Peroxisomal Disorders and their Diagnosis

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What is a peroxisome?

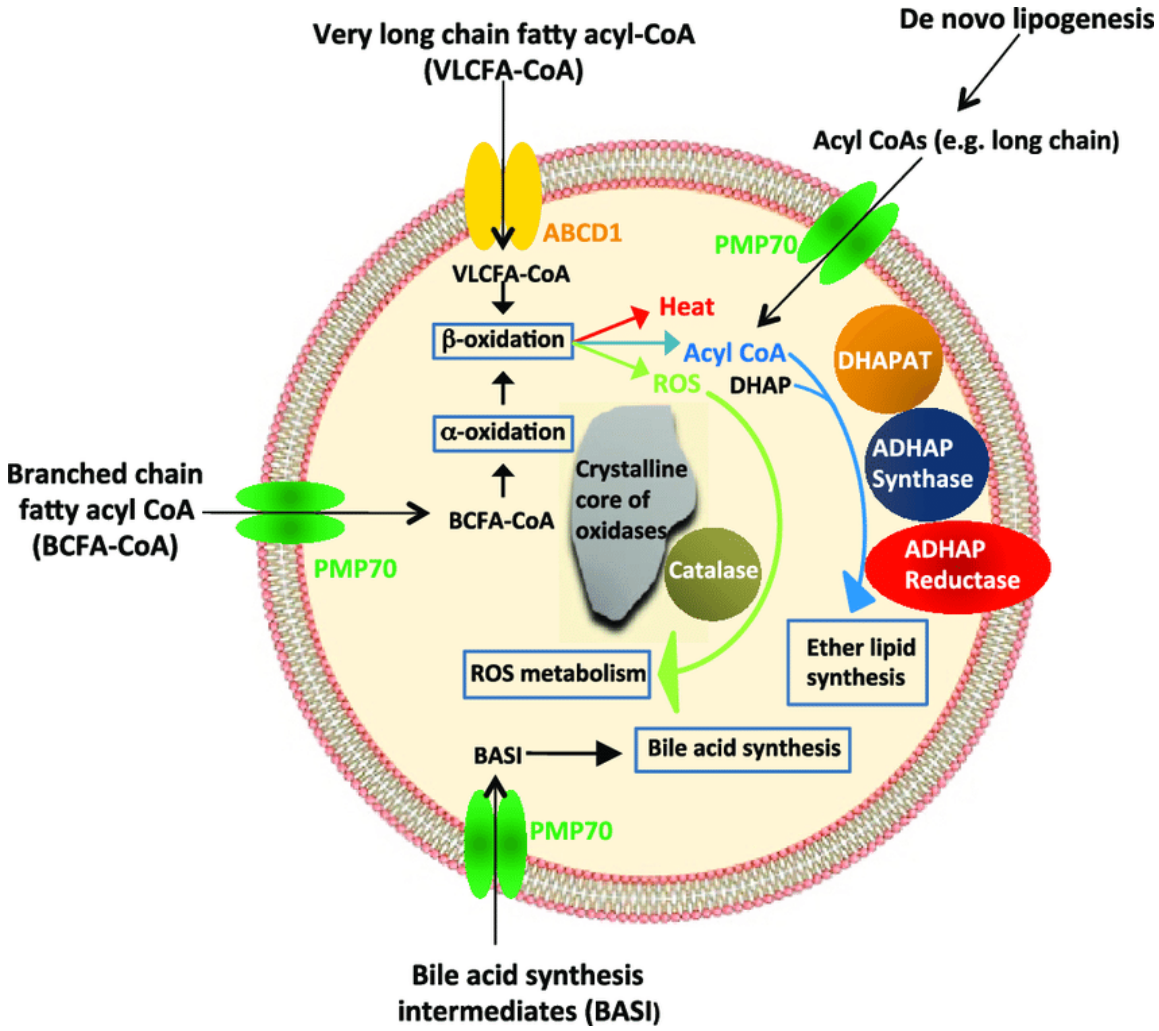
- A peroxisome is a single membrane bound organelle found in the cytoplasm of eukaryotic cells

