Workshop 1 – Diagnosis and management of peroxisomal and purines and pyrimidines disorders

Patient 1

Clinical information

- Man of 38 years with no previous medical history
- Complaints of gait disorder: poor balance (worse in the dark and in case of uneven surface); sometimes also falls
- Urge incontinence (urinary)
- In retrospect, slight gait disorder already present for many years (difficulty running past 5 years), slowly progressive
- Also fatigue, for many years

Examination

- Increased tone in both legs, reduced vibration sense in the hallux on both sides, brisk deep tendon reflexes in the legs, bilateral Babinski sign

- Findings are compatible with gait disorder and incontinence due to slowly progressive spinal cord disease with involvement of the corticospinal tracts and dorsal columns

- Not very specific, many possible causes (both acquired and genetic)

What is your differential diagnosis?

Differential Diagnosis

- Multiple sclerosis
- Hereditary spastic paraplegia
- Spinocerebellar ataxia
- Vitamin B_{12} deficiency
- etc

What (besides NGS) do you order at the lab?

Relevant results

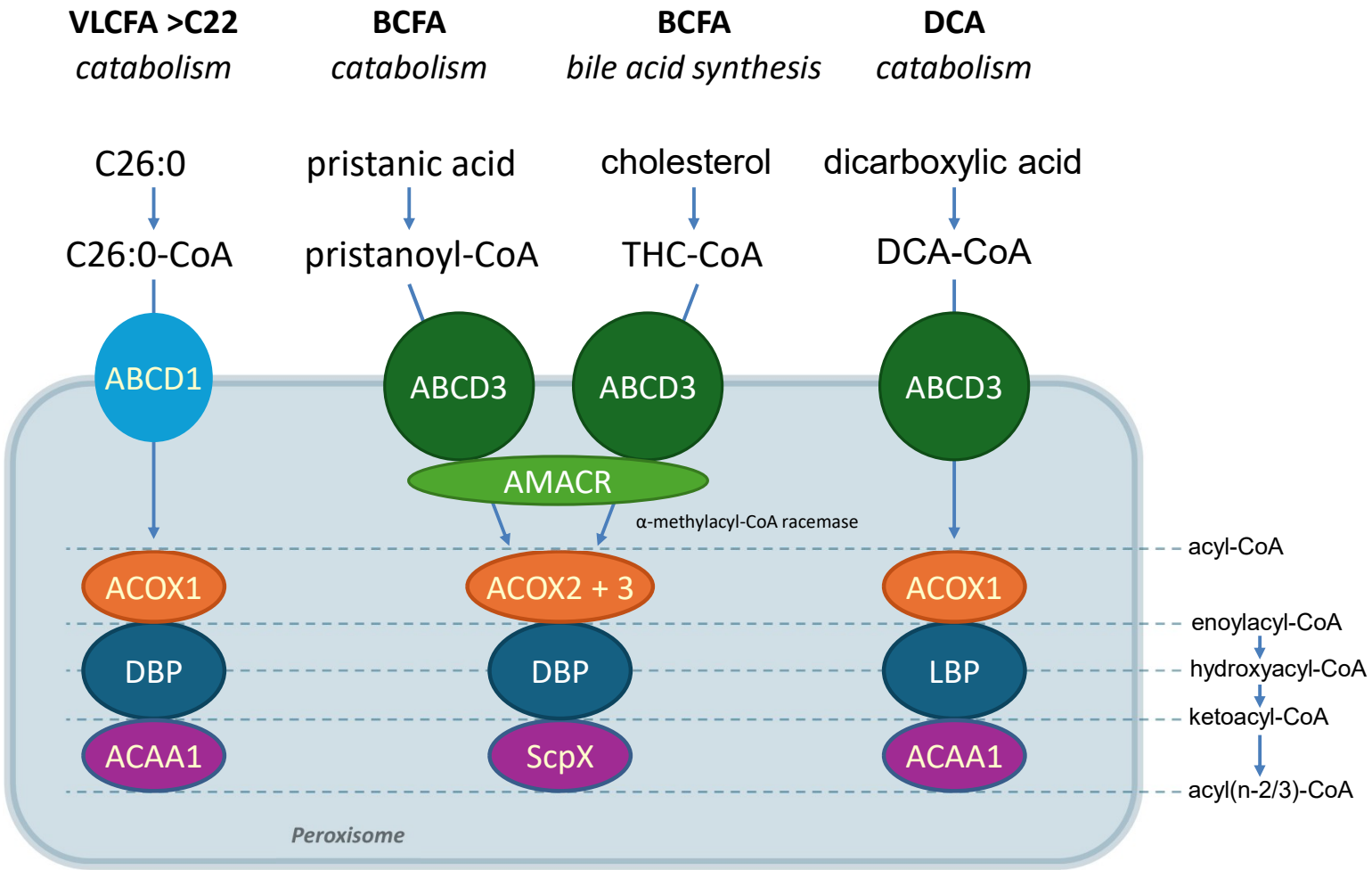
Metabolite	Results	Control range
C26:0	**3.43** µmol/L	0.43 – 1.06
C22:0	47 µmol/L	40 - 119
C24:0	79 µmol/L	33 - 84
C26:0 / C22:0	**0.073**	0.006 – 0.019
C24:0 / C22:0	**1.68**	0.69 – 0.99
Phytanic acid	2.6 µmol/L	< 5.0
Pristanic acid	0.35 µmol/L	< 1.0
Pipecolic acid	0.6 µmol/L	0.5 – 5.0
Sodium	**131 mmol/L**	135-145

