Early Onset
Lysosomal Acid Lipase (LAL) Deficiency
-- A Rare Disorder With a Possible New Treatment

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Current Enzyme Replacement Therapies (ERT) at WCHOB

- MPS I (Hurler, Scheie) – Aldurazyme (alpha-L-iduronidase)
- MPS II (Hunter) - Elaprase (idursulfase)
- Pompe disease (GSD II) – Lumizyme (alglucosidase alfa)
- Fabry disease – Fabrazyme (alpha-galactosidase A)
- Gaucher disease type 1 - Cerezyme (imiglucerase)
Case History

• The patient is presented at age 6 and was the family’s 1st child. He now has a younger sister and brother.
• He was well until age 10 days when he developed lethargy, rash and fever, and subsequently “stopped breathing.”
• At WCHOB, chronic constipation led to testing that showed elevated liver transaminases [ALT 77 units/L (ref 5-50), AST 69 units/L (ref 5-50)], lipid abnormalities [cholesterol HDL 37 mg/dL (ref 40-60), cholesterol LDL 186 mg/dL (ref 0-100), triglycerides 83 mg/dL (ref 30-100)] and ultrasonographic evidence of hepatomegaly (14.3 cm).
• He was subsequently followed by several gastroenterologists who performed several laboratory tests and ultimately a liver biopsy on 2/8/12.
• He was followed with a descriptive diagnosis of hepatitis but studies had not disclosed an etiological diagnosis. He had no similarly affected relative. He is referred to Genetics for possible inherited metabolic liver disease.
MICROVESICULAR
Blood Spot Lysosomal Acid Lipase (LAL, LIPA, cholesterol ester hydrolase) Assay

- Lysosomal Acid Lipase (LAL) Deficiency/Cholesterol Ester Storage Disease Test Results (Enzyme Analysis).
  - Patient history/indication: Hepatosplenomegaly, microvascular stenosis/mild fibrosis suggesting CESD.
- RESULT: LAL (LIPA) lysosomal acid lipase (cholesterol ester hydrolase) <0.02 mM/punch*ht. Normal range: 79.9-378.6 nM/punch*hr.
  - Normal control sample: 186.74 nM/punch*hr. Deficient control sample: <0.02 nM/punch*hr.
- INTERPRETATION: In this sample, LAL enzyme levels are deficient. LAL enzyme deficiency is consistent with a defect in the LIPA gene and a diagnosis of Wolman disease (early onset) and Cholesterol Ester Storage Disease (late onset).

8/10/12
Lysosomal Acidic Lipase (LAL) Deficiency/ LIPA Gene Mutation Analysis

• RESULTS:
  – Heterozygous exon 7 c.796G>T, p.Gly266X
  – Heterozygous exon 8 c.894G>A

• INTERPRETATION: Two presumed pathogenic mutations were identified by sequencing, an exon 7 nonsense mutation and an exon 8 synonymous mutation. Although the exon 8 mutation is silent, this change occurs at the last nucleotide of the exon and interferes with the normal splicing process. This mutation (E8SJM) has been previously reported in patients with CESD.

8/10/12
The following slides are from a slide set provided by Synageva BioPharma and have not been altered.

http://www.clinicaltrials.gov
(search Synageva)
An Overview of Late Onset Lysosomal Acid Lipase (LAL) Deficiency

June 2012
Discussion

• Overview of Lysosomal Acid Lipase (LAL) Deficiency

• Biology of LAL Deficiency

• Overview of late onset LAL Deficiency

• Analysis of late onset LAL Deficiency in the medical literature

• Diagnosis of LAL Deficiency

• Clinical options in late onset LAL Deficiency
LAL Deficiency Terminology

**LAL Deficiency is sometimes called:**
- Cholesterol Ester Storage Disease or CESD (late onset LAL Deficiency)
- Wolman disease (early onset LAL Deficiency)
- Acid Cholesteryl Ester Hydrolase Deficiency, Type 2
- Acid Lipase Disease
- Cholesteryl Ester Hydrolase Deficiency Storage Disease
- LIPA Deficiency