- Many oxygen–dependent reactions take place in the peroxisomes to protect the cell against oxygen radicals – it contains catalase to remove the resulting H_2O_2

- Main functions are;
  - Involved in the synthesis of etherlipids (e.g. plasmalogens) and bile acids
  - Catabolism of very long chain fatty acids and branched chain fatty acids e.g. phytanate

- Various PEROXIN proteins (encoded by the PEX genes) are required for peroxisomal biogenesis and transmembrane transport (bringing enzymes into the peroxisome where they can function)

Diagram of a Peroxisome



Categorisation of Peroxisomal Disorders

- Simplest version is;
  - **Peroxisomal Biogenesis Disorders** – disorders which affect the formation and functioning of the whole peroxisome and result in disruption of all synthetic and catabolic functions to a greater or lesser extent
  - **Single enzyme or transport protein defects** – result in the loss of a specific synthetic or catabolic function while other functions of the peroxisome are unaffected

Peroxisomal Biogenesis Disorders

- In the severest forms the peroxisomes may be entirely absent from the cell or only “ghost” peroxisomes present. In milder cases peroxisomes may be present but dysfunctional

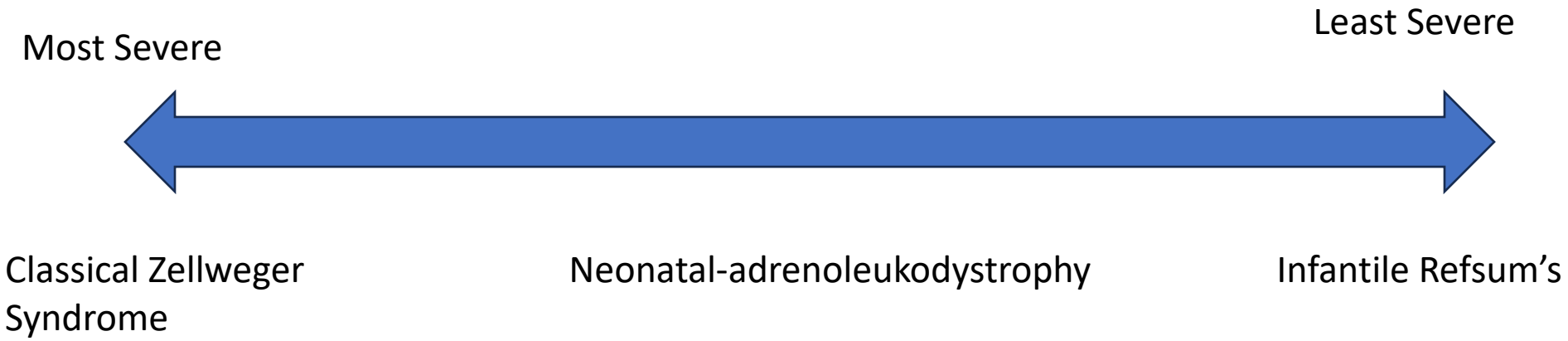
- At least 29 proteins denoted as PEROXINS are required for biogenesis, fission, and protein import to form full functional peroxisomes

- These are coded for by the PEX genes

- Defective peroxisome assembly is most frequently caused by mutations in PEX1 but other known causative genes are PEX2, 3, 5, 6, 10, 11b, 12, 13, 14, 16, 19, 26

Nomenclature: Zellweger Spectrum Disorder vs Peroxisomal Biogenesis Disorder

- Historically the PBDs were categorised in to syndromes according to clinical severity



But important to remember that these are clinical descriptors – and DO NOT correlate with causative gene
You can have a PEX1 mutation and have Classical Zellweger or Infantile Refsum's depending on severity of mutation(s) OR you can have Classical Zellweger and have a defect in one of many PEX genes

Cont…

- Preferred terminology now is to refer to them all as PEROXISOMAL BIOGENESIS DISORDERS (due to specific PEX defect) and consider patients more individually
- Avoids a few of problems;
- Not all patients easily put in to 1 of 3 categories when severity seems to be a continuum
- There are clearly some patients who are less severely affected than the classical Infantile Refsum's type (which is actually still pretty severe it's just that you usually survive more than a few yrs)
- The term "Zellweger Spectrum disorder" is easily confused with Classical Zellwegers and can cause misunderstandings about severity of condition / life expectancy
- People confuse Infantile Refsum's and Adult Refsums's / Refsum's disease and think they are infantile and later presenting forms of the same disorder!