What is your diagnosis?

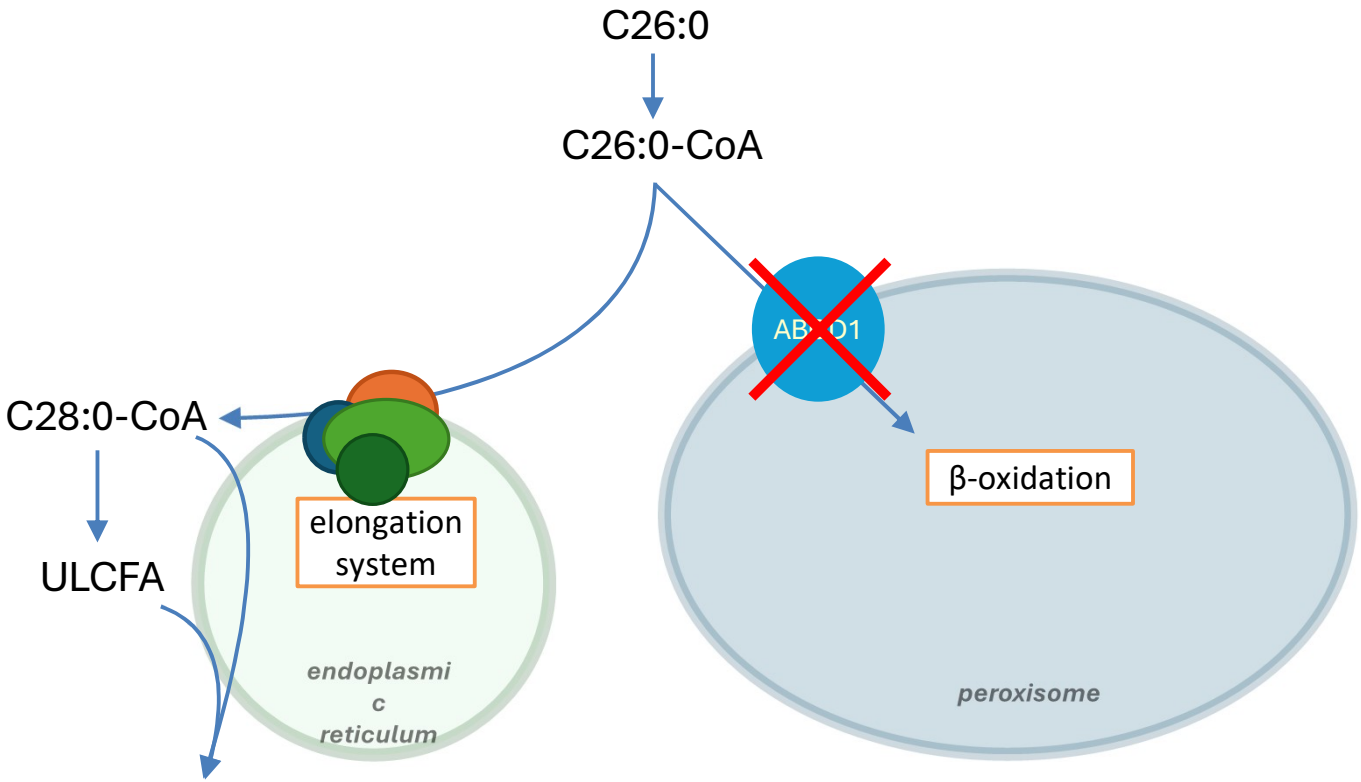
ABCD1 = ALDP = adrenoleukodystrophy protein
ABCD3 = PMP70 = peroxisomal membrane protein 70
ACOX = acyl-CoA oxidase
AMACR = α-methylacyl-CoA racemase
DBP = D-bifunctional protein
LBP = L-bifunctional protein
ACAA1 = 3-Ketoacyl-CoA thiolase
ScpX = sterol carrier protein X