**Differential Diagnosis:**
- Non-Alcoholic Fatty Liver Disease (NAFLD)
- Non-Alcoholic Steatohepatitis (NASH)
- Alcoholic Liver Disease
- Cryptogenic Cirrhosis
- Niemann-Pick Disease (NPD) Type C
- Chanarin-Dorfman Syndrome
Lysosomal Acid Lipase (LAL) Deficiency

- LAL Deficiency is a rare, autosomal recessive disorder caused by a decrease or absence of the LAL enzyme.
- Leads to an accumulation of cholesteryl esters and triglycerides in various tissues which results in hepatomegaly, splenomegaly, and liver fibrosis/cirrhosis.
- Two major phenotypes presenting as a clinical continuum:
  - Early onset (also referred to as Wolman disease) which occurs in infants
  - Late onset (also referred to as Cholesteryl Ester Storage Disease [CESD]) affecting children and adults
Lysosomal Acid Lipase (LAL) Biology

**Endocytosis**

- LDL binds to LDL receptors
- LDL is internalized into the cell through coated vesicles
- LDL is then directed to lysosomes

**Liver lipid content in a rat model of LAL Deficiency**

- **Cholesteryl Ester**
  - LAL Deficient: High
  - Normal: Low

- **Triglyceride**
  - LAL Deficient: High
  - Normal: Low

**Free cholesterol and fatty acids are key regulators of lipid synthesis**
LAL Deficiency
Single Disease with Early and Late Onset Phenotypes

Liver Complications
- Fatty liver/steatosis
- Fibrosis
- Liver failure
- Liver transplant
- Death

GI Complications
- Persistent vomiting
- Diarrhea
- Severe Malabsorption
- Growth failure
- Death

Cardiovascular Risk
- Accelerated atherosclerosis
- MI
- CVA
- > Mortality risk

Impaired QoL
- Reduced physical functioning
- Fatigue

Possible Differential Diagnosis:
LAL Deficiency (Wolman and Cholesterol Ester Storage Disease)

Rare Disease Embedded in a Common Phenotype: NAFLD

When should a diagnosis of CESD be considered in a patient with Fatty Liver and/or Abnormal Transaminases?
One or more of the following Clues can help narrow down the Phenotype:
• BMI < 30 (non-obese)
• Onset of liver abnormalities in childhood
• Very low HDL (< 10 mg/dl)
• Disproportionate spleen enlargement for degree of liver disease

To Check for ongoing Clinical Trials of a novel enzyme replacement therapy:
www.clinicaltrials.gov

Estimated Prevalence:
25 per million*

Testing:
www.genetests.com

For more information:
Eugene Schneider, MD, Medical Director
Eugene.Schneider@Synageva.com
781-357-9920

Late Onset LAL Deficiency: a rare disease with a common phenotype

- Estimated prevalence of 25:1 million\(^1\)
  - Similar to other Lysosomal Storage Disorders (e.g., Gaucher, Fabry, Pompe)
- Prominent hepatic manifestations
  - Fatty liver
  - Hepatomegaly
  - Elevated transaminases
- Cardiovascular involvement
  - Type IIB hyperlipoproteinemia
  - Low HDL has been observed

\(^1\) Muntoni, et al; “Prevalence of Cholesteryl Ester Storage Disease”, Arteriosclerosis, Thrombosis, and Vascular Biology, July 19, 2010
Late Onset LAL Deficiency Presents Across the Age Continuum with Significant Morbidity

