Overview on peroxisomal disorders

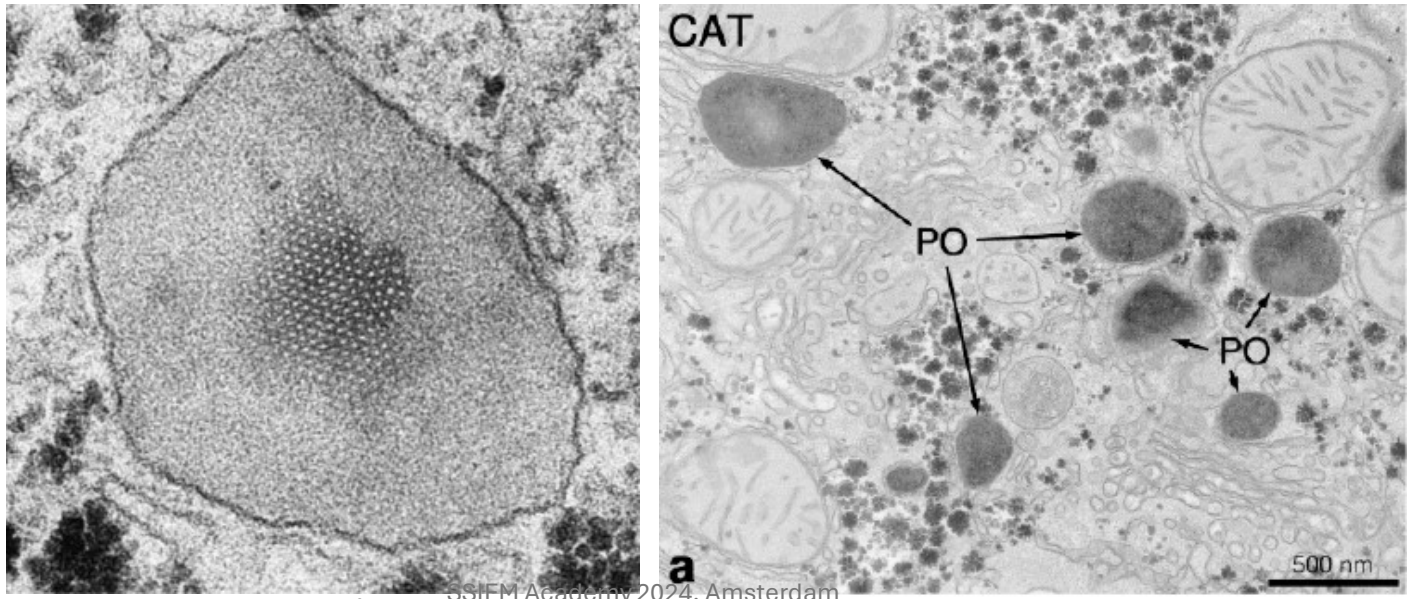
Metabolic and clinical aspects

Elaine Murphy/Fred Vaz

SSIEM Academy 2024, Amsterdam

Peroxisomes

- Subcellular organelles
- Size : 0.1 to 1 μm
- Ubiquitous distribution: abundant in tissues active in lipid metabolism (liver, adipose tissue, kidney, …), absent in erythrocytes

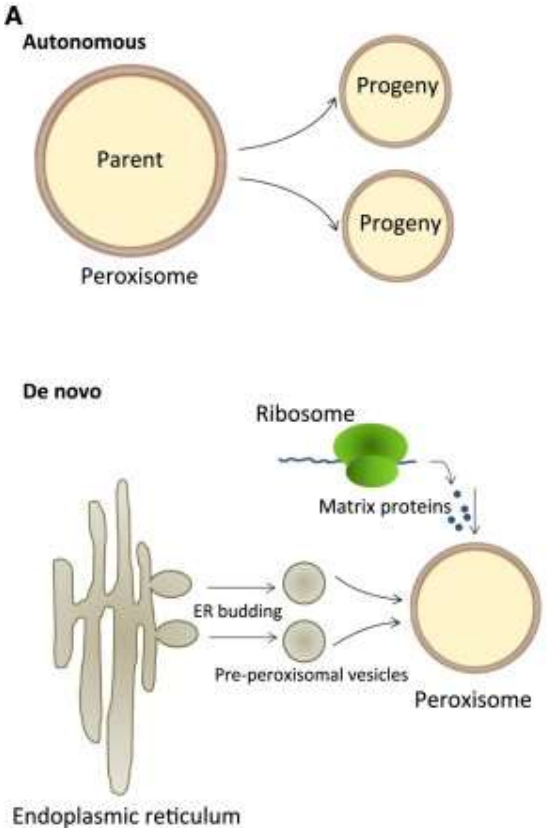


EM: peroxisome

Peroxisomal biogenesis

- Growth and fission of pre-existing organelles

- De novo process involving
  - Budding from smooth endoplasmic reticulum
  - Import of matrix proteins



From Lodhi & Semenkovich, Cell Metabolism 2014;19:380

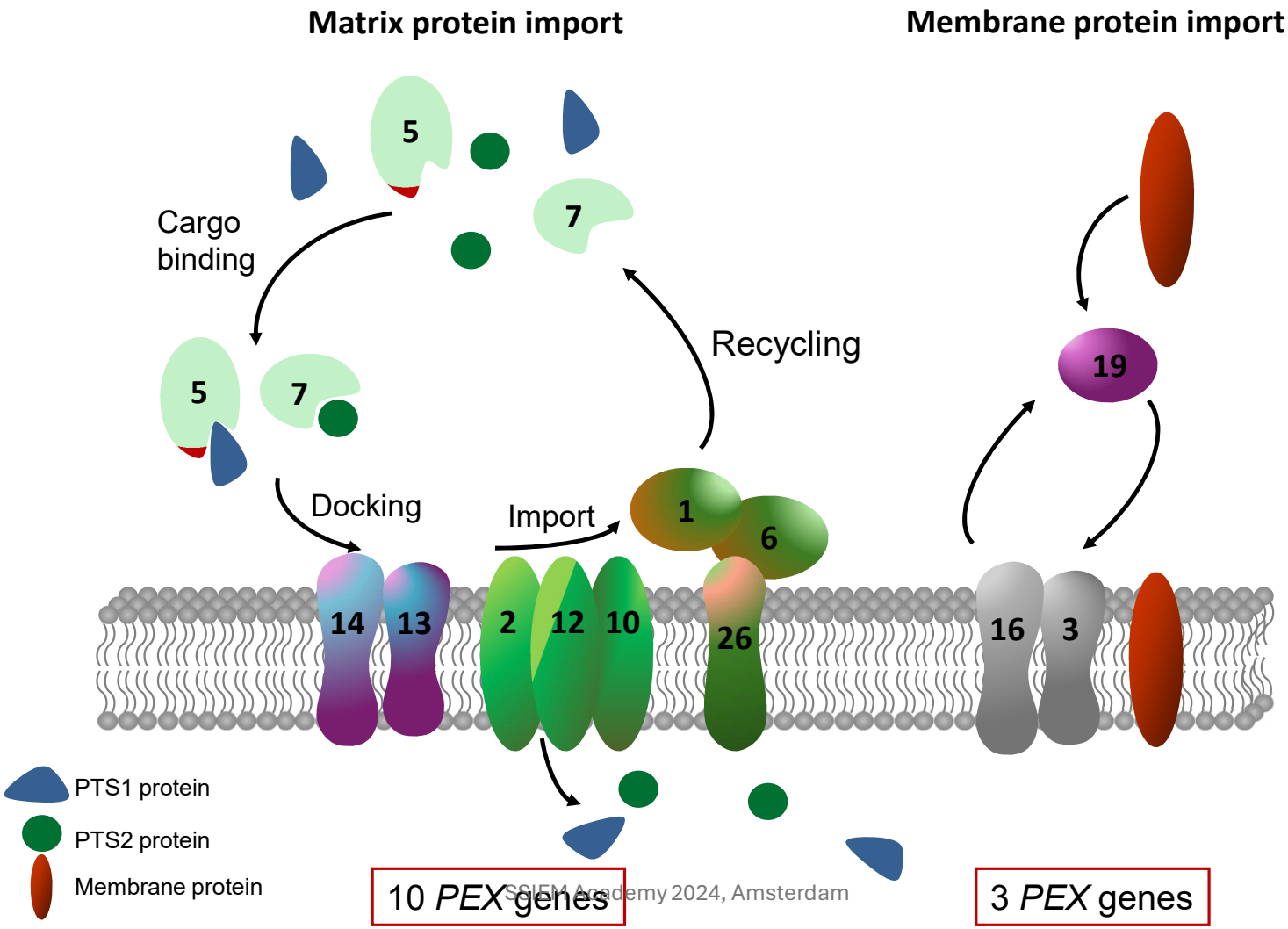
Biogenesis of peroxisomes

- 3 steps
  - Constitution of the lipid membrane which derives from the endoplasmic reticulum
  - Insertion of peroxisomal membrane proteins (PMP) into the membrane
  - Import of the matrix proteins through the membrane

- PMP, also called peroxins, are involved in
  - The biosynthesis of the peroxisomal membrane
  - The import of matrix proteins (enzymes)

- PMP and matrix proteins are synthesized in free polyribosomes and are specifically targeted to peroxisome

peroxisomal protein import



Import of peroxisomal proteins

- 4 steps
  - Recognition of the targeting signal of the protein to be imported by a specific peroxin : complex peroxin – protein
  - Stabilization of the complex at the outer face of peroxisomal membrane
  - Translocation of the protein in the peroxisomal matrix
  - Recycling of the peroxin receptor