peroxisomal β-oxidation

import and degradation



adrenoleukodystrophy

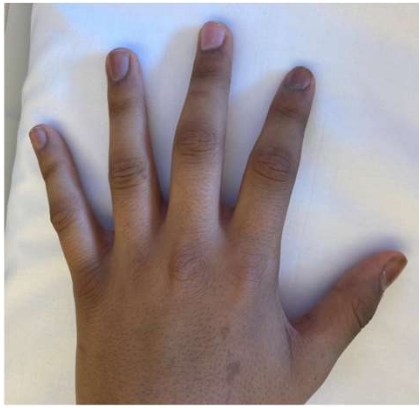


VLCFA and ULCFA incorporation in membrane lipids

Biomarkers: C26:0-lysoPC and total VLCFA

Patient 2

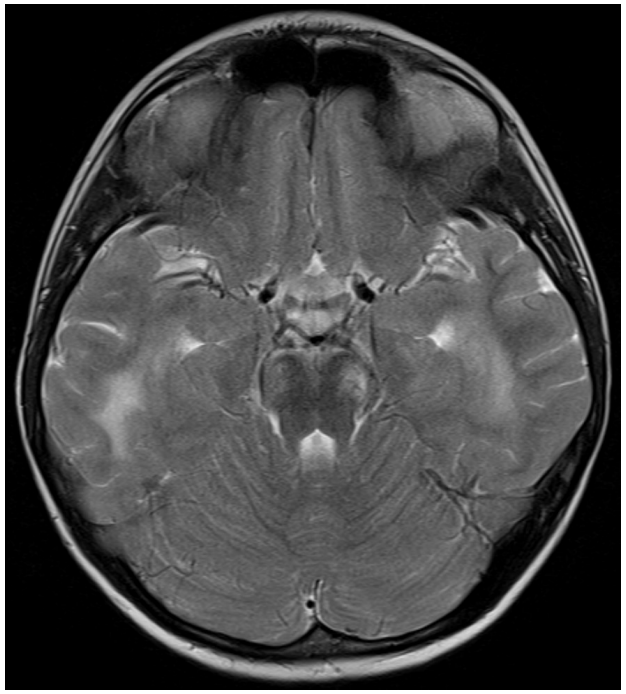
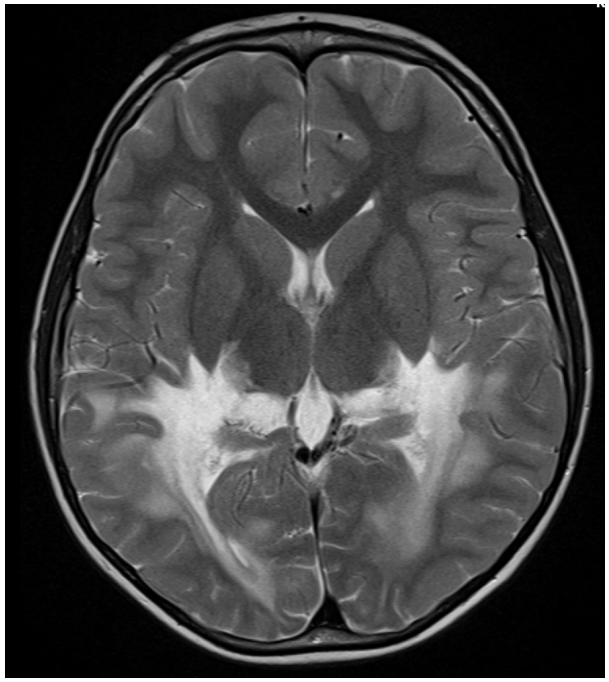
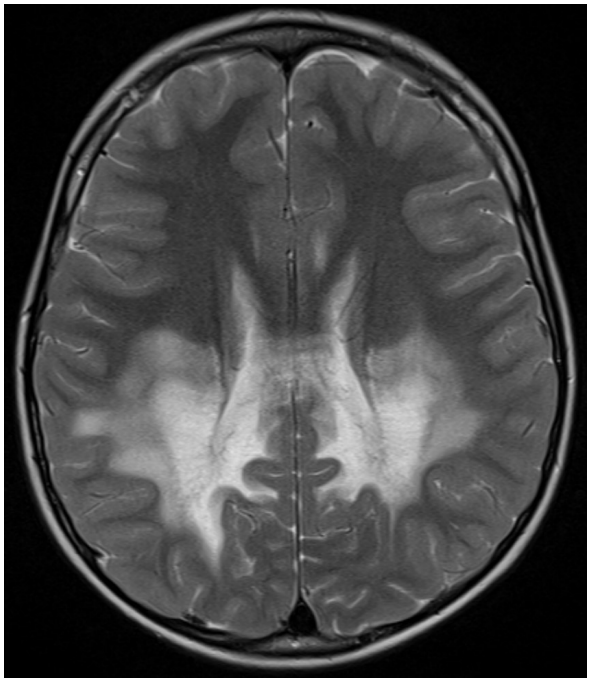
Clinical information