<table>
<thead>
<tr>
<th>Age</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 years</td>
<td>Hepatosplenomegaly, hyperlipidemia and hypertransaminasemia determined incidentally, orange-yellow liver</td>
</tr>
<tr>
<td>4 years</td>
<td>Hepatomegaly, splenomegaly, hypertriglyceridemia, hypercholesterolemia. Liver biopsy showed steatosis, infiltration of hepatocytes, and macrophages with lipids</td>
</tr>
<tr>
<td>5 years</td>
<td>Hyperlipoproteinemia, prolonged diarrhea, and hepatosplenomegaly, cirrhotic liver, enlargement of adrenal glands with calcification</td>
</tr>
<tr>
<td>5 years</td>
<td>Hypercholesterolemia and hepatosplenomegaly</td>
</tr>
<tr>
<td>7 &amp; 9 years</td>
<td>Hepatic fibrosis and portal hypertension. Esophageal varices and aortic plaques in the older child, death at 7 (siblings) and 9 yrs old</td>
</tr>
<tr>
<td>9 years (Twins)</td>
<td>High liver enzymes, hyperlipidemia, hepatosplenomegaly, ~5% LAL activity</td>
</tr>
<tr>
<td>11 years</td>
<td>Hyperlipidemia (cholesterol 323, triglycerides 259 md/dl), high liver enzymes</td>
</tr>
<tr>
<td>13 years</td>
<td>Hepatomegaly</td>
</tr>
<tr>
<td>18 years</td>
<td>Hepatomegaly, elevated triglycerides, low HDL-C</td>
</tr>
<tr>
<td>26 years</td>
<td>Abdominal pain, elevated LFTs, compound heterozygote with common allele</td>
</tr>
<tr>
<td>36 years</td>
<td>Hepatosplenomegaly and anemia, elevated chitotriosidase, accumulation of cholesterol esters in skin fibroblasts</td>
</tr>
<tr>
<td>41 years</td>
<td>Chronic diarrhea which caused deterioration due to weight loss, high LFTs, increase Kupffer cells in liver (sinusoids and portal tracts)</td>
</tr>
<tr>
<td>51 years</td>
<td>Hypercholesterolemia, accelerated atherosclerosis and liver cancer, no hepatomegaly</td>
</tr>
<tr>
<td>80 years</td>
<td>Severe hyperlipidemia/low LDL, compound heterozygote of two rare alleles</td>
</tr>
</tbody>
</table>
## Analysis of Late Onset LAL Deficiency Cases in Medical Literature

### n=75 cases

<table>
<thead>
<tr>
<th>Gender</th>
<th>n=50 (66.7%)</th>
<th>Age at Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>31 (41.3%)</td>
<td>Mean 14.3 yo</td>
</tr>
<tr>
<td>Female</td>
<td>43 (57.3%)</td>
<td>SD ±15.0</td>
</tr>
<tr>
<td>Alive</td>
<td>64 (85.3%)</td>
<td>Median 9</td>
</tr>
<tr>
<td>Deceased</td>
<td>6 (8.0%)</td>
<td>min-max 2-80</td>
</tr>
</tbody>
</table>

| Data not recorded | 1 (1.3%) | 5 (6.7%) | 25 (33.3%) |

### n=75 cases

<table>
<thead>
<tr>
<th>Hepatomegaly or abnormal transaminases</th>
<th>Splenomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data available</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>74 (98.7%)</td>
</tr>
<tr>
<td>No</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Yes</td>
<td>37 (49.3%)</td>
</tr>
<tr>
<td>No</td>
<td>5 (0.7%)</td>
</tr>
</tbody>
</table>

| Data not recorded | 1 (1.3%) | 33 (44.0%) |
Gallbladder Dysfunction in LAL Deficiency

JPGN 50 (5) 555 2010

- Late onset LAL deficiency (CESD) is not generally appreciated as a cause of RUQ/abdominal pain and gallbladder disease

- Mechanism unclear but may be parallels with findings in Gaucher disease where the overall prevalence has been shown to be 32% (Taddei TT et al, 2010)
Cerebrovascular Risk in Patients with Late Onset LAL Deficiency

- Cerebrovascular disease described as incidental finding in other case reports

- No systematic analysis of prevalence of LAL Deficiency in “young” stroke patients

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**Case 2**

A female adolescent presented at the age of 13 years with 2 episodes of transient loss of right-sided vision, facial weakness, and mild right hemiparesis. A cranial magnetic resonance imaging showed a small left frontal infarction. An extensive diagnostic workup was inconclusive apart from elevated cholesterol and triglycerides, which was unexplained. She was empirically started on a low-fat diet.