Clinical Presentation…is multisystemic…

- Classic neonatal presentation: **severe hypotonia, areactivity, seizures**, **severe cholestasis / liver dysfunction, dysmorphic** (high forehead, large fontanels) with **skeletal abnormalities** (e.g. short proximal limbs, calcific stippling), **sensorineural deafness, retinopathy, microgyria** (“folds” in cerebral cortex are smaller than usual)

- Common features in milder cases are **retinopathy, sensorineural deafness, hypotonia (milder), cognitive deficiencies**, - less likely to have liver disease / notable skeletal abnormalities

Transport Protein Defects / Single Enzyme Defects

Transport Protein / Receptor Defects:

- **X-linked adrenoleukodystrophy (ABCD1 gene)** – ATP-binding cassette transporter for import of straight chain saturated VLCFAs – presents with behavioural change / regression / visual and hearing impairment, sometimes isolated adrenal insufficiency- (also adrenomyeloneuropathy in adults – spastic paraparesis / peripheral neuropathy)

- **ABCD3 Transporter Deficiency** – transporter for branched chain fatty acids and bile acids - presents with severe liver disease , abnormal bile acids (single patient)

- **Rhizomelic Chondrodysplasia Punctata type 1 (PEX7 gene)** – PTS2 receptor for ADHAPS enzyme (plasmalogen synthesis) and Phytanoyl-CoA hydroxylase (phytanate catabolism) - presents with proximal shortening of limbs, calcific stippling, facial dysmorphism, microcephaly, cataracts, spasticity, intellectual disability, ichthyosis

Transport Protein Defects / Single Enzyme Defects

Single Enzyme defects:

- **AcylCoA oxidase deficiency (ACOX gene)** – affects VLCFA metabolism only – resembles moderately severe PBD
- **D-bifunctional Protein deficiency (HSD17B4)** – catalyses 2nd and 3rd steps of peroxisomal b-oxidation - can present very similar to severe PBD but may also present with hypotonia and seizures as most prominent features
- **Phytanoyl-CoA hydroxylase deficiency (PHYH gene)** –affects metabolism of the branched chain fatty acid **phytanic acid** - predominant feature is retinitis pigmentosa, but also presents with ataxia, deafness, anosmia, polyneuropathy (but normal intelligence) – also referred to as Adult Refsum's
- **α-Methyl-CoA racemase deficiency (AMACR gene)** – predominantly affects metabolism of pristanic acid (branched chain fatty acid) – similar presentation to Phytanoyl-CoA hydroxylase deficiency
- **Catalase deficiency (CAT gene)** – presents with chronic mouth ulcers
- **RCDP Type 2 / DHAP acyltransferase deficiency (GNPAT gene)** – step in plasmalogen synthesis - presents like RCDP 1
- **RCDP Type 3 / Alkyl -DHAP synthesis deficiency (AGPS gene)** – step in plasmalogen synthesis (same enzyme as RCDP 1)