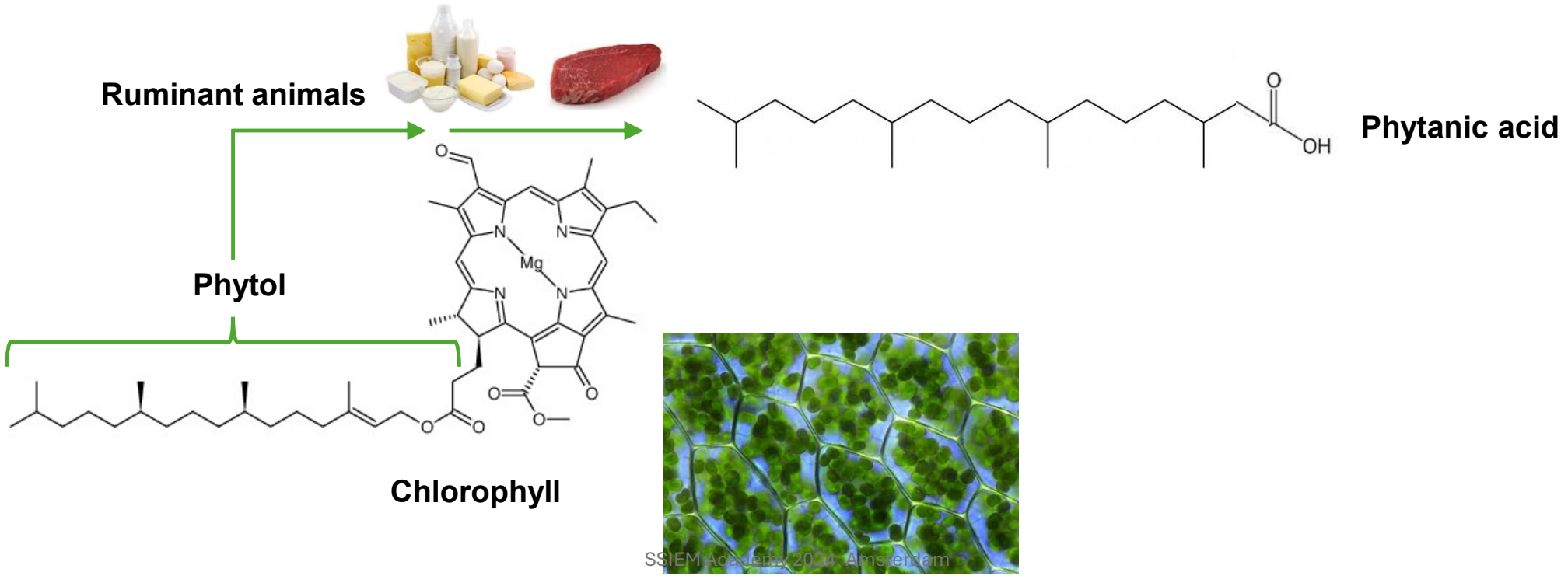
- Targeting signals of the peroxisomal matrix proteins:
  - Peroxisomal targeting signal 1 (**PTS1**): 90% of proteins, receptor: peroxin 5 (**Pex5**)
  - Peroxisomal targeting signal 2 (**PTS2**): alkyl-DHAP synthase, phytanoyl-CoA hydroxylase, and ACAA1 (thiolase), receptor: peroxin 7 (**Pex7**)

Metabolic functions of peroxisomes

- Catabolism
  - α-oxidation of phytanic acid (BCFA)
  - β-oxidation of
    - very-long-chain fatty acids (VLCFA)
    - pristanic acid (BCFA)
    - dicarboxylic acids
  - Detoxification of glyoxylate
  - Pipecolic acid degradation
  - H_2O_2 detoxification (catalase, peroxidase, …)
  - D-amino acids, polyamines, some leukotrienes and prostaglandins, ….
- Biosynthesis
  - Bile acids
  - Ether(phospho)lipids (including plasmalogens)
  - PUFA synthesis (e.g. docosahexaenoic acid (C22:6ω3 or DHA))

Phytanic acid α-oxidation

- Catabolism
  - α-oxidation of phytanic acid (BCFA)



Phytanic acid α-oxidation

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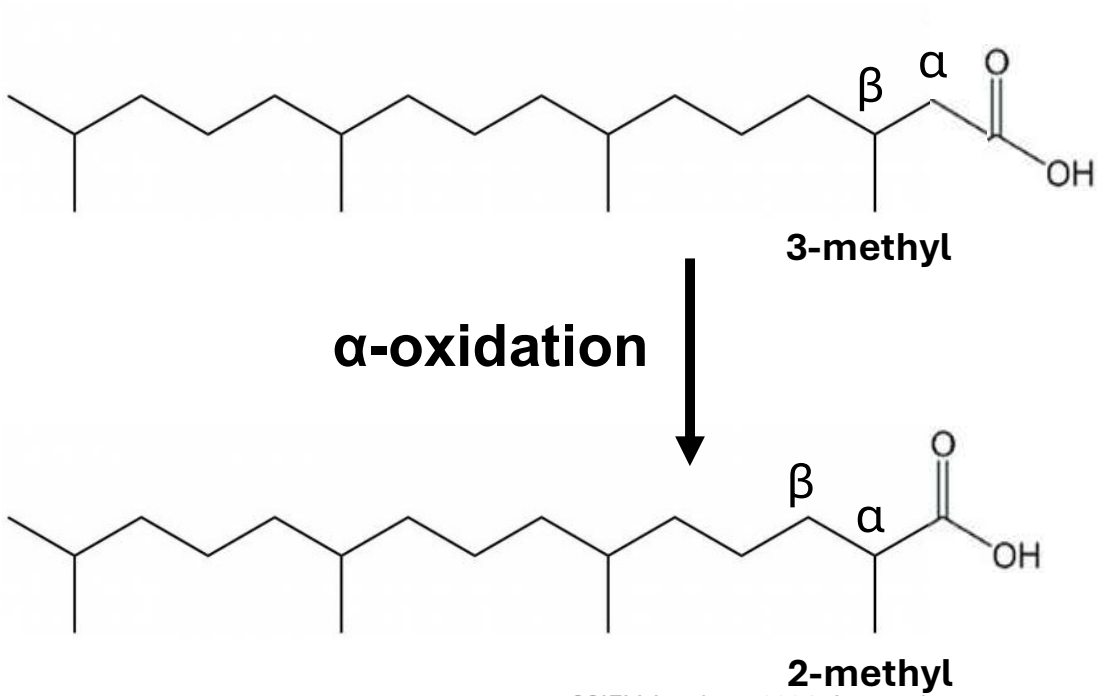
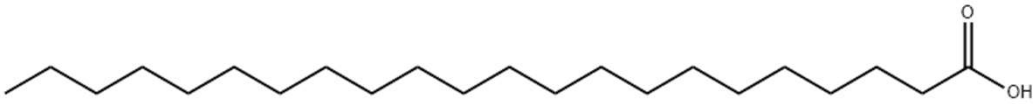


Phytanic acid (C20:0)

Pristanic acid (C19:0)

Fatty acid β-oxidation

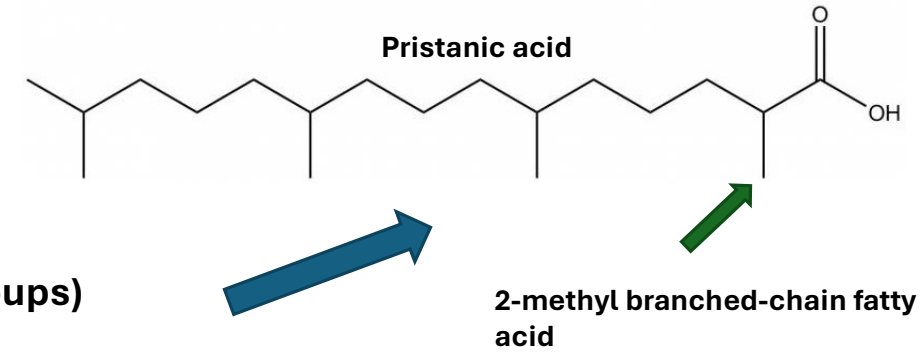
- Catabolism
  - **Very long-chain fatty acids (VLCFA)**
  - Branced-chain fatty acids (BCFA)
  - Dicarboxylic acids



C22 and longer, up to C40!

Fatty acid β-oxidation

- Catabolism
  - Very long-chain fatty acids (VLCFA)
  - **Branched-chain fatty acids (BCFA)**
    - **Pristanic acid (C19:0 = C15:0 with 4 methyl groups)**
    - C_{27}-bile acid intermediates (THCA and DHCA)
  - Dicarboxylic acids



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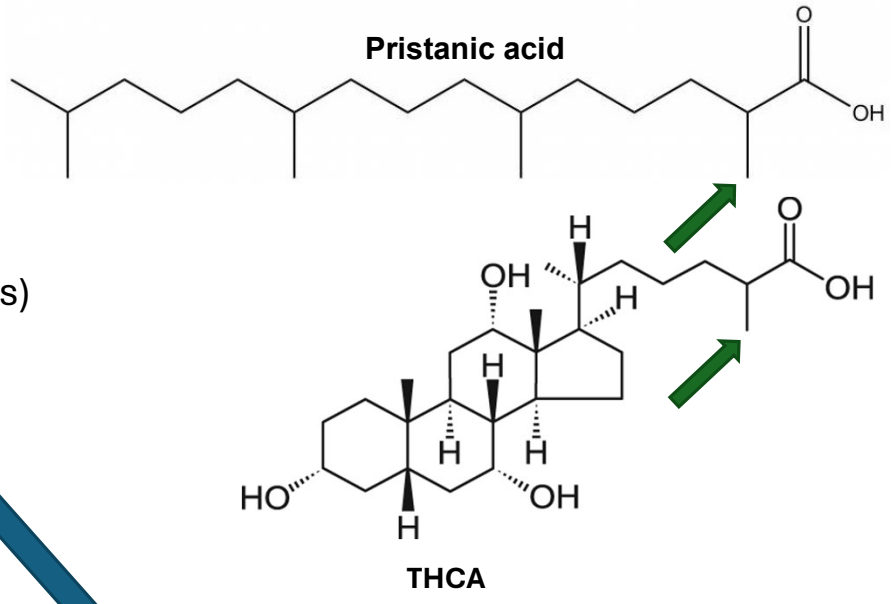
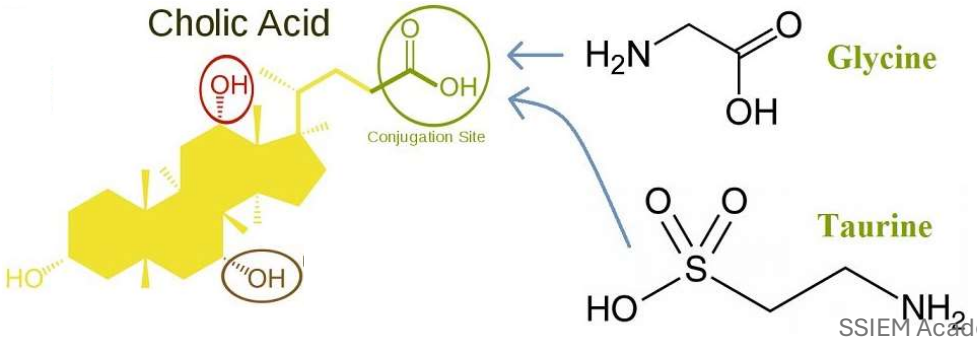


THCA

Conjugation to taurine/glycine



Precursors of the primary bile acids (C_{24}):
cholic acid (CA) and *chenodeoxycholic acid (CDCA)*

So actually bile acid synthesis by oxidation!

