Case 2 History

- Two month old male admitted for G-tube and fundoplication because of failure to thrive (he weighed 3745 g; length was 57 cm). Shortly after birth at term, hypotonia was evident and seizures began. Dysmorphic features included flat facies, high forehead and inner epicanthal folds. Fontanelles were wide open and there was ventriculomegaly. The left optic nerve was hypoplastic. Karotyping was 46XY with no chromosomal defects.

- Because of rhinorrhea, cough, and fever, surgery was cancelled and antibiotics given. He was jaundiced with hepatomegaly.

- Lab values: AST 688, ALT 287, ALK phos 315, GGT 108, Conjugated bilirubin 7.6, Albumin 3.4. He developed respiratory insufficiency, apneic spells and desaturation and died within 48 hours.

- Autopsy limited to chest and abdomen: liver enlarged and firm (340 g vs 140 expected), splenomegaly (40 g vs 14), small subcapsular cysts in both kidneys, atrophic thymus, pneumonia.
Very Long Fatty Acids in Fibroblasts
(Kennedy-Krieger Institute – H & A Moser)

<table>
<thead>
<tr>
<th>Very Long Fatty Acids in Fibroblasts</th>
<th>Comparison Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acid</td>
<td>Liver</td>
</tr>
<tr>
<td>( \text{TFA} )</td>
<td>0.500</td>
</tr>
<tr>
<td>( \text{C}18:0 )</td>
<td>1.000</td>
</tr>
</tbody>
</table>


**improvements:**
- Increased the amount of data presented.
- Included a comparison of liver samples.
- Added a note on the significance of peroxisomal disorders.

**Next slide:**

**CATALASE**

- **normal liver**
- **patient**
Two main categories of Peroxisomal Disorders

- Biogenesis Disorders, Zellweger Syndrome Spectrum (ZSS)
  - Classic Zellweger Syndrome
  - Neonatal Adrenoleukodystrophy
  - Infantile Refsum Disease
  - Rhizomelic Chondrodysplasia punctata

- Single Enzyme Defects – (most common)
  - X-linked Adrenoleukodystrophy
  - Refsum Disease

The Clinical spectrum of Peroxisomal Biogenesis Disorders (PBD)

<table>
<thead>
<tr>
<th>Disease Phenotype</th>
<th>Biochemical Phenotype</th>
<th>PEX Gene Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic Zellweger</td>
<td>Diffuse SKL protein Localization</td>
<td>Nonsense, Frame shift</td>
</tr>
<tr>
<td>Neonatal Adrenoleukodystrophy</td>
<td>Absent or deficient peroxisomes</td>
<td>In-frame deletions, insertions</td>
</tr>
<tr>
<td>Infantile Refsum</td>
<td>Punctate SKL protein Localization</td>
<td>Missense</td>
</tr>
</tbody>
</table>

Peroxisomal Biogenesis Disorders (PBD) Are Multisystem Diseases due to abnormal peroxisome assembly

- Feeding problems
- Severe Hypotonia
- Dysmorphic facial features

Peroxisomes in historical perspective

- 1954: Swedish Graduate Student Rhodin describes “microbodies”
- 1964: Hans Zellweger describes Cerebrotegmental Syndrome
- 1970: De Duve associates absent peroxisomes in Zellweger Syndrome
- 1973: Biochemical pathways in peroxisome elucidated
- 1980: Disease gene cloned in yeast
- 2000: Additional disease (Adrenoleukodystrophy, Refsum disease) associated to peroxisomes
- 2012: Clinical Biochemical and Molecular Diagnosis available

Peroxisomal Biogenesis Disorders Lead to devastating Neurologic disease

- Patient with Zellweger
- Normal Brain
- Patient with Zellweger
- Severe Hypotonia
- Severe polymicrogyria

Historical Perspective

- 1917-present: De Duve fractionates lysosomal and peroxisomal enzymes
- 1980: De Duve receives Nobel Prize
- 1985: Goldfischer demonstrates absent peroxisomes in Zellweger Syndrome
- 1990: Biochemical pathways in peroxisome elucidated
- 2000: Disease gene cloned in yeast
- 2010: Additional diseases (Adrenoleukodystrophy, Refsum disease) associated to peroxisomes
- 2012: Clinical Biochemical and Molecular Diagnosis available