Tests of Peroxisomal Function

- **Very long chain fatty acids (VLCFA)** – these are straight chain long chain fatty acids primarily **C26 & C24, - C22** is also measured to allow calculation of **C26/C22 and C24/C22 ratios** (more diagnostic than absolute values) – increased where there is deficient β-oxidation
- **Phytanic acid** – branched chain fatty acid, elevation indicates deficiency in α-oxidation (dietary in origin)
- **Pristanic Acid** – low in isolated α-oxidation disorders (phytanate high), isolated increase in racemase deficiency (dietary in origin)
- **Plasmalogens (etherphospholipids)** – low in severe PBDs (normal in milder cases), low in isolated disorders of plasmalogen synthesis (and FAR1 deficiency)
- **Bile Acid intermediates (urine and plasma)** – increases in various specific bile acid intermediates seen in various disorders (PBD, D-bifunctional PD, Sterol carrier protein 2, α -Methylacyl-CoA racemase deficiency
- **Pipecolic Acid** –breakdown product of lysine which is metabolised in the peroxisome, increased in PBDs and sometimes D-bifunctional protein deficiency

Diagnostic Patterns in Biochemical Results

Disease	VLCFA	Plasmalogens	Phytanic acid	Pristanic acid	Bile acids
Disorders of peroxisomal biogenesis	↑	↓	↑*	↑*	↑
Rhizomelic chondrodysplasia punctata types 1 and 5	*n*	↓	↑*	(↓)	*n*
Rhizomelic chondrodysplasia punctata types 2 and 3	*n*	↓	*n*	*n*	*n*
X-linked adrenoleukodystrophy	↑	*n*	*n*	*n*	*n*
Refsum disease	*n*	*n*	↑	(↓)	*n*
α-Methyl-acyl-CoA racemase deficiency	*n*	*n*	(↑)	↑*	↑
D-bifunctional protein deficiency	↑	*n*	↑*	↑*	↑
Acyl-CoA oxidase deficiency	↑	*n*	*n*	*n*	*n*
Sterol carrier protein-2 deficiency	(↑)	*n*	(↑)	↑*	↑

Example of a Classic Severe PBD (Classical Zellweger phenotype)

Initial VLCFA/phytanate/pristanate results at 3 days of age – "preterm baby with significant hypotonia"

- C22 = 19 µmol/L (15-112)
- C24 = 35 µmol/L (14-80)
- C26 = 12.65 µmol/L (0.33-1.50)
- C24 / C22 ratio = 1.84 (0.44-0.97)
- C26 / C22 ratio = 0.666 (0.005-0.030)
- Phytanate = 3.6 µmol/L (<19.3)
- Pristanate = 1.12 µmol/L (<1.88)

Repeat samples taken at 3 weeks

- C22 = 22 µmol/L (15-112)
- C24 = 30 µmol/L (14-80)
- C26 = 7.74 µmol/L (0.33-1.50)
- C24 / C22 ratio = 1.36 (0.44-0.97)
- C26 / C22 ratio = 0.352 (0.005-0.030)
- Phytanate = 72.8 µmol/L (<19.3)
- Pristanate = 33.94 µmol/L (<1.88)

Classic severe PBD cont…

- Plasma bile acids = Increase in the primary bile salts indicating cholestasis and an increase in the bile acid intermediates tauro-trihydroxycholestanoate and tauro-tetrahydroxycholestanoate
- Plasma Pipecolic Acid = 12.08 µmol/L (<2.46)
- Plasmalogens
  - C16 / Palmitate = 0.001 (ref. 0.082-0.140, - consistent with CZ = <0.025)
  - C18 / Stearate = 0.001 (0.176-0.280, - consistent with CZ = <0.050)

Milder PBD

- 4 yr old child with “hypotonia and developmental impairment”
  - C22 = 20 µmol/L (15-112)
  - C24 = 25 µmol/L (14-80)
  - C26 = 2.26 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 1.25 (0.44-0.97)
  - C26 / C22 ratio = 0.113 (0.005-0.030)
  - Phytanate = 89.6 µmol/L (<19.3)
  - Pristanate = 22.4 µmol/L (<1.88)
- Plasma bile acids = Normal primary bile salts but with a small increase in the bile acid intermediates tauro-trihydroxycholestanoate and tauro-tetrahydroxycholestanoate
- Plasma Pipecolic Acid = 39.9 µmol/L (<2.46)
- Plasmalogens
  - C16 / Palmitate = 0.098 (ref. 0.082-0.140, - consistent with CZ = <0.025)
  - C18 / Stearate = 0.206 (0.176-0.280, - consistent with CZ = <0.050)