Fatty acid β-oxidation

- Catabolism
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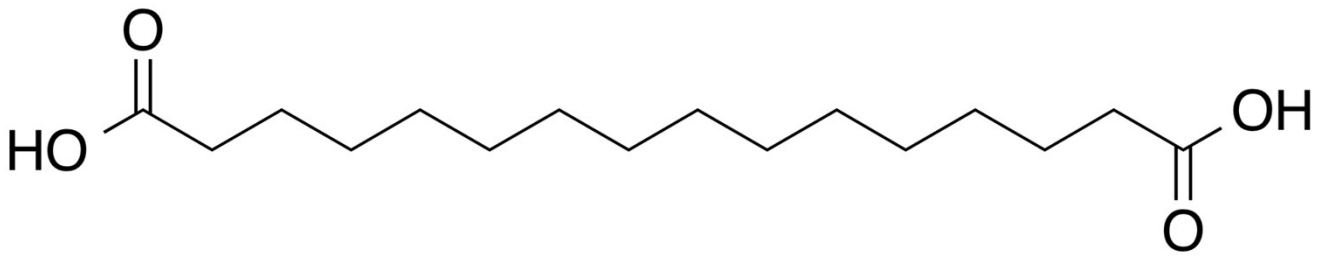
  - Pristanic acid (BCFA)
  - **Dicarboxylic acids**

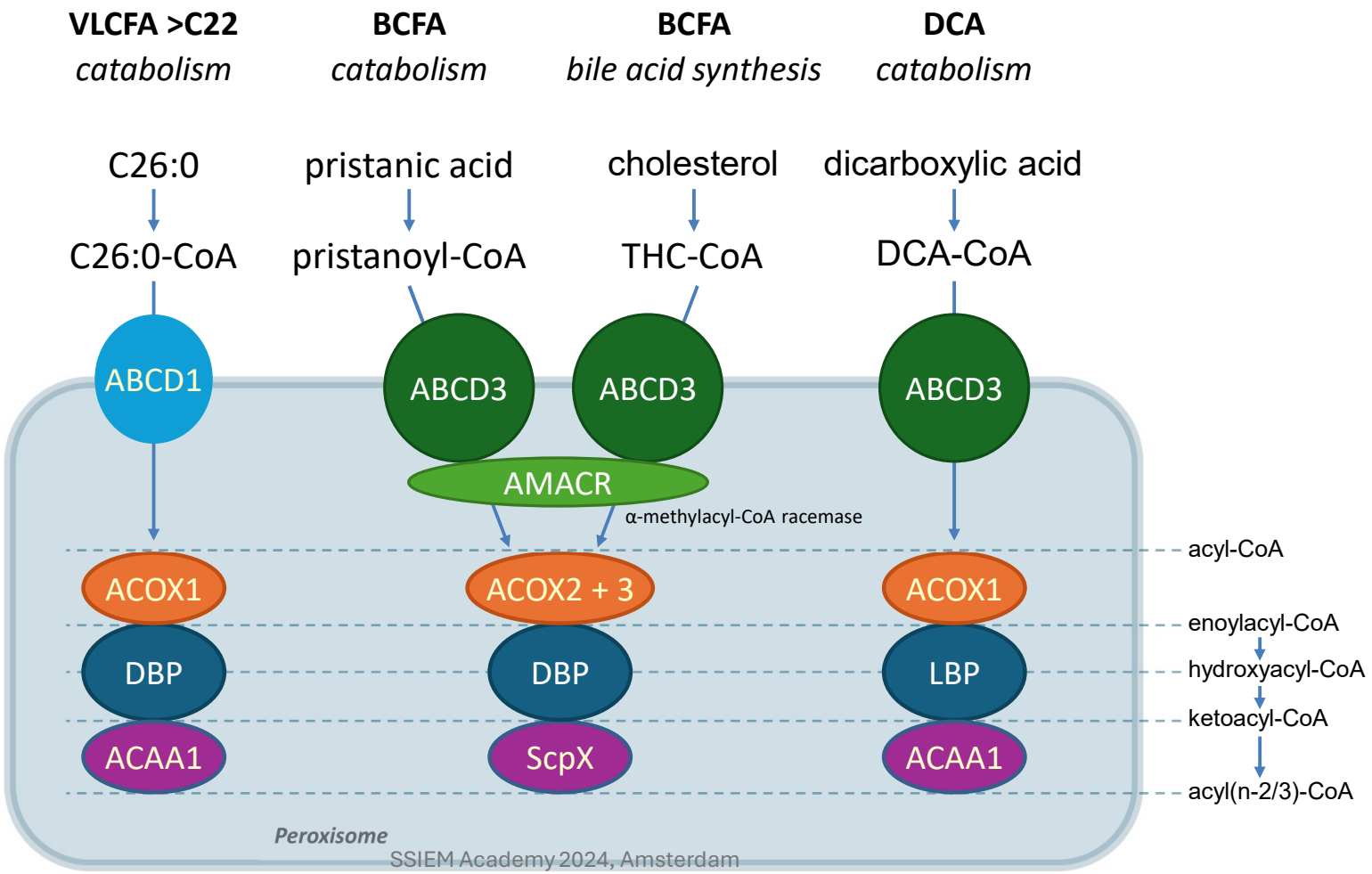


- Products of ω-oxidation in endoplasmic reticulum
- Partially broken down in the peroxisome (then mitochondrion)

ABCD1 = ALDP = adrenoleukodystrophy protein
ABCD3 = PMP70 = peroxisomal membrane protein 70
ACOX = acyl-CoA oxidase
AMACR = α-methylacyl-CoA racemase
DBP = D-bifunctional protein
LBP = L-bifunctional protein
ACAA1 = 3-Ketoacyl-CoA thiolase
ScpX = sterol carrier protein X