**Case Report**

A 36-year-old female patient was admitted because of hepatosplenomegaly and anemia. At the age of 2 years, icterus and slightly raised brownish skin spots had developed and hepatomegaly was diagnosed. A lipid storage disease was suspected and a fat-restricted diet was recommended. Five years before admission, subtotal thyroid gland resection had been performed for goiter. Neurological evaluation showed slight hemiparesis of the left arm, suggestive of mild stroke in the past.

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Haller W et al, JPGN 50 (5) 555 2010

Late Onset LAL Deficiency: Differential Diagnosis

**Signs and Symptoms**

- Liver
  - Microvesicular Steatosis
  - Hepatomegaly with or without Splenomegaly
  - Elevated Transaminases
  - Unexplained Cirrhosis

- Lipid
  - Elevated LDL
  - Elevated Triglycerides
  - Low HDL
  - Elevated Total Cholesterol

  - Other
    - Malabsorption
    - Unexplained Short Stature

**Differential Diagnosis**

- Late onset LAL Deficiency (CESD)
- Non-Alcoholic Fatty Liver Disease (NAFLD)
- Non-Alcoholic Steatohepatitis (NASH)
- Glycogen Storage Disorder
- Alcoholic Liver Disease (ALD)
- Cryptogenic Cirrhosis

**Diagnostic Testing Resources**

- [orpha.net](http://www.orpha.net/consor/cgi-bin/ClinicalLabs.php)
- [GENETests™](http://www.ncbi.nlm.nih.gov/sites/GeneTests/)

Dried blood spot (DBS) enzyme activity assay is also available.¹


For more information on the DBS assay or to request supplies, please contact Synageva at info@synageva.com.
Dried Blood Spot (DBS) Enzyme Activity Assay Can Expedite Testing for LAL Deficiency

- Allows for the possibility of testing high risk populations
- A highly specific LAL inhibitor is critical to the methods
  - LAL activity is determined by measurement of total lipase activity and lipase activity in the presence of the inhibitor

Late Onset LAL Deficiency: Where Can I Find Information About Testing?

Sources of information regarding laboratories who offer diagnostic testing:

- **www.genetests.org**: Click laboratory directory. Enter the search term “Cholesterol Ester Storage Disease” in the Disease Name category. Click on the “Testing” tab.

- **www.orpha.net**: Click the tab “Diagnostic Tests”. Enter the search term “LAL Deficiency” under the Simple Search category with “Disease name” selected. Click on “Lysosomal Acid Lipase (LAL) Deficiency”.

- Dried blood spot testing for LAL Deficiency via Yorkhill Laboratory in Scotland (John.Hamilton2@ggc.scot.nhs.uk) and Massachusetts General Hospital (MGH) in Boston (kagoss@partners.org)
Late Onset LAL Deficiency: Treatments?

• Currently, there is no approved treatment
• High blood lipid levels are treated with a combination of low-fat diet and lipid-lowering medications although there is no evidence that they improve the underlying disease
An Overview of SBC-102

- SBC-102 (recombinant human lysosomal acid lipase (LAL) enzyme), is an investigational enzyme replacement therapy for LAL Deficiency, a lysosomal storage disorder (LSD)
- Received orphan drug designations in both the US and EU in 2010 for SBC-102 for LAL Deficiency
- SBC-102 is designed to replace the functionality of the LAL enzyme, which is responsible for the metabolism of cholesteryl esters and triglycerides that are delivered to lysosomes by a variety of routes including low-density lipoprotein (LDL) receptor mediated endocytosis.
SBC-102