NOTE THE COMPLETELY NORMAL PLASMALOGENS IN THIS PATIENT

D-bifunctional Protein Deficiency- straightforward case

- **2 week old baby – "? Zellweger syndrome"**
  - C22 = 45 µmol/L (15-112)
  - C24 = 84 µmol/L (14-80)
  - C26 = 15.52 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 1.87 (0.44-0.97)
  - C26 / C22 ratio = 0.345 (0.005-0.030)
  - Phytanate = 1.7 µmol/L (<19.3)
  - Pristanate = 0.39 µmol/L (<1.88)
- Plasma Pipecolic Acid = 4.75 µmol/L (<2.46)
- Plasma bile acids – Normal concentration of primary bile acids and no abnormal bile acid intermediates
- Plasmalogens
  - C16 / Palmitate = 0.079 (ref. 0.082-0.140, - consistent with CZ = <0.025)
  - C18 / Stearate = 0.188 (0.176-0.280, - consistent with CZ = <0.050)

You couldn't exclude AcylCoA oxidase deficiency from these results - but baby was confirmed to have mutations in HSD17B4 (D-bifunctional PD)

Alternative D-bifunctional PD patient…

6 week old baby - “seizures -?D-bifunctional”

- C22 = 121 µmol/L (15-112)
- C24 = 128 µmol/L (14-80)
- C26 = 6.03 µmol/L (0.33-1.50)
- C24 / C22 ratio = 1.06 (0.44-0.97)
- C26 / C22 ratio = 0.050 (0.005-0.030)
- Phytanate = 39.0 µmol/L (<19.3)
- Pristanate = 6.21 µmol/L (<1.88)

- Plasma Pipecolic Acid = 9.65 µmol/L (<2.46)

- FASTING repeat sample
  - C22 = 70 µmol/L (15-112)
  - C24 = 80 µmol/L (14-80)
  - C26 = 2.04 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 1.14 (0.44-0.97)
  - C26 / C22 ratio = 0.029 (0.005-0.030)
  - Phytanate = 1.4 µmol/L (<19.3)
  - Pristanate = <0.15 µmol/L (<1.88)

- Plasma Pipecolic Acid = 9.32 µmol/L (<2.46)
- Bile Acids – no abnormality
- Plasmalogens - normal

This patient was severely affected, made no developmental progress and died at about 18 months of age

Alternative D-bifunctional PD patient cont…

- D-functional protein deficiency patients can be VERY tricky
- **Degree of abnormality of biochemistry** DOES NOT seem to correlate with **severity of clinical presentation** and they are reported in the literature as having extremely variable biochemistry, from completely obvious to more or less normal
- Some have raised pipecolic acid, some don't
- Some have abnormal bile acid intermediates – some don't
- They will ALL have normal plasmalogen results as this aspect of peroxisomal function is not affected
- This case also shows the problem sometimes encountered with non-fasting samples - diets high in long chain fats and / or dairy products can distort results. Fasting samples may be necessary.

X-Linked Adrenoleukodystrophy

- **7 yr old boy with "?X-ALD, suspicious MRI"**

  VLCFA / Phyt/ Prist

  - C22 = 56 µmol/L (15-112)
  - C24 = 81 µmol/L (14-80)
  - C26 = 3.48 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 1.45 (0.44-0.97)
  - C26 / C22 ratio = 0.062(0.005-0.030)
  - Phytanate = 3.4 µmol/L (<19.3)
  - Pristanate = 1.09 µmol/L (<1.88)

Context of the patient and results are typical for X-ALD – but important to realise that in isolation they are not specifically diagnostic (you could in theory have the same set of results from a mild PBD / D-bifunctional)

X-ALD Carriers (females)