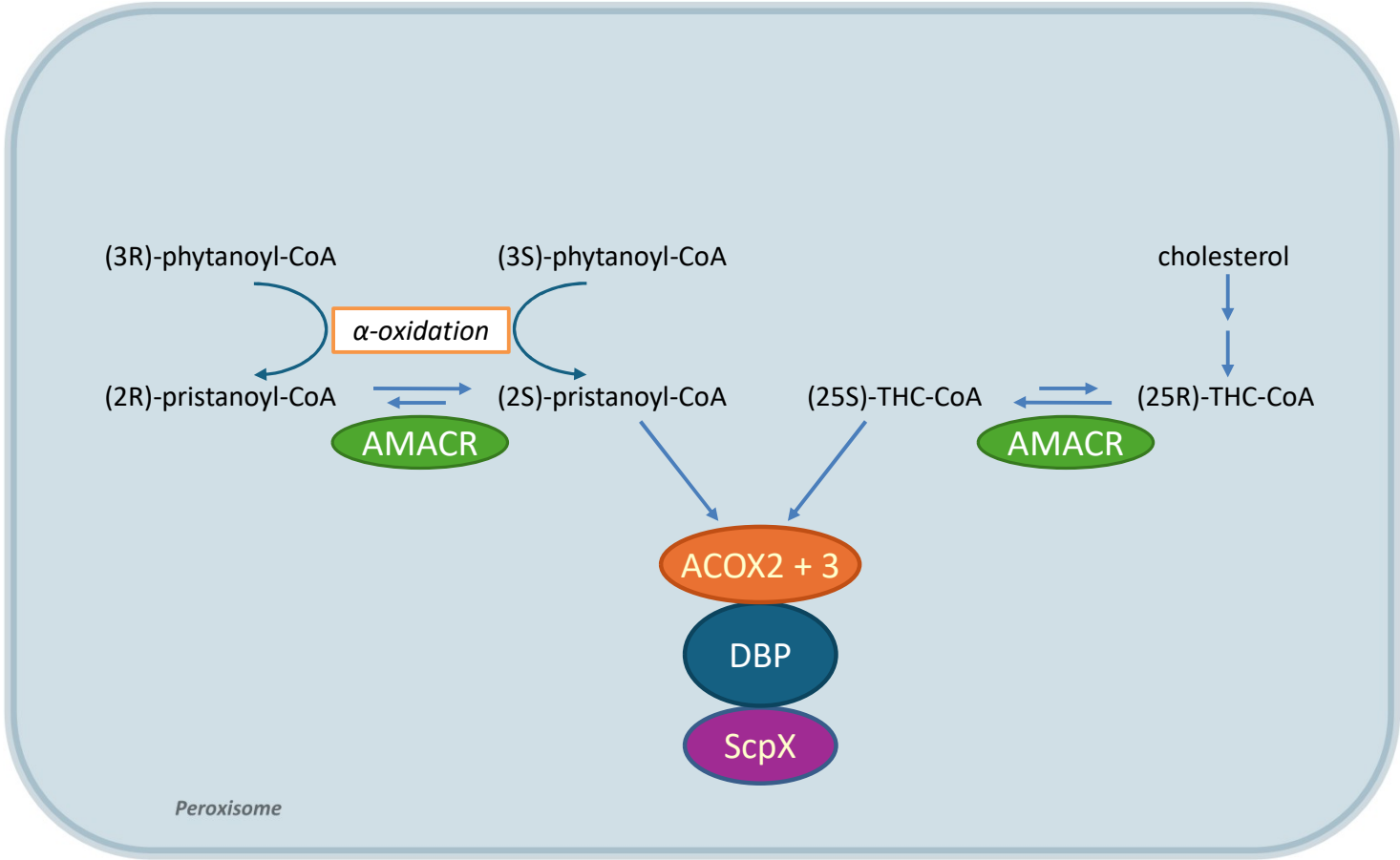
peroxisomal β-oxidation

import and degradation



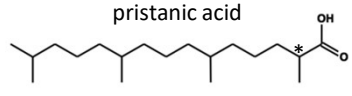
peroxisomal fatty acid metabolism

α-methylacyl-CoA racemase

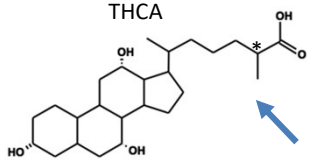


2-methyl branched-chain

pristanic acid

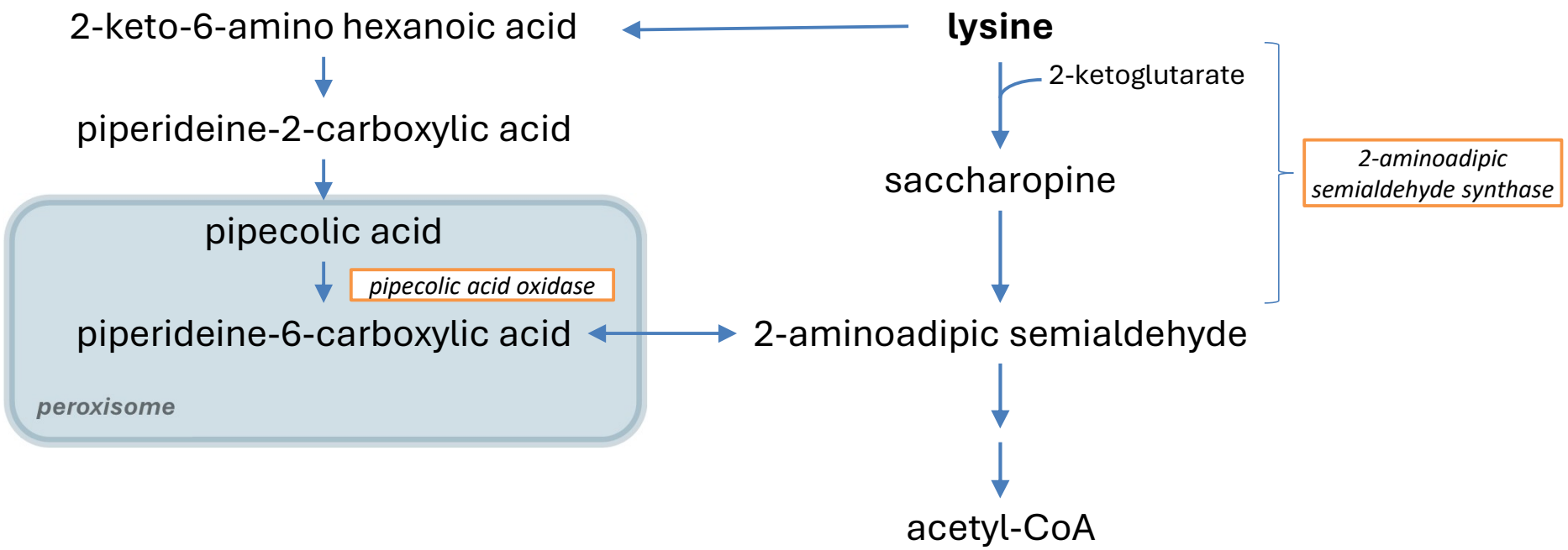


THCA



*asymmetric carbon atom

Pipecolic acid degradation



- Pipecolic acid oxidase is a peroxisomal enzyme

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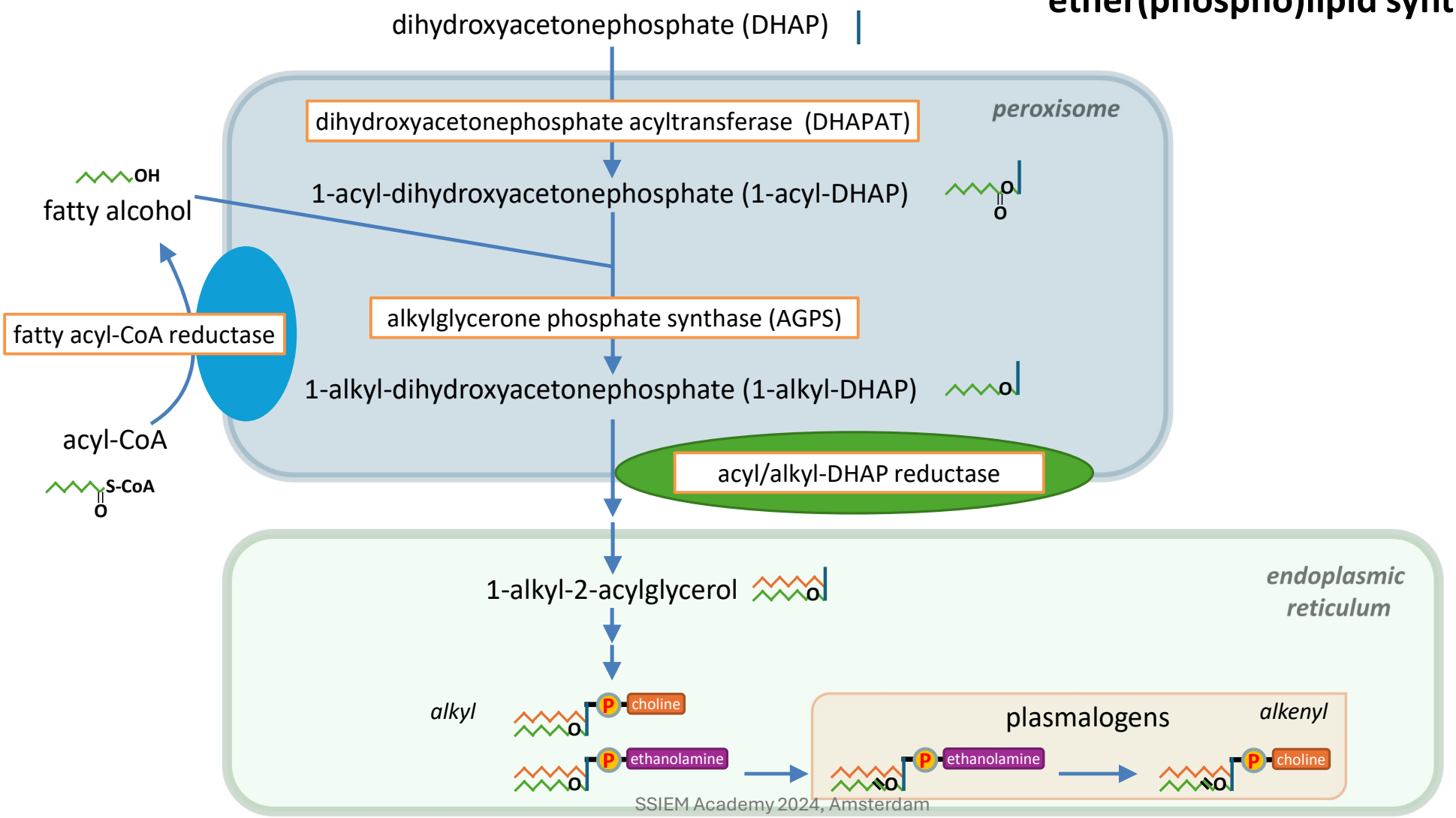
ether(phospho)lipid synthesis



Biomarkers

Blood, urine and fibroblasts

Biochemical investigation in blood (1)

- **Peroxisomal β-oxidation**
  - Very long-chain fatty acids
    - C26:0 hexacosanoic or cerotic acid
    - C24:0 tetracosanoic or lignoceric acid
    - C22:0 docosanoic or behenic acid
    - Ratio C26:0 / C22:0 and C24:0 / C22:0

    → GC, GC/MS (stable isotope dilution) or LC-MS/MS
  - C26:0-lysoPC (VLCFA in a lysophospholipid)

    → LC-MS/MS
  - Pristanic acid

    → GC, GC/MS or LC-MS/MS
  - Bile acid intermediates: dihydroxy- and trihydroxycholestanoic acids (DHCA/THCA)

    → GC, GC/MS (after hydrolysis of Gly and Tau conjugates) or LC-MS/MS
- **Peroxisomal α-oxidation**
  - Phytanic acid

    → GC, GC/MS or LC-MS/MS

Not age-dependent
VLCFA → diet/hyperlipidemia/peanut
C26:0-lysoPC → no dietary influence

Diet-derived! = age-dependent

Biochemical investigation in blood (2)

- **Plasmalogen (<u>erythrocytes</u>)**
  - C16:0- and C18:0-dimethylacetal (after transmethylation)
  - Ratio to the corresponding FA

- **Pipecolic acid**
  - →GC/MS stable isotope dilution
  - →Amino acid analysis: lack of sensitivity if ninhydrine without methylcellosolve
  - →LC-MS/MS

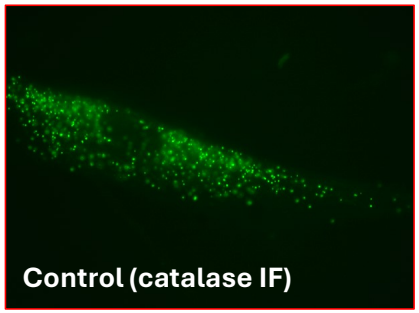
Biochemical investigation in urine

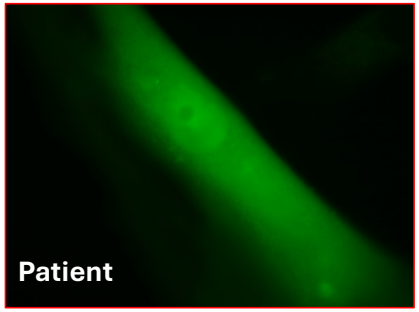
- **Bile acids**
  - LC-MS/MS or direct infusion MS

- **Detoxification of glyoxylate**
  - Oxalic and glycolic acid (GC/MS stable isotope dilution)

(Functional) confirmation of diagnosis

- **Fibroblasts**
  - β-oxidation of C24:0 or C26:0 or d3-C22:0 loading test
  - Measurement of VLCFA and/or C26:0-lysoPC
  - Immunofluorescence microscopy analysis (catalase/ABCD1)
  - β-oxidation of pristanic acid and α-oxidation of phytanic acid
  - DHAPAT activity
  - Immunoblotting for thiolase
  - Complementation analyses





- **DNA**
  - NGS : panel or genes or exome/genome sequencing

Conclusion (1)

Peroxisomal investigation in blood

- At minimum:
  - VLCFA
  - Phytanic and pristanic acid
- If possible:
  - C26-lysoPC in bloodspot
  - C_{27} bile acids: DHCA and THCA (+plasma and urine spectrum)
  - Plasmalogens in erythrocytes
  - Pipecolic acid

⇒Analysis of VLCFA, phytanic, pristanic acid and plasmalogens allows to detect almost all peroxisomal disorders

Conclusion (2)

- NGS / mutation analyses
- Further investigation in fibroblasts: oxidation studies, enzyme activities, western blot, …
  - Specialized labs
- Essential to provide an accurate genetic counselling and prenatal diagnosis

Clinical aspects

Peroxisomal biogenesis disorders

1. Zellweger type

2. Rhizomelic chondrodysplasia punctata type

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PBD: Zellweger spectrum (ZS) type

