
</tbody>
</table>

Valayannopoulos et al. WORLD 2014

Growth Curve of Subject 1

Subject 1 (M)

Survival at 12 months

Safety

- Majority of SAEs:
  - Were not related to sebelipase alfa
  - Related to documented central line infections or hospitalizations for empirical treatment with antibiotics
- 3 related SAEs
  - Occurred in a single subject in association with the same infusion
    - Fever (malaise)
    - Malaise with tachycardia
    - Tachycardia
  - Resulted in overnight hospitalization
    - Treated with IV antibiotics empirically for possible line bacteremia (all cultures negative)
- Majority of IRRs have been mild
  - Fever
  - Vomiting

Valayannopoulos et al. WORLD 2014
4 subjects developed anti-drug antibody (ADA)

- Subject 3 - ADA positive after 7 weeks on treatment
- Subject 5 - ADA positive after 8 weeks on treatment
- Subject 6 - ADA positive after 16 weeks on treatment
- Subject 1 - ADA positive after 58 weeks on treatment

No apparent change in clinical response after ADA development

Sebelipase Alfa:
Experience in Adults

102 wk data
R. Tripuraneni et al. Effect of Sebelipase in Adults with Lysosomal Acid Lipase Deficiency Oral Presentation EAS 2014

Characteristics and Baseline Test Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population</th>
<th>Lab Analyte</th>
<th>Reference Range</th>
<th>Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, yrs)</td>
<td>29</td>
<td>ALT (U/L)</td>
<td>≤67 (22 to 119)</td>
<td>76 (22 to 119)</td>
</tr>
<tr>
<td>Male:Female (n)</td>
<td>6:3</td>
<td>AST (U/L)</td>
<td>≤60 (37 to 69)</td>
<td>56 (37 to 69)</td>
</tr>
<tr>
<td>White (n)</td>
<td>9</td>
<td>Total Chol (mg/dL)</td>
<td>69-232</td>
<td>182 (116 to 291)</td>
</tr>
<tr>
<td>BMI (median, kg/m²)</td>
<td>25.2</td>
<td>LDL (mg/dL)</td>
<td>≤162 (70 to 300)</td>
<td>135 (70 to 300)</td>
</tr>
<tr>
<td>Hepatomegaly (n)</td>
<td>8</td>
<td>HDL (mg/dL)</td>
<td>≥35 (22 to 49)</td>
<td>39 (22 to 49)</td>
</tr>
<tr>
<td>Lipid lowering meds (n)</td>
<td>7</td>
<td>Triglycerides (mg/dL)</td>
<td>≤199 (80 to 277)</td>
<td>108 (80 to 277)</td>
</tr>
<tr>
<td>Elevated* ALT or AST (n)</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Elevated: ≥1.5xULN and <3xULN.

RED = abnormal value

Improvement in Transaminases
Mean percent decrease: 58% ALT and 28% AST (wk 104)

Improvement in Dyslipidemia Profile
Mean percent decrease: 54% LDL, 31% TG (wk 104)
Mean percent increase: 18.4% HDL (wk 104)
Rapid, Sustained Reduction in Liver Volume and Fat Fraction (MRI)

Percent Change From CL04 Baseline

<table>
<thead>
<tr>
<th>Week 10</th>
<th>Week 24</th>
<th>Week 52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver Volume</td>
<td>Liver Fat Fraction</td>
<td></td>
</tr>
<tr>
<td>-10%</td>
<td>-30%</td>
<td>0%</td>
</tr>
<tr>
<td>-5%</td>
<td>-12%</td>
<td>0%</td>
</tr>
<tr>
<td>-3%</td>
<td>-4%</td>
<td>0%</td>
</tr>
<tr>
<td>-2%</td>
<td>-5%</td>
<td>0%</td>
</tr>
<tr>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Safety Profile

- No drug-related serious adverse events (SAE) in this trial
- One unrelated SAE: cholecystitis and cholelithiasis
  - The subject underwent elective cholecystectomy.
  - The subject has continued in the study.
- No evidence of anti-drug antibodies in subjects tested to date in this study
- Most infusion-related reactions (IRR) were mild, mainly GI related (diarrhea, abdominal cramping).

ARISE Trial

Study Design
- Randomized, double-blind, placebo controlled
- Multi-center global trial
- N = 50

Endpoints
- Normalization in ALT
- Change in LDL, non-HDL, TG, HDL
- Change in liver volume and liver fat content
- Improvement in liver histopathology

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Which Patient to Test for LAL D?

Hepatologists
- All non-obese* patients with persistent hepatomegaly OR
- Unexplained elevation in transaminase
- Cryptogenic cirrhosis
- Microvesicular steatosis or macro/microvesicular steatosis
- Can also do additional IHC staining

Lipidologists
- Non-obese: LDL >=160mg/dL & HDL <40mg/dL (males) or <50mg/dL (females)
- Presumed familial hypercholesterolemia (FH) patients with unclear family history
- Presumed FH patients who have negative genetic testing for the genes encoding LDLR, APO B, and PCSK9 genes

Recent Development of Dry Blood Spot Assay Allows for Easy Testing

Blood Test for LAL D

- LAL activity is determined by via a LAL specific inhibitor
- Allows for the possibility of testing from blood spots

Labs That Perform LAL D Testing (US)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Contact Information</th>
<th>Data</th>
<th>Reference</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massachusetts General Hospital, Boston, MA</td>
<td>Phone: 1-617-732-5690</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Seattle Children’s Hospital and University of Washington</td>
<td>Phone: 1-206-882-5210</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Baylor College of Medicine</td>
<td>Phone: 1-816-416-8080</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>GeneDx, Gaithersburg, MD</td>
<td>Phone: 1-301-519-2100</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>Thomas Jefferson University, Philadelphia, PA</td>
<td>Phone: 1-215-955-4923</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Laboratory Corporation of America</td>
<td>Phone: 1-800-345-4363</td>
<td>X</td>
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<td>X</td>
</tr>
</tbody>
</table>

Recent Development of Dry Blood Spot Assay Allows for Easy Testing

LAL Activity

<table>
<thead>
<tr>
<th>LAL Activity</th>
<th>LAL Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Elevated</td>
<td>Elevated</td>
</tr>
</tbody>
</table>

LAL deficiency is an under-recognized cause of cirrhosis, accelerated atherosclerosis, and early death. It usually presents in childhood. Key signs in children and adults include: (not all are required)
- Elevated transaminases, hepatomegaly and/or microvesicular steatosis
- Dyslipidemia (elevated LDL and low HDL)
Diagnosis can be made via a simple blood test. Analysis of sebelipase alfa in the ongoing clinical trials:
- Improved growth & survival in infants
- Produces sustained improvements in the biochemical markers of liver damage, and the dyslipidemia in the adults
- Safety and tolerability profile is encouraging after administration of more than >300 infusions.

US and European Guidelines: Endorse Testing to Rule Out LAL D
- Symptoms overlap with hereditary disorders
- Rule out LAL D in microvesicular steatosis cases

Liver Disease: Two Rare Diseases

Wilson disease
- Symptoms >3y of age
- Acute presentation at any age >3y
- Chronic progressive disease
- Autosomal recessive

LAL Deficiency
- Symptoms from infancy
- Acute presentation in infancy
- Chronic progressive disease >2y of age
- Autosomal recessive

Is tested in each child with liver disease >3y

Should we routinely test for LAL D in children with liver disease?

Thank you!