References:
Peroxisomes are ubiquitous eukaryotic organelles

- Composed of single lipid bilayer containing matrix proteins
- First noted in 1954 by Rhodin as 0.5-1.0 µm diameter vesicles in renal tissue
- Number between 100s to 1000s in eukaryotic cells
- Sequester oxidizing biochemical processes from the cytoplasm
- Identified by immunofluorescence using catalase antibody, or peroxisomal targeted fluorescent proteins

Catalase Immuno-electron microscopy

Zellweger Syndrome
Recommended Clinical Studies

• Complete family history
• Photos of facial features, skull
• Dermatoglyphics
• Growth parameters, serial
• Whole body x-ray, serial
• CT of brain

Biochemical abnormalities of Zellweger syndrome reflect global peroxisome dysfunction

<table>
<thead>
<tr>
<th>Biochemical Function</th>
<th>General Purpose</th>
<th>Abnormality in ZSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Acid Oxidation of very long chain fatty acids</td>
<td>Energy metabolism, membrane composition, Docosahexanoic acid biosynthesis</td>
<td>↑C26 and C24, ↑C26/C22 and C26/C24 ratio, ↓DNA</td>
</tr>
<tr>
<td>Plasmalogen Biosynthesis</td>
<td>Membrane composition</td>
<td>Absent RBC Plasmalogens</td>
</tr>
<tr>
<td>Phytic Acid Oxidation</td>
<td>Catabolism of a dietary toxin</td>
<td>↑Phytic Acid</td>
</tr>
<tr>
<td>Bile Acid Biosynthesis</td>
<td>Enzymatic synthesis</td>
<td>↓Bile acids with resultant fat soluble vitamin malabsorption</td>
</tr>
<tr>
<td>H2O2 detoxification</td>
<td>Detoxify reactive oxygen species</td>
<td>Evidence of oxidative stress</td>
</tr>
<tr>
<td>Catalase Solubility</td>
<td>Reflects underlying ability to localize to peroxisomes</td>
<td>Increased cytosolic localization (&quot;solubility&quot;)</td>
</tr>
</tbody>
</table>

Diagnostic Studies

• ↑Carbon 26, Carbon 24 fatty acids in plasma and in skin fibroblasts
• ↓Red Blood Cell Plasmalogens
• ↑Pipecolic acid in plasma
• ↓Phytic acid oxidation activity in skin fibroblasts
• ↑Cytosolic Catalase distribution in skin fibroblasts
• Mutations in a Pex gene found on sequencing
Two common 5' polymorphisms in PEX1 correlation with survival in PEX1 peroxisome biogenesis disorder patients

Genetics of Peroxisomal Biogenesis Disorders

Complementation Groups of Peroxisomal Biogenesis Disorders in Humans

<table>
<thead>
<tr>
<th>Gene</th>
<th>Human Phenotypes Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pex1</td>
<td>Zellweger, Neonatal Adrenoleukodystrophy, Infantile Refsum</td>
</tr>
<tr>
<td>Pex2</td>
<td>Zellweger</td>
</tr>
<tr>
<td>Pex3</td>
<td>Neonatal Adrenoleukodystrophy</td>
</tr>
<tr>
<td>Pex5</td>
<td>Zellweger, Neonatal Adrenoleukodystrophy, Infantile Refsum</td>
</tr>
<tr>
<td>Pex6</td>
<td>Zellweger, Neonatal Adrenoleukodystrophy, Infantile Refsum</td>
</tr>
<tr>
<td>Pex10</td>
<td>Zellweger, Neonatal Adrenoleukodystrophy, Infantile Refsum</td>
</tr>
<tr>
<td>Pex12</td>
<td>Zellweger, Neonatal Adrenoleukodystrophy, Infantile Refsum</td>
</tr>
<tr>
<td>Pex13</td>
<td>Zellweger, Neonatal Adrenoleukodystrophy</td>
</tr>
<tr>
<td>Pex16</td>
<td>Zellweger</td>
</tr>
<tr>
<td>Pex26</td>
<td>Zellweger, Neonatal Adrenoleukodystrophy, Infantile Refsum</td>
</tr>
</tbody>
</table>

Wanders 2004 AJMG 126(A): 355-375

Clinical Features of Zellweger Syndrome
68 Cases

<table>
<thead>
<tr>
<th>General</th>
<th>Facial Features (Open Face)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history</td>
<td>50/70 Pale facies, 15/50</td>
</tr>
<tr>
<td>Congenital anomalies</td>
<td>50/70 Hair hypoplasia, 15/50 Feeding difficulties</td>
</tr>
<tr>
<td>Central nervous system</td>
<td>50/70 Prenatal diagnosis</td>
</tr>
<tr>
<td>Difficult breathing</td>
<td>30/30</td>
</tr>
<tr>
<td>Heart defects</td>
<td>20/50</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>15/50</td>
</tr>
<tr>
<td>Heterotopias</td>
<td>10/50</td>
</tr>
<tr>
<td>Gyrinal abnormalities</td>
<td>10/50</td>
</tr>
<tr>
<td>Hemosiderosis</td>
<td>10/50 Hemorrhage</td>
</tr>
<tr>
<td>Biliary dysgenesis - cholestasis</td>
<td>10/50</td>
</tr>
<tr>
<td>Fibrosis, cirrhosis</td>
<td>10/50</td>
</tr>
<tr>
<td>Lack of peroxisomes, undetectable catalase</td>
<td></td>
</tr>
</tbody>
</table>

Zellweger (Cerebro-Hepato-Renal) Syndrome

1. Central Nervous System
   - Gyral abnormalities
   - Heterotopias
   - Glosis

2. Liver
   - Hemosiderosis
   - Biliary Dysgenesis - cholestasis
   - Fibrosis, cirrhosis
   - Lack of peroxisomes, undetectable catalase

3. Kidneys – Tubular & Glomerular Cysts

4. Dysmorphic Features
Back to our Patient

Pathology of the Liver

Open tight Junctions

Open tight junction

Normal bile canaliculus

The Role of the Tight Junction

Epithelial integrity; Paracellular pathway protection; Polarity
Separation of Apical and Basolateral membrane functions
Isolated bile canaliculi
Tight Junctions are strong

Tight Junction Defect in Navaho Neuro-Hepatopathy

3 year old twin with history of Reye syndrome at 10 months
Self-mutilation
Corneal ulcerations
Sensory neuropathy
Hepatomegaly
MPV 17 mutation

Tight Junctions in Other Cholestatic Livers

Bile duct ligation in rats alters ZO1, Claudins, microfilaments
Rahner C. Gastroent 1996, 110: 1564
Histochem Cell Biol 1996;106: 573 and Moly DP 2008; 129: 289

Chemical colitis in rats causes cholestasis, increased tight junction permeability and decreased organic anion transport in canaliculi
Lee L. Gastroent 1997; 113: 1347
Kawaguchi T. Hepatol 2000; 31: 1285

Modulation of TJ barrier function in human disease

Classification
- Cancer
- Immunological disease
- Inflammatory bowel disease
- Hemolytic diseases
- Nephrotic syndrome
- Retinopathy
- Vision loss
- Acquired or hereditary cholestatic hyperbilirubinemia
- Bacterial or viral hepatitis
- Genetic or idiopathic cholestasis

Tight junction protein affected
- Claudin-1, 14
- Claudin-2, 5, 7, 8
- Claudin-3
- Occludin, ZO-1
- Memebrane-associated guanylate kinase (MAGUK)

What’s the Connection?

Without Catalase, H2O2 accumulates

Many other influences on Tight junction integrity –
e.g. TNFα, INFgamma, Calcium depletion, Phosphorylation of Myosin II regulatory protein (MLC), EGF, TGFβ, HGF, PLGF-1, VEGF

Evidence that peroxisomal defects affect mitochondria in human disease

- Goldfischer 1973 depicted mitochondrial abnormalities as well as absent peroxisomes by EM + focus on non-heme iron of resp chain
- Mathis et al 1980 identified defective mitochondrial metabolism of bile acids in liver from Zellweger syndrome patients
- Trijbeals et al 1983 demonstrated an oxygen consumption defect in muscle mitochondria for ZS patients
- Hughes et al. 1990 described hepatic crystalline mitochondrial inclusions

DLP1 deficiency (dynamin-like related protein)

Infant with severe CNE maldevelopment, optic atrophy
Elevated lactate and VLCFA
Elongated and constricted peroxisomes and mitochondria – failure of fission (reproduction) of both organelles

Structural defects found in mice with DLP1 knock-out

Mitochondrial Alterations “Caused by” Defective Peroxisomal Biogenesis in a Mouse Model for Zellweger Syndrome (PEX5 Knockout Mouse)