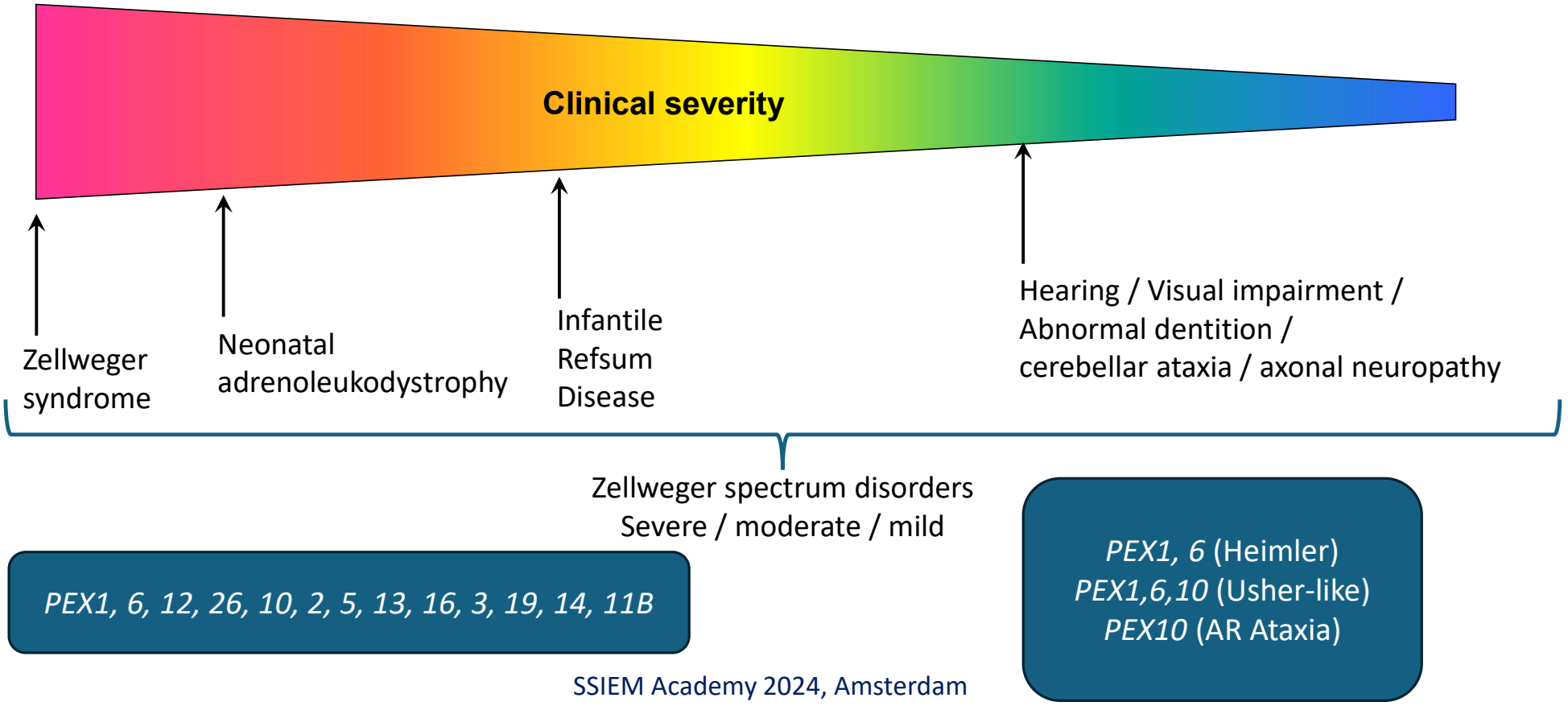
- Autosomal recessive, loss of function variants (13 genes)
  (exception heterozygous p.Arg860Trp variant in *PEX6*)

- >80% of cases caused by *PEX1, PEX6, PEX12 variants* (> 60% PEX1)

- Phenotype relates to the genotype and not the gene (e.g. *PEX1* and *PEX6* disorders can be associated with the full Zellweger spectrum or with a milder Heimler Syndrome)

- Incidence ≈1 in 100,000

PBD-ZS: Continuum of clinical phenotype

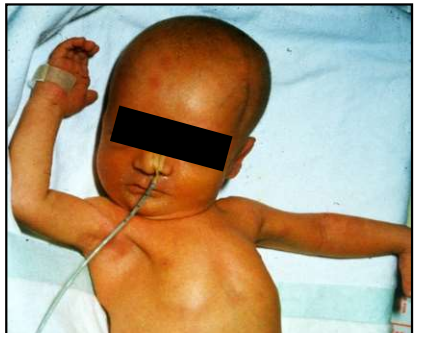


PBD-ZS: Neonatal presentation

'severe'

Central nervous system
Hypotonia / Brain malformations / Seizures

Dysmorphism
Flat face / Large anterior fontanelle / Broad nasal bridge / Widely split sutures



Gastrointestinal / Hepatic
Poor feeding
Hepatomegaly / cholestasis / Jaundice / hepatic dysfunction

Other
Renal cysts

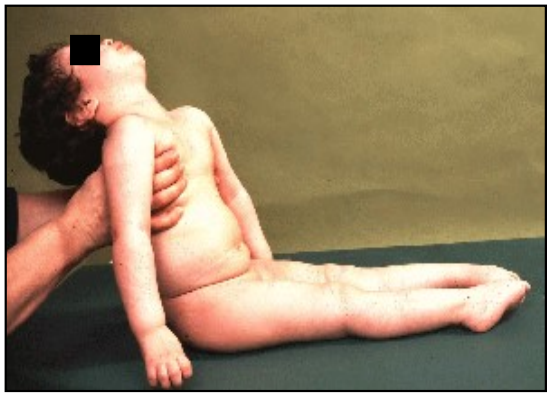
The most severe die within 1 year

PBD-ZS: Presentation in an older child

'intermediate / milder'

Sensory
Retinal dystrophy
Sensorineural hearing loss

Nervous system
Hypotonia
Developmental delay
Leukodystrophy



Gastrointestinal / Hepatic
Hepatic dysfunction – vitamin K responsive coagulopathy

Other
Adrenal insufficiency
Reduced bone mineral density

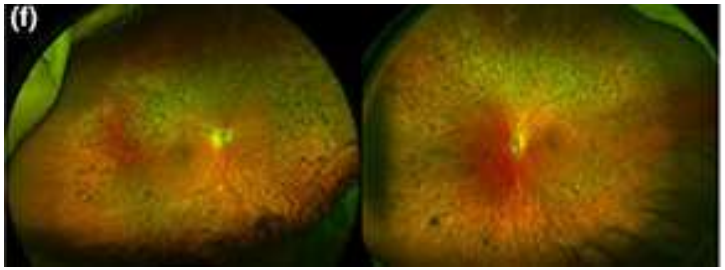
Very variable life expectancy

PBD-ZS: Presentation / diagnosis in an adult 'mildest'

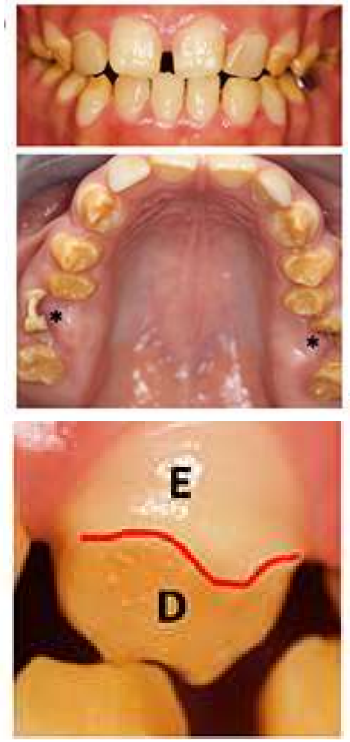
Sensory
Vision impairment (retinal dystrophy) / Cataracts / Hearing loss (sensorineural)

Dental
Ameliogenesis imperfecta

Nervous system
Cerebellar ataxia
Peripheral neuropathy



Retinal image: increased pigment, thinning of the vessels



doi: 10.1002/ajmg.c.31823

PBD-ZS: Treatment

- Largely symptomatic
- May include vitamin K, adrenal replacement
- Low phytanate diet – not proven
- Cholic acid supplementation – not proven

PBD: Rhizomelic chondrodysplasia punctata (RCDP) type

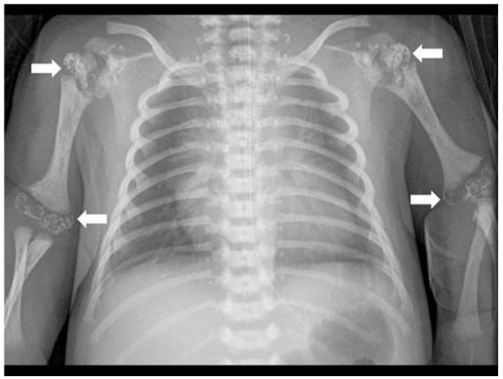
- Peroxisomal biogenesis defects or single enzyme deficiencies – all involved in plasmalogen biosynthesis (etherlipid metabolism disorders)
- Mild / moderate / Severe 'classic' disease
- Incidence <1 in 100,000
- NOT all have rhizomelia!

Number	Gene	Type
RCDP1	***PEX7****	**Biogenesis defect**
RCDP2	*GNPAT*	Single enzyme
RCDP3	*AGPS*	Single enzyme
RCDP4	*FAR1*	Single enzyme
RCDP5	*PEX5*	Biogenesis defect

PBD-RCDP1: Presentation / Progression

Skeletal abnormalities
Shortening of the humerus / femur / stiff painful joints (contractures) / epiphyseal stippling / spinal stenosis

Congenital cardiac disease
Septal defects / tetralogy of Fallot / Pulmonary stenosis



Other
Congenital cataracts eczema / icthyosis / rashes / respiratory infections

Nervous system
Developmental delay
Seizures
Cerebral / cerebellar atrophy

The most severe die before the age of 10 years

PBD-RCDP: Treatment

- Largely supportive
- May include spinal cord decompression, orthopaedic surgery, treatment of congenital cardiac disease
- Low phytanate diet – consider for those with milder forms

Clinical aspects

Import disorders

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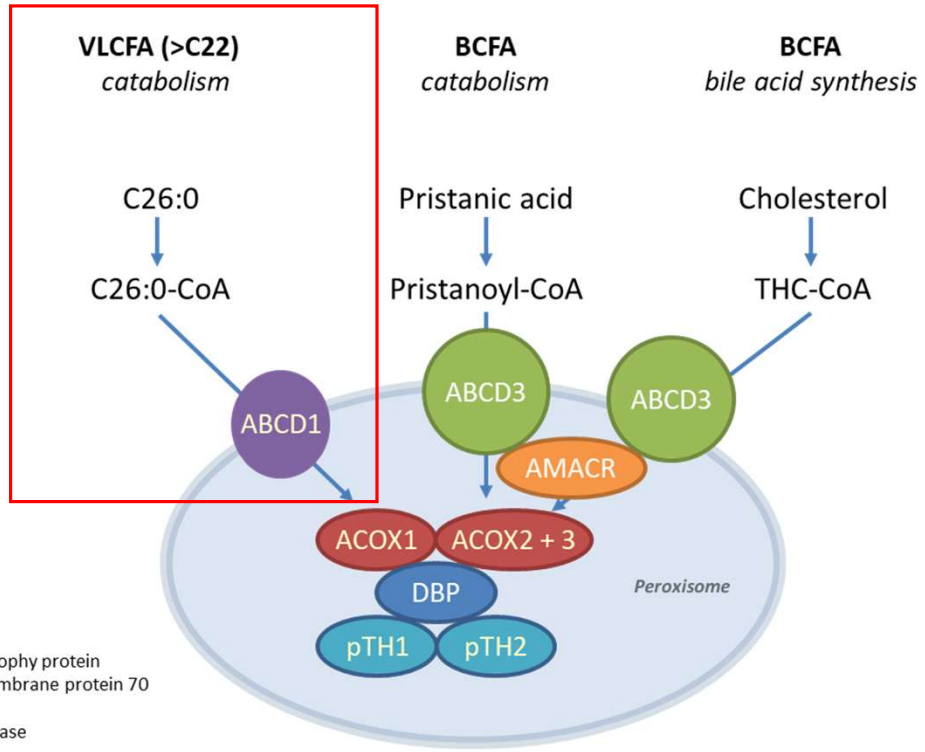
Peroxisomal functions
Import and degradation



ABCD1 = ALDP = adrenoleukodystrophy protein
ABCD3 = PMP70 = peroxisomal membrane protein 70
ACOX = acyl-CoA oxidase
AMACR = α-methylacyl-CoA reductase
DBP = D-bifunctional protein
pTH = peroxisomal thiolase