- Boy with no previous medical history, now 7 years old
- Used to do well in school, past 6 months sharp decline in school performance, mostly due to attention-deficit problems
- Seems to be “deaf” sometimes, neurological examination unremarkable
- Parents noted brownish pigmentation over the knuckles
- There is dysfunction at school, with a wide differential diagnosis, also non-neurological causes
- “Red flags” are the abrupt onset, and possible auditory inattention

What do you order ASAP?

Brain MRI

Loes Score: parieto-occipital WM 3, anterior temporal WM 1, corpus callosum 2, visual pathway 4, auditory pathway 2, corticospinal frontopontine fibres 2
total 14

MRI interpretation and further course

- Extensive white matter lesions compatible with leukodystrophy, with typical gadolinium enhancement beyond the leading edge of the lesion
- Pathognomonic for adrenoleukodystrophy
- Confirmed by *ABCD1* mutation analysis and increased C26:0-LPC
- No longer eligible for HSCT (outcome very poor in advanced disease)
- Supportive care, now (3 years later) bedridden and requiring tube feeding

Adrenoleukodystrophy – clinical phenotypes

Childhood cerebral form (boys 4-12 years) 40%

- School failure, cognitive regression, behavioural changes, ataxia, adrenal insufficiency
- Leukodystrophy → decerebration within 2-4 years

Adrenomyeloneuropathy (AMN) (males early adults) 55%

- Progressive spastic paraparesis, impotence, peripheral neuropathy, adrenal insufficiency
- Survival: decades

«Adrenal insufficiency only» (boys childhood-adult) <5%

- Maybe only adrenal insufficiency

Female heterozygotes: >50% with AMN-like symptoms, white matter not involved

Virtually all males eventually develop AMN

☞ *Lesson to learn from these patients*

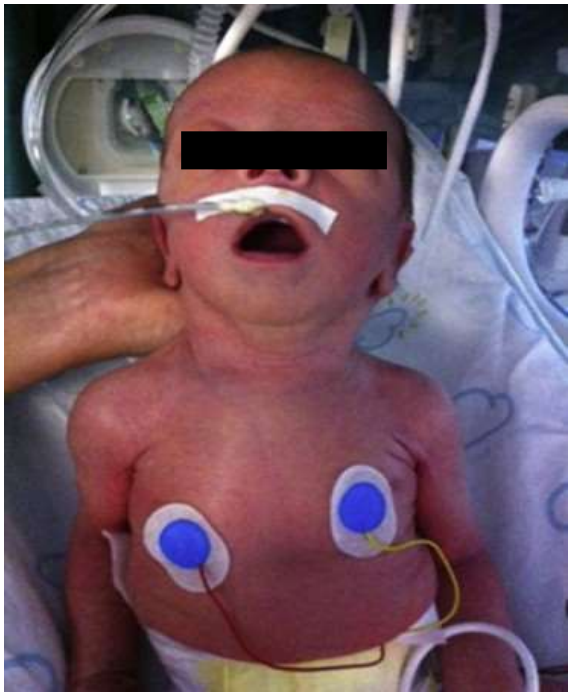
- ALD is the most frequent peroxisomal disorder: prevalence 1 in 14.000
- X-linked disorder, female carriers (heterozygotes) can be symptomatic
- Neurological and endocrine symptoms
- Several phenotypes
  - Childhood cerebral form with demyelination (usually <10 y)
  - Early adulthood and middle age form with adrenomyeloneuropathy
  - At any age: adrenal insufficiency
- Peroxisomal investigation: abnormal VLCFA (normal in approximately 15% of heterozygote/carriers)

A newborn with severe hypotonia

SSIEM Academy 2024, Amsterdam

Clinical presentation

- Girl born from non-consanguineous parents
- At birth
  - Generalized severe hypotonia
  - Dysmorphic features: high forