CLINICAL OVERVIEW OF REFSUM
Today’s Agenda

Opening by Kristie DeMarco, President & Founder of Global DARE Foundation

Clinical Overview of Refsum Disease by Professor Anthony Wierzbicki, PhD

Question & Answer Session
Webinar Housekeeping Details

- All participants are in listen only mode
- How to ask a question during the Q&A:
  - Participants following on Zoom can type their questions in the Q&A box at any time during the presentation or by raising their hand by pressing Alt+Y on your keyboard or clicking on the hand icon on your toolbar.
  - Participants joining by phone can press *9 on their phone to raise their hand.
- Questions will be answered in the following order:
  - Q&A box in Zoom
  - Dial in participants
  - Online participants
- Today’s session will be recorded for later viewing on Global DARE Foundation Website (www.defeatadulttrefsumeverwhere.com)
Global DARE Foundation's mission is to promote world-wide awareness and better quality of life for all who are diagnosed with Adult Refsum Disease.
Refsum disease and Peroxisomal alpha-oxidation disorders

Anthony S. Wierzbicki
Consultant in Metabolic Medicine/Chemical Pathology
Guy’s & St Thomas Hospitals
London UK
The peroxisome

• Ancient organelle
• Present in plants, fungi and animals
  • Proteins ≈ 310; Enzymes ≈150
• Functions in animals
  • Fatty acid beta-oxidation (minus 2 carbon units)
  • Fatty acid alpha-oxidation (minus 1 carbon unit)
  • VLCFA synthesis (add 2 carbon unit)
  • Cholesterol synthesis (HMG-CoA to FPP & GPP)
  • Complex alcohol metabolism
  • Nucleotide degradation (uric acid pathway)
  • Vitamin metabolism (vitamin A (?), C, E)
  • Oxidative environment (catalase) ?utility

Gronemayer T et al; PLoS 2013; 8: e57395
Animal Peroxisomes

(a) Wild-type cells

PMP70  Catalase

Stained for PMP70

Stained for catalase

(b) Pex1 mutants (deficient in matrix-protein import)

(c) Pex3 mutants (deficient in membrane-protein insertion)
Groups of peroxisomal disorders

- Peroxisomal biogenesis disorders
  - PTS-1
    - Zellweger syndrome
    - Neonatal adrenoleukodystrophy
    - Infantile Refsum disease
  - PTS-2
    - Rhizomelic chondrodysplasia

- Single enzyme/protein deficiencies
  - Approximately 30 known including
    - X-linked adrenoleukodystrophy
    - Refsum’s disease
Refsum’s disease: Progress over the years

- Clinical description 1947
- Identification of phytanic acid 1953
- Dietary treatment 1967
- Role of peroxisome 1973
- Identification as PTS-2 dependent enzyme 1982
- Localisation in peroxisome 1992
- Identification & cloning of enzyme 1996
- Identification of heterogeneity 2000
- Second locus – RCDP variant (Pex 7) 2003
- Phenocopy/Third locus- AMACR 2000
- Phenocopy/Fourth locus- PHARC 2010
Refsum’s disease

- Autosomal recessive
  - Prevalence: 1 in $10^6$
- 90% due to deficiency phytanoyl CoA hydroxylase (chromosome 10p13)
- 5% due to variants rhizomelic chondrodysplasia (RCDP) due to defect in import of PTS-2 signal containing enzymes to peroxisome
- 3% due to alpha-methyl-acyl racemase (AMACR) phenocopy
- 1% due to PHARC (ABDH12) deficiency
- 1% unknown
Clinical symptoms in Refsum’s disease

Wierzbicki AS et al; J Neurochem 2002; 80; 727
Refsum’s disease: eye changes
Refsum disease:
hands and feet
Revenge of the hamburger
Phytanic acid alpha-oxidation

Jansen GA & Wanders RJ. BBA 2006; 1763: 1403
Metabolic pathways

• **Alpha-oxidation**
  - (minus 1 carbon @ acid end)
  - Peroxisomal
  - Enzyme pathway in bacteria, algae, plants, animals
  - High basal capacity (>100mg/day; m > 32µmols)

• **Omega-oxidation**
  - (oxidation; minus 2 carbon @ methyl end)
  - Microsomal
  - Enzymes CYP4A in plants. Humans- ?CYP4F
  - Variable capacity (0-50mg/day; 0-16µmols)

Wierzbicki AS et al; J Neurochem 2002; 80; 727; Mukherji M et al; Prog Lipid Res 2003; 42; 359
Mutations in PhyH

Three key elements

Phytanic acid bound to carrier protein SCP-2 (through alpha-end)