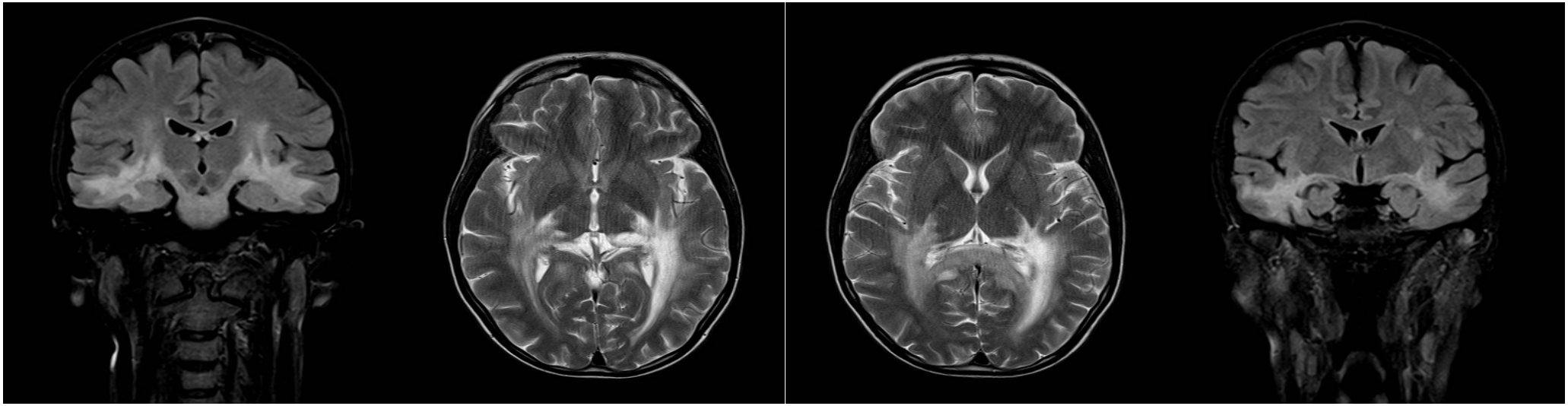
X-linked adrenoleukodystrophy / adrenomyeloneuropathy (X-ALD/AMN)

- X-linked pathogenic variants in the *ABCD1* gene
- Prevalence ≈ 1 in 14,000 male births
- Phenotype cannot be predicted by the VLCFA concentration or by the nature of the pathogenic *ABCD1* gene variant.
- Diagnosis
  - (sex-specific) newborn screening (USA, Taiwan, Netherlands, Japan);
  - clinical presentation;
  - family screening

X-ALD/AMN: phenotypic heterogeneity

Adrenal insufficiency	**Adrenoleukodystrophy**	**Adrenomyeloneuropathy**
Males	Males	Males and (≈80%) females
Any age (typically 2-7 years)	Any age (typically 3-12 years)	Typically from 20s onwards
Adrenal autoantibody negative	Behavioural change Cognitive change Focal neurological deficits Seizures	Leg weakness, spasticity, pain, bladder & bowel dysfunction
Overlap of all X3 phenotypes		

X-ALD/AMN: Cerebral form



Age at death 25 years. These images aged 23 years.

Cerebral X-ALD: Management

Steroid replacement / Sick-day rules

Surveillance

Clinical review / MRI every 3-6 months until aged 10 years, then yearly thereafter

Treatment

Haematopoietic stem cell transplant (HSCT)

Ex vivo gene therapy (Bluebird Bio: Skysona, Lenti-D vector – phase III)

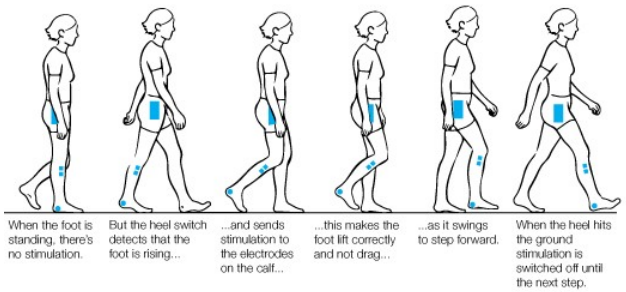
Early stage of disease

(Near) normal neurological examination)(NFS < or =1)

Low lesion load on MRI (Loes score 0.5-9.0)

Adrenomyeloneuropathy: Management

- Physiotherapy, orthotics
- Muscle relaxants – baclofen
- Pain management
- Functional electrical stimulation



- Bladder dysfunction – medications; intermittent self-catherisation; botulinum toxin; sacral nerve stimulation
- Bowel dysfunction – medications; enemas; sacral nerve stimulation; transanal irrigation
- Falls management, mobility aids, home adaptations etc

X-ALD/AMN: Very active area of research

Minoryx Therapeutics – Leriglitazone (oral PPARγ agonist, metabolite of pioglitazone)

Did not meet primary goal in AMN clinical trial

Cerebral ALD – in clinical trial NCT05819866 (men) and NCT04528706 (boys)

Poxel – Clinical trials NCT05146284 PXL770 (activates AMPK) and NCT05200104 PXL065 (R-stereoisomer of pioglitazone)

Not yet recruiting

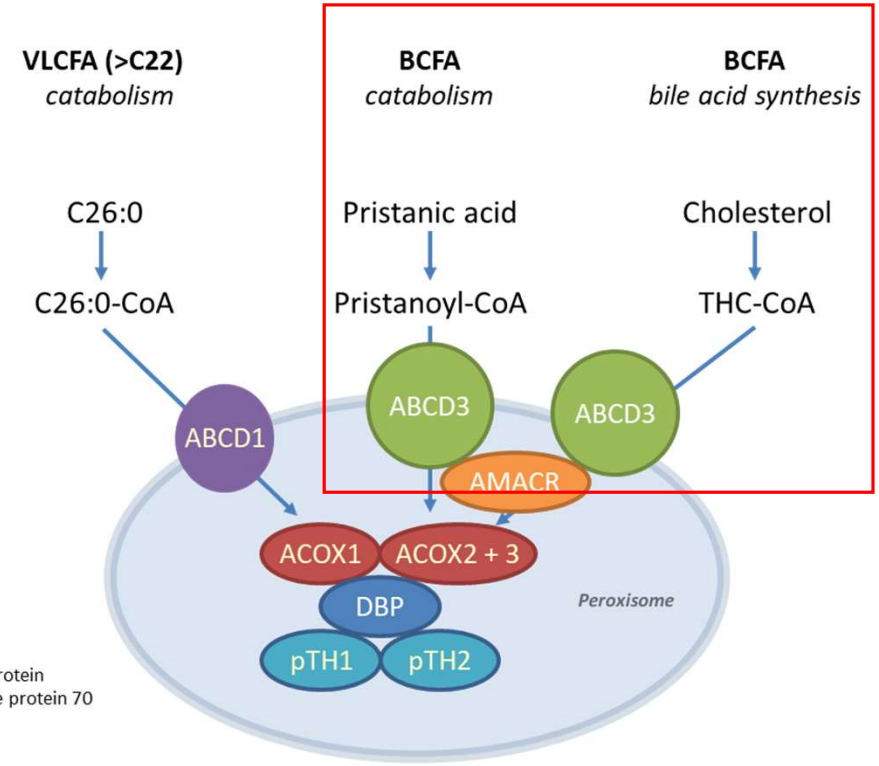
Viking Therapeutics - Clinical trial NCT04973657 VK0214 - thyroid hormone receptor agonist – upregulates ABCD2 – primary completion expected 2024

Swanbio Therapeutics – SBT101 (AAV gene therapy)

A Study to Evaluate Administration of SBT101 Gene Therapy in Adult Patients With Adrenomyeloneuropathy (AMN) (PROPEL)

Clinical trial NCT05394064 – primary completion expected in 2026

Peroxisomal functions
Import and degradation



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ABCD3 deficiency

Very rare, autosomal recessive, first described in 2015

Hepatosplenomegaly + impaired hepatic function

Biochemical abnormalities
- C27 bile acid intermediates in plasma
- Impaired beta-oxidation of pristanic acid

Clinical aspects

Single enzyme disorders

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(Adult) Refsum disease

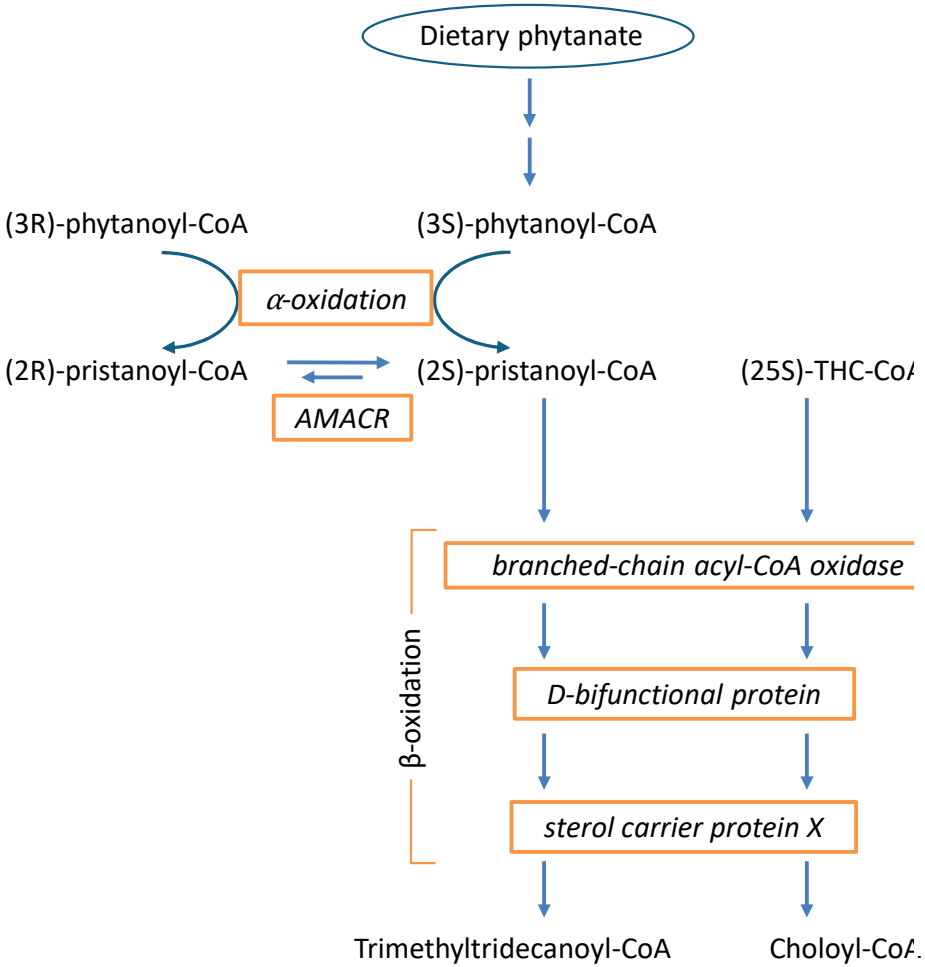
- ***NOT*** to be confused with infantile Refsum disease (the peroxisomal biogenesis disorder)