- **Mother of X-ALD patient (obligate heterozygote)**
  - C22 = 57 µmol/L (15-112)
  - C24 = 67 µmol/L (14-80)
  - C26 = 1.80 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 1.18 (0.44-0.97)
  - C26 / C22 ratio = 0.032 (0.005-0.030)
  - Phytanate = 1.2 µmol/L (<19.3)
  - Pristanate = <0.15 µmol/L (<1.88)
- MOST X-ALD carriers will have mildy abnormal VLCFA (to a greater or lesser extent) but around 10% will have completely normal results so when doing family studies it is essential to do gene testing
- Carriers can be fairly easy to spot / deal with in the context of an affected family member but when screening a woman with “?MS, rule out X-ALD” it can be trickier

α-Methyl-Acyl-CoA racemase deficiency

- 45 yr old woman presenting with dystonia
- **VLCFA/ Phyt / Prist**
  - C22 = 38 µmol/L (15-112)
  - C24 = 35 µmol/L (14-80)
  - C26 = 0.42 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 0.92 (0.44-0.97)
  - C26 / C22 ratio = 0.011 (0.005-0.030)
  - Phytanate = 21.3 µmol/L (<19.3)
  - Pristanate = 142.4 µmol/L (<1.88)
- Bile Acids – small signal consistent with Tauro-trihydroxycholestanoate
- In conjunction with raised pristanate this is consistent with **α-Methyl-Acyl-CoA racemase deficiency**
- **Sterol Carrier Protein 2 deficiency** patients also have elevated pristanate but different bile acid intermediates (bile alcohol glucuronides)

Phytanoyl-CoA hydroxylase Deficiency (Refsum / Adult Refsum disease)

- 37 yr old woman (no clinical details)
- **VLCFA/ Phyt / Prist**
  - C22 = 44 µmol/L (15-112)
  - C24 = 37 µmol/L (14-80)
  - C26 = 1.43 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 0.84 (0.44-0.97)
  - C26 / C22 ratio = 0.033 (0.005-0.030)
  - Phytanate = 495.3 µmol/L (<19.3)
  - Pristanate = 0.03 µmol/L (<1.88)

- **Isolated massive increase in phytanate** typical of Phytanoyl-CoA hydroxylase deficiency

RCDP (Rhizomelic Chondrodysplasia Punctata) Type 1

- 5 yr old girl “short stature, developmental delay, 1 pathogenic and 1 VUS mutation in PEX7”
- **VLCFA/ Phyt / Prist**
  - C22 = 71 µmol/L (15-112)
  - C24 = 58 µmol/L (14-80)
  - C26 = 1.70 µmol/L (0.33-1.50)
  - C24 / C22 ratio = 0.82 (0.44-0.97)
  - C26 / C22 ratio = 0.024 (0.005-0.030)
  - Phytanate = 124.9 µmol/L (<19.3)
  - Pristanate = 0.63 µmol/L (<1.88)

Plasmalogens

- C16 / Palmitate = 0.026 (0.082-0.140)
- C18 / Stearate = 0.035 (0.176-0.280)

- Child does NOT have rhizomelia (short proximal limbs) but on skeletal survey did have some skeletal abnormalities consistent with CDP
- Classic RCDP patients have ZERO plasmalogens and do not survive beyond 1-2 yrs – **clearly an attenuated case**

Making a diagnosis – things to be aware of…

- **VLCFA / phytanate / pristanate** – typically gets used as a type of screening test for peroxisomal disorders but you have to understand what patients you might not pick up i.e. RCDP type 2/3 (abnormal plasmalogens only) or atypical mild PBDs
- If your first line test is VLCFA only (with phyt / prist a separate test) you are potentially compounding the problem
- Patients with PEX11b mutations notoriously have completely normal biochemistry
- Diets high in long chain fats and / or dairy products (phytanate) can give misleading / atypical results, particularly non-fasting samples. A repeat fasting sample may help. Patients on ketogenic diets and who love peanut butter are most likely to give problems.
- Neonatal or mild PBD, D-bifunctional, AcylCoA oxidase deficiency & X-ALD - may all show same pattern of raised C26, C24 and ratios – only degree of elevation and patient context point you in the right direction - appropriate further testing may then provide the specific diagnosis

Any Questions??