Oxygen donor binding site
• 2-oxo-glutarate

Oxygen carrier site
• HXD motif

McDonough M et al; J Biol Chem 2005; 280; 41101
Types of Refsum Syndrome

- Classical
  - Mutations in PhyH (total loss of function)
- PhyH import (PTS-2 signal) defects
  - RCDP variants
  - Peroxisomal biosynthesis disorders (PTS-1&2)
    - Infantile Refsum disease- Zellweger spectrum
- Other downstream alpha-oxidation defects
  - AMACR
- Other
  - PHARC
Rhizomelic chondrodysplasia type 1

- Short upper arm and thigh
- Calcifications in growth cartilages
- Cataracts at birth
- Mild to severe growth retardation
- Severe mental deficiency
- Seizures.
- Most die age < 10 years

- Milder version known
Matrix protein import into the peroxisome

1. Receptor-ligand binding
   - PTS1 proteins
   - Pex5
   - Pex7
   - PTS2 proteins

2. Transport to the peroxisome

3. Receptor docking
   - 17, 14, 13

4. Dissociation and translocation
   - 12, 10, 2, 8

5. Receptor recycling
   - 1, 6, 4
   - 22
   - Cytosol
   - Matrix
### Signs & Symptoms

- Peripheral neuropathy
- Cataract
- Retinitis pigmentosa
- Leukodystrophy
- Ataxia
- Vertical gaze palsy
- Jaundice
- Hepatosplenomegaly
- Hypotonia

### Function of AMACR

![Diagram showing the function of AMACR](image)

Ferdinandusse S et al; Nature Genet 2000; 24 : 188
PHARC: a Refsum phenocopy

Fiskerstrand T et al; Am J Hum Genet 2010; 87 : 410
Treatment of Refsum disease syndromes

• Taking phytanic acid out:
  • Plasmapheresis/apheresis

• Stopping phytanic acid getting in:
  • Low phytanic acid diet
    • Removal of herbivore meat; carnivorous fish
    • Avoid dairy products- use soy alternatives
  • Reduce fat absorption
    • orlistat

• Stopping phytanic acid release
  • Metabolic stress management
    • Dextrose infusions
    • Fortisip etc supplements
Phytanic acid apheresis

Straube R et al; Tranfus Apher Sci 2003; 29 : 85
## Components of a Refsum Disease Diet

<table>
<thead>
<tr>
<th>Foods to Avoid</th>
<th>High Phytanic Acid Foods</th>
<th>Low Phytanic Acid Food Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat Products</strong></td>
<td></td>
<td><strong>Meat Products and Meat Substitutes</strong></td>
</tr>
<tr>
<td>Beef</td>
<td></td>
<td>Pork, Ham, Bacon</td>
</tr>
<tr>
<td>Veal</td>
<td></td>
<td>Chicken; Chicken livers</td>
</tr>
<tr>
<td>Calves Liver and Kidneys</td>
<td></td>
<td>Duck</td>
</tr>
<tr>
<td>Lamb</td>
<td></td>
<td>Turkey</td>
</tr>
<tr>
<td>Mutton</td>
<td></td>
<td>Soya Meat substitutes</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td>Quorn</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tofu (Bean curd)</td>
</tr>
<tr>
<td><strong>Dairy Products</strong></td>
<td></td>
<td><strong>Dairy Products</strong></td>
</tr>
<tr>
<td>Full cream /semi-skimmed cow’s milk</td>
<td></td>
<td>Skimmed milk</td>
</tr>
<tr>
<td>Sheep’s Milk</td>
<td></td>
<td>Soya milk</td>
</tr>
<tr>
<td>Goats Milk</td>
<td></td>
<td>Soya yoghurt, fat-free yoghurt</td>
</tr>
<tr>
<td>Full cream or greek yoghurt</td>
<td></td>
<td>Soya cheeses</td>
</tr>
<tr>
<td>Cheese made from cows, sheep or goats milk</td>
<td></td>
<td>Swedish Glace, Sorbet</td>
</tr>
<tr>
<td>Ice-cream</td>
<td></td>
<td>Alpro Soya /SoyaToo dairy-free products</td>
</tr>
<tr>
<td>Cream</td>
<td></td>
<td>Dark chocolate, carob</td>
</tr>
<tr>
<td>Milk Chocolate</td>
<td></td>
<td>Fat free sweets</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Phytanic acid profiles in patients on dietary therapy

Harley C et al; CIEM 2005
Refsum disease: Phytanic acid pre- & post-dietary therapy

Baldwin EJ et al; J Neurol Neurosurg Psychiatr 2010; 81: 954-7
Phytol metabolism

Phytol conversion

Metabolic map of phytol

Wanders RJA et al. BBA 2011; 1811: 498
## Green vegetables & Refsum disease diet

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Phytol (µmol/kg)</th>
<th>Phytanic acid (µmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>1.3</td>
<td>6.4</td>
</tr>
<tr>
<td>Asparagus</td>
<td>0</td>
<td>2.3</td>
</tr>
<tr>
<td>Lentils</td>
<td>19.2</td>
<td>0</td>
</tr>
<tr>
<td>Broad Beans</td>
<td>9.6</td>
<td>6.7</td>
</tr>
<tr>
<td>Grapes</td>
<td>9.6</td>
<td>0</td>
</tr>
<tr>
<td>Prunes</td>
<td>35.2</td>
<td>0</td>
</tr>
<tr>
<td>Peanuts</td>
<td>1312</td>
<td>0</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Coppack SW et al; BMJ 1988; 296 : 828
## Plasma phytanic and urine 3-MAA response to fasting