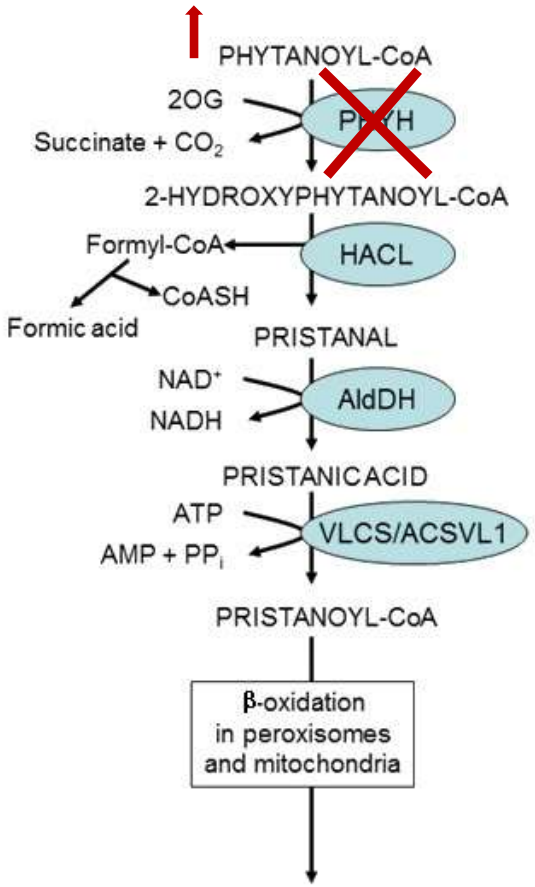
- Caused by:
  - Single enzyme deficiency – phytanoyl-CoA hydroxylase (≈90% of cases)(Gene – *PHYH*)
  - *PEX7* gene variants (≈10% of cases)(targets phytanoyl-CoA hydroxylase to where it needs to be)

- Autosomal recessive

- Onset: late childhood to adulthood

peroxisomal fatty acid metabolism





THC(A) = di/trihydroxycholestanoic (acid)

Adult (Refsum disease)

Clinical features: chronic

Retinitis pigmentosa (100%) +/-

- Anosmia (>85%)
- Polyneuropathy (sensory and motor)
- Sensorineural hearing loss
- Ataxia
- Ichthyosis
- Short metacarpals and metatarsals
- Cardiac arrhythmias and cardiomyopathy

Clinical features: acute

Triggered by weight loss, trauma, infections

Weakness, ataxia, sudden visual or auditory deterioration, ichthyosis

ie. a stroke-like presentation

Adult (Refsum disease): Management

Management: Chronic

- Low phytanate diet
- Ruminant (cow, sheep, and goat) products and certain fish (cod) products
- Check that parentral / entral feeds are phytanate free

Management: Acute

- Plasmapheresis / lipid apheresis
- For significant weakness, cardiac disease
- Avoid fasting (acute mobilisation of lipids)

Treatment goal: Maintain plasma phytanic acid 100 – 300 µmol/L

Alpha-methylacyl CoA racemase deficiency

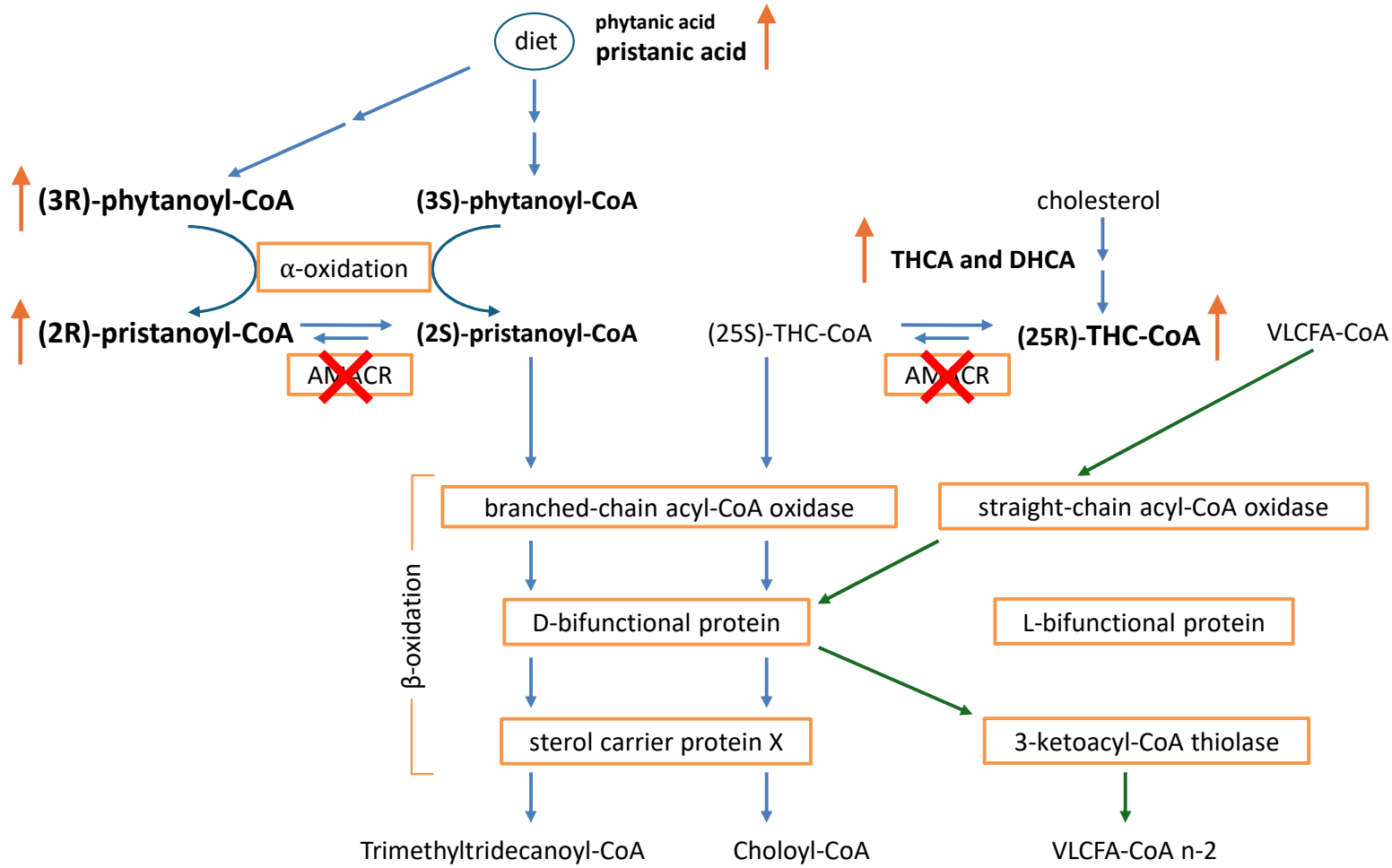
Autosomal recessive variants in *AMACR* gene

Clinically very heterogenous

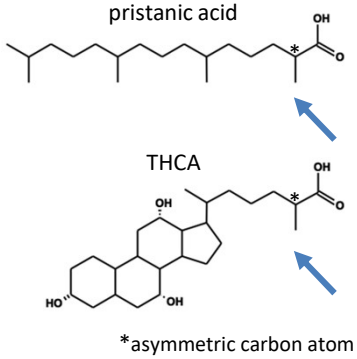
More commonly presents in adults than children

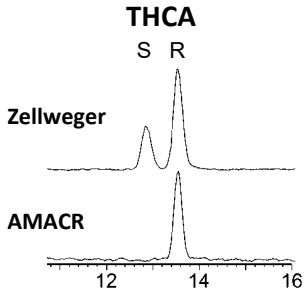
α-methylacyl-CoA racemase deficiency

chirality matters



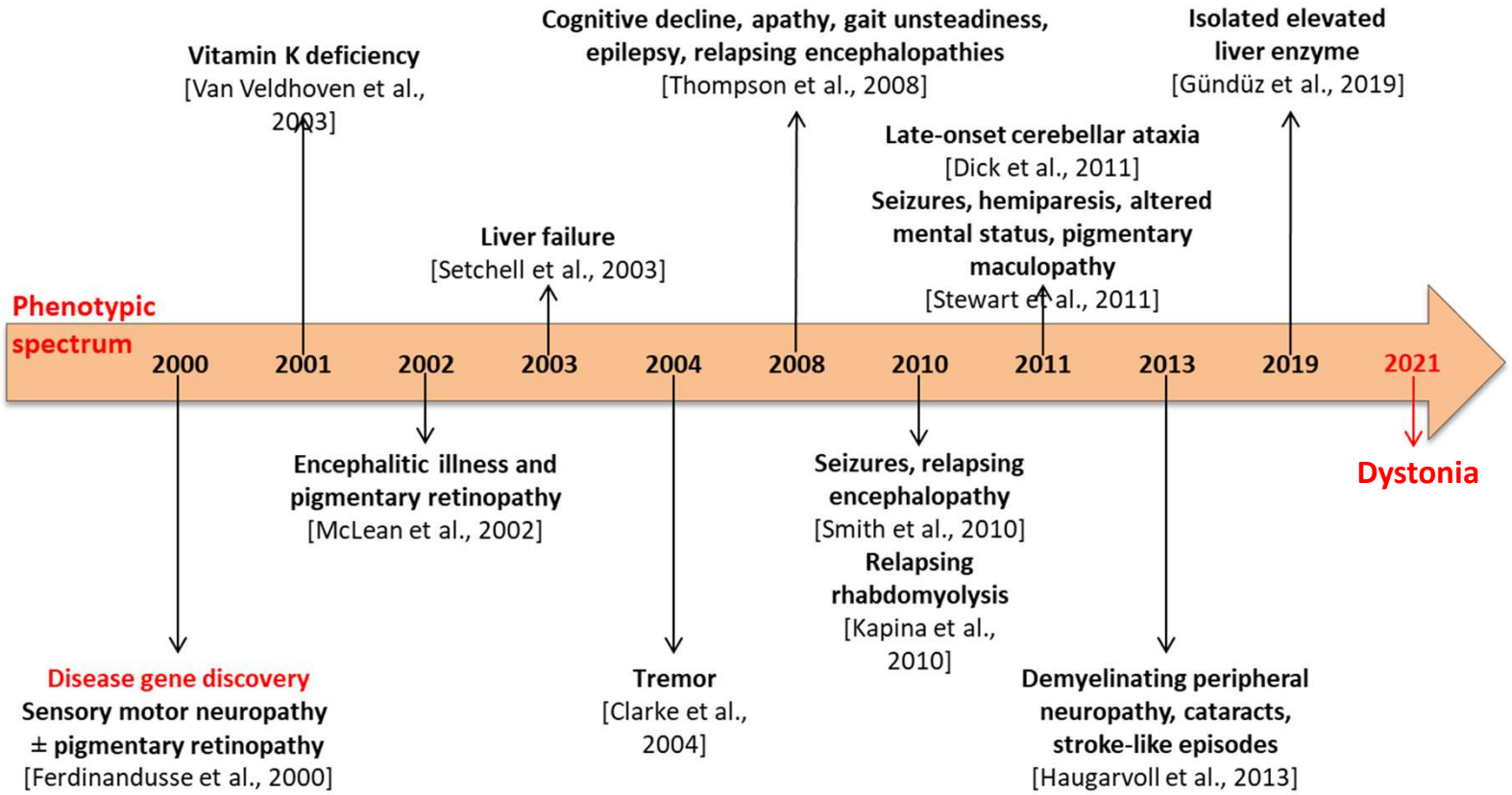
2-methyl branched-chain





THCA = trihydroxycholestanoic acid

AMACR deficiency: clinical heterogeneity



Slide thanks to Francesca Magrinelli

AMACR deficiency: Management

Low phytanate (pristanate) diet

Cholic acid supplementation

Avoid fasting, rapid weight loss

Very limited long-term follow up on effectiveness

Other single enzyme deficiencies

(that can mimic PBD-ZS disorders)