Mice with Peroxisomal biogenesis defects

Mitochondrial Alterations “Caused by” Defective Peroxisomal Biogenesis in a Mouse Model for Zellweger Syndrome (PEX5 Knockout Mouse)

Mice with Peroxisomal biogenesis defects

Peroxisomal Biosynthesis
Organelle interactions – Endoplasmic Reticulum
Mitochondria
Peroxisomes and Aging

Summary

An infant with classic Zellweger syndrome
Spectrum of Peroxisomal disorders
Clinical and Laboratory studies
Pathologic findings
The Tight Junction
Navaho neuro-hepatopathy
Peroxisomal – Mitochondrial interplay


Paris L, Tonutti L, Vannini C, Bazzoni G. Structural organization of the tight junctions. Biochim

mitochondrial DNA depletion: new patients and novel mutations. Mol Genet Metab. 2010; 99:300-8

Acknowledgments: Michael Wangler, Assistant Professor, Molecular and Human Genetics, College of
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Supplemental Information
Peroxisomes are ubiquitous eukaryotic organelles

- Composed of single lipid bilayer containing matrix proteins
- First noted in 1954 by Rhodin as 0.5-1.0 µm diameter vesicles in renal tissue
- Number between 100s to 1000s in eukaryotic cells
- Importance of organelle thought to be questionable until discovery of related diseases
- Identified by immunofluorescence using catalase antibody, or peroxisomal targeted fluorescent proteins
- Identified on Electron Microscopy using diaminobenzidine (alkaline DAB).

A peroxisome’s life cycle

ER-peroxisome connection

Membrane origin vs Endosymbiosis from primitive micro-organism

The interdependence of peroxisomes, ER, and mitochondria in lipid metabolism.

Peroxisome diversity across the eukaryotic tree of life. Red circles indicate extensive biochemical data as well as comprehensive proteomics and bioinformatics surveys are available. Orange circles indicate an intermediate level of information on peroxisomal composition, mostly based on biochemical studies of individual proteins or pathways coupled with comprehensive sequence analyses to predict peroxisomal localization. Yellow circles indicate that the presence of peroxisomes in that group is well established but characterization of their function and diversity within the group is very scarce. White circles indicate that an apparent absence of these organelles in all the members studied (only a white circle is associated with the group) or in some of the samples (circles with different colours are associated with the group). Absence of a circle next to the group indicates that the presence or absence of peroxisomes or their enzymatic content in this group remains to be clearly established. (Adapted from a modified version of fig. 1 of flavellin et al. 2005)

### Contrasting Severe Mitochondrial and Peroxisomal Human Phenotypes

<table>
<thead>
<tr>
<th>Human Disease</th>
<th>Zellweger Syndrome</th>
<th>Pyruvate Dehydrogenase Deficiency (Neonatal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiology</td>
<td>Defect in Peroxisomal Biogenesis</td>
<td>Defect in entry of pyruvate into mitochondrial metabolism</td>
</tr>
<tr>
<td>Metabolic Effects</td>
<td>Increased Very-long chain fatty acids (VLCFAs), absent plasmalogens, oxidative stress</td>
<td>Lactic acidemia, Increased Alanine, CHO utilization defect</td>
</tr>
<tr>
<td>Nervous system</td>
<td>Polymicrogyria, intractable seizures, hypotonia</td>
<td>Agenesis of the corpus callosum, hypotonia</td>
</tr>
<tr>
<td>Other organ systems</td>
<td>Liver, Renal cysts</td>
<td>Cardiac, muscle</td>
</tr>
</tbody>
</table>

### Peroxisomal and Mitochondrial Cooperation

- **Glycolysis**
- **AAO**
- **Acetyl-CoA Oxidation**
- **Pyruvate Oxidation**
- **Oxidative Stresses**
- **Mitochondrial ATP Synthesis**
- **Protein Synthesis**
- **Fat Acid Oxidation**
- **VLCFAs Metabolism**
- **Plasmalogens Metabolism**
- **Aging**

### Caloric Restriction on Mitochondria

- **Mitochondrial Failure**
- **Aging**
- **Calorie Intake Reduction**
- **Mitochondrial Dysfunction**
- **Proteostasis**

### Front Physiol 2012, 3: 283