<table>
<thead>
<tr>
<th></th>
<th>Plasma phytanic acid</th>
<th>Urine 3-MAA</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/L</td>
<td>µg/mg creatinine</td>
<td>hours</td>
</tr>
<tr>
<td>N</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>1</td>
<td>151</td>
<td>632</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>258</td>
<td>409</td>
<td>4.8</td>
</tr>
<tr>
<td>3</td>
<td>388</td>
<td>1028</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>410</td>
<td>474</td>
<td>2.7</td>
</tr>
<tr>
<td>5</td>
<td>1210</td>
<td>1417</td>
<td>13.0</td>
</tr>
<tr>
<td>Median</td>
<td>388</td>
<td>632</td>
<td>4.8</td>
</tr>
</tbody>
</table>

% change | $163(115 - 419)$ | $219 (-16 – +235)$

Wierzbicki AS et al; J Lipid Res 2003; 44 : 1481
Consequences of fasting

• 1 patient severe elevation of PA – asymptomatic

• 3 patients – moderate elevation of PA
  • One re-admitted for 1 week generalised weakness after 12 weeks
  • One - 8 kg weight loss
  • One – leg cramps for 3 days

Wierzbicki AS et al; J Lipid Res 2003; 44 : 1481
Treatment of Refsum disease syndromes

- Enzyme replacement therapy
  - Problem of correct localisation
- Exploiting structure changes in PhyH
  - Alternate oxygen donor compounds
  - Works in cells not in animals
- Drug therapy
  - Alternative pathways of phytanic acid oxidation
  - Omega-oxidation induction
- Gene therapy
  - Replace PhyH
    - Liver - deals with intake
    - Eye – deals with visual deterioration
- Stem cell therapy
  - Eye – replace damaged retinal cells
Omega-oxidation

Potential drug target
- an inducible overflow mechanism
- Has moderate capacity
- Relies of pre-existing enzyme proteins
- Role in PA surge protection
- Good candidate for inducer-based drug therapy
Phytanic acid excretion in a new patient

Wierzbicki AS et al; J Lipid Res 2003; 44 : 1481
Treating the PhyH (-/-) mouse

<table>
<thead>
<tr>
<th>Omega-oxidation</th>
<th>Liver homogenates</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Cytochrome P450</td>
<td></td>
</tr>
<tr>
<td>• 4F3A</td>
<td>0.7</td>
</tr>
<tr>
<td>• 4F3B</td>
<td>0.6</td>
</tr>
<tr>
<td>• 4F2</td>
<td>0.5</td>
</tr>
<tr>
<td>• 4A11</td>
<td>0.4</td>
</tr>
<tr>
<td>• What inducers?</td>
<td></td>
</tr>
</tbody>
</table>

• But no cytochrome or alpha-oxidation in brain (cerebellum)

Wanders RJA et al. BBA 2011; 1811: 498
hESC transplant to Cobalt or Laser retina-ablated primates

hESC transplant survival
90 day data

hESC photoreceptor integration

Shirai H et al; PNAS 2016; in press
Conclusions

• Alpha-oxidation disorders
  • Rare partially treatable disorders
  • Unknown diseases
    • HACL deficiency phenotype unknown
    • Milder phenotype of PhyH mutations

• Treatment
  • Current
    • Diet–based therapy & apheresis
  • Novel
    • Potential drug candidates e.g. omega-oxidation
    • Gene or iPS ectodermal-retinal therapies
Q&A

For more information contact:

- Global DARE Foundation
- info@globaldarefoundation.org
- www.defeatadultrefsumeverywhere.org
UPCOMING REFSUM WEBINARS

Global DARE Foundation will be holding additional webinars throughout the summer. Registration can be accessed through our website at https://www.defeatadultrefsumeverywhere.org/dare-events

7/1/20, 7:00AM EST
Science behind Refsum Disease
Ronald JA Wanders, PhD & Sacha Ferdinandussa, PhD from Academic Medical Center, UMC will be providing the Science behind Refsum Disease

7/24/20, 7:00AM EST
Refsum Diet Overview & Discussion
Eleanor Baldwin & Sarah Firman, the clinical dietitians at Guy's & St. Thomas Hospital in London will be providing an overview of the specialized diet for Refsum Disease

8/7/20, 8:00 PM EST
Gene Therapy - A Potential Therapy for Refsum Disease
Ryan Butler, PhD from UT Southwestern will provide an overview of Gene Therapy as a potential future therapy for Refsum Disease