Gene	Protein / enzyme	Clinical
HSD17B4	D-bifunctional protein	Hearing loss, ataxia, hypogonadism, neuropathy
ACOX2	Branched chain acyl-CoA oxidase	Liver dysfunction
ACOX1	Acyl-CoA oxidase	Hypotonia, seizures, failure to thrive, developmental delay, and neurological regression
SCP2	Sterol carrier protein-2	Dystonia, neuropathy
ABCD5	Acyl-CoA binding domain containing protein 5	Spastic paraparesis, ataxia, leukodystrophy, retinal dystrophy

Clinical aspects

Disorders of glycoxylate / oxalate metabolism

SSIEM Academy 2024, Amsterdam

Hyperoxaluria

Autosomal recessive variants in:

AGXT (type 1): alanine:glyoxylate aminotransferase deficiency

GR/HPR (type 2): glyoxylate/hydroxypyruvate reductase (GR/HPR) deficiency

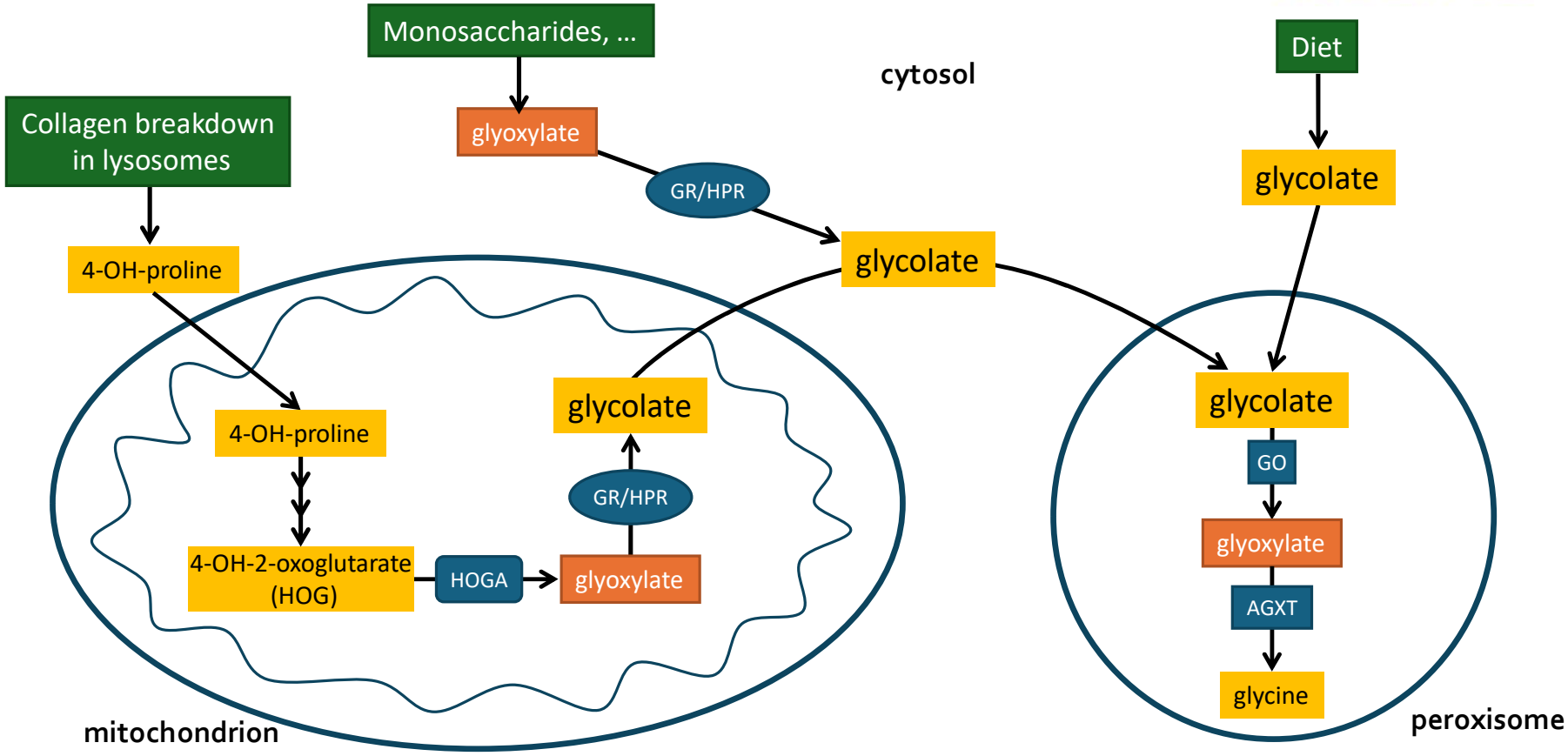
HOGA1 (type 3): 4-hydroxy-2-oxoglutarate aldolase (HOGA) deficiency

All lead to elevated oxalate – needs to be excreted in urine – binds to calcium - insoluble calcium oxalate crystals (calcium oxalate monohydrate)

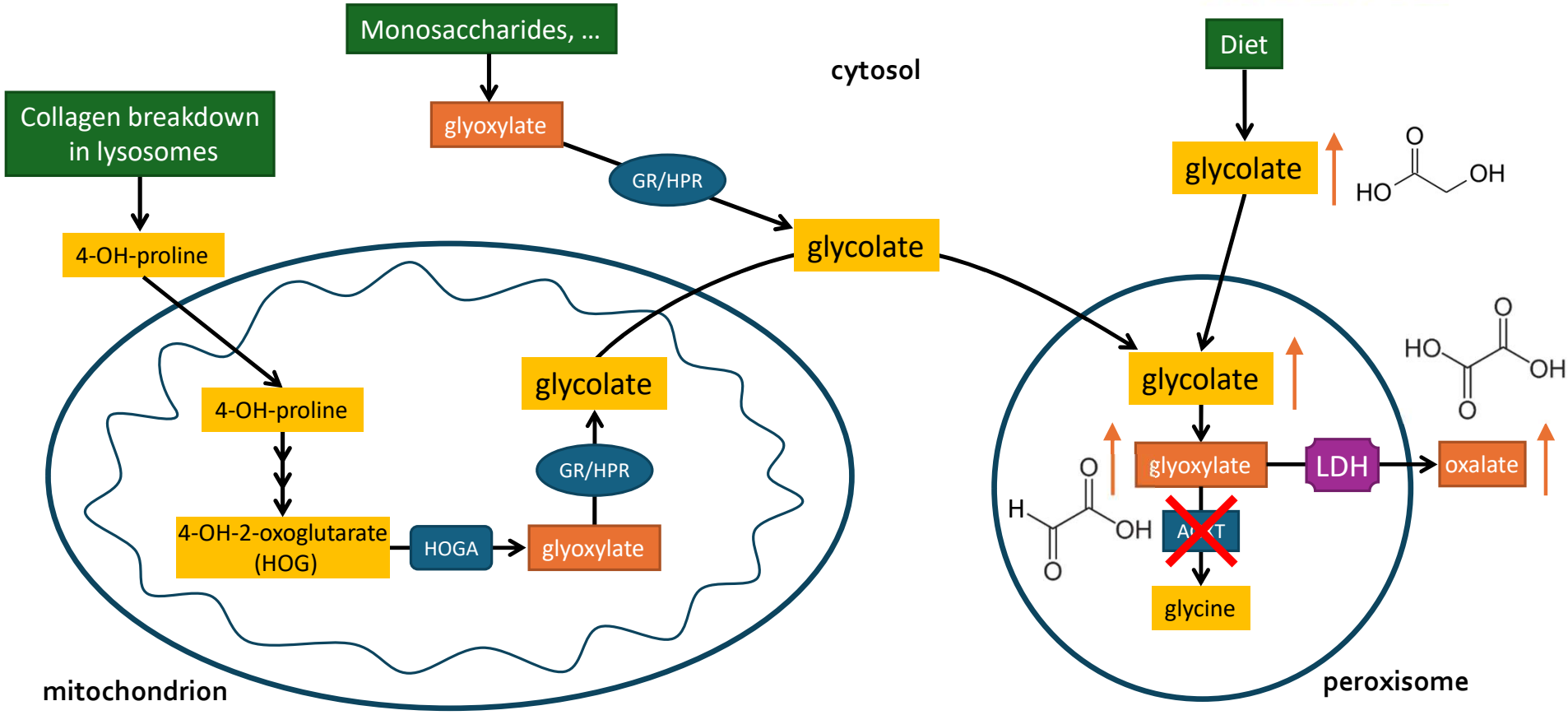
Clinical features: nephrocalcinosis, nephrolithiasis, end-stage renal disease

Onset: Infancy - adulthood

Glyoxylate metabolism



Hyperoxaluria type 1: AGXT deficiency



Hyperoxaluria type 1: Disease progression

As GFR falls, calcium oxalate is deposited outside the kidneys

Retina: Visual disturbance.

Heart: Cardiac conduction disturbances and cardiomyopathy.

Vessels: Vascular involvement can lead to ischemia, infarcts, non-healing cutaneous ulcers.

Bone: Oxalate osteodystrophy - bone pain and pathologic fractures, refractory anemia.

Nerves: Peripheral neuropathy.

Management: Pyridoxine, liver transplantation, renal transplantation

Lumasiran (Oxlumo®), an mRNAi therapeutic agent

Peroxisomal disorders: Conclusions

- Mostly autosomal recessive (exception: X-ALD/AMN)
- Multisystem → single system disorders; presentation at ANY age
- Significant biochemical derangements → no/limited biochemical abnormalities
- Neonatally fatal → no impact on life expectancy (mild/moderate/severe)
- Limited specific treatments (exceptions: X-ALD/AMN; Refsum disease; hyperoxaluria; bile acid or hormone replacement)