Preclinical Targeting and Activity

Cellular Targeting & Effects

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

• Uptake into key cells, including macrophages

• Lysosomal localization demonstrated

• Corrects enzyme deficiency

In Vivo Activity

* SBC-102 5mgkg⁻¹ once weekly for 4 weeks
SBC-102 in Preclinical Disease Model

Growth failure-dose response qw dosing

Body weight (grams)

Age (days)

Wild type

3 mgkg\(^{-1}\) qw

5 mgkg\(^{-1}\) qw

1 mgkg\(^{-1}\) qw

0.35 mgkg\(^{-1}\) qw

LAL Deficient
## SBC-102
### Clinical Development in Late Onset LAL Deficiency

#### Phase I/II Open-Label
- Adult patients with liver dysfunction due to LAL Deficiency
- Open label dose escalation
- 4 week dosing
- 3 dose levels
- Multicenter
- $N = 9$
- **Study Completed**
- Patients continue to be treated in extension study

#### Randomized, Double-Blind, Placebo-Controlled Trial*
- Patients with liver dysfunction due to LAL Deficiency
- Randomized, placebo-controlled study
- Multicenter
- $N = \text{TBD}$

### Trial Measurements:
- Pharmacodynamic markers
- Liver Function Tests

### Endpoints:
- Safety, Efficacy and Pharmacokinetics

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* Late Onset Natural History Study On-going

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* subject to discussions with regulatory agencies
SBC-102: Phase I/II (Late Onset) Results

• SBC-102 was well tolerated at all dose levels

• Once weekly infusion of SBC-102 for 4 weeks demonstrates biological activity at all dose levels as evidenced by:
  • Rapid decreases in serum transaminases
  • Increased blood lipid levels due to mobilization of tissue lipid

• Patients continue to be treated in extension study
  • Assessments will include liver fat fraction, serum transaminases and lipids
LAL-CL01

Effects on Transaminases

<table>
<thead>
<tr>
<th></th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>D14</td>
</tr>
<tr>
<td></td>
<td>p=0.021</td>
<td>p=0.008</td>
</tr>
<tr>
<td>Normal (N)</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>ULN</td>
<td>70</td>
<td>55</td>
</tr>
<tr>
<td>25th Percentile</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>75th Percentile</td>
<td>80</td>
<td>60</td>
</tr>
</tbody>
</table>

Medians with 25th and 75th Percentiles
### Summary

<table>
<thead>
<tr>
<th><strong>Severity</strong></th>
<th>• Late onset LAL Deficiency is a progressive disease with significant morbidity and mortality in pediatric and adult cases</th>
</tr>
</thead>
</table>
| **Presentation** | • The presenting signs and symptoms are relatively common  
• Good disease awareness is essential for early recognition and diagnosis |
| **Diagnosis** | • Advances in DBS diagnostic testing have the potential to simplify LO LALD diagnosis  
• Differential liver pathology compared to NAFLD/NASH  
• IHC staining for lysosomal markers such as Cathepsin D, LAMP 1, LAMP 2 and Integral Membrane Protein for late onset LAL Deficiency |
| **Investigational modalities** | • SBC-102 is well-tolerated at all dose levels (Phase I/II study) and demonstrates biological activity  
• Study of investigational enzyme replacement therapy is ongoing  
  [http://www.clinicaltrials.gov](http://www.clinicaltrials.gov) (search Synageva) |
Resources

• Patient Organizations
  – LAL Solace, www.lalsolace.org
  – National Information Centre for Metabolic Diseases (CLIMB), www.climb.org.uk/
  – Vaincres les Maladies Lysosomales, www.vml-asso.org
  – National Organization for Rare Disorders, www.rarediseases.org
  – American Liver Foundation, www.liverfoundation.org

• Healthcare Organizations
  – NASPGHAN, www.naspghan.org
  – American Association for the Study of Liver Diseases, www.aasld.org
  – American Gastroenterological Association, www.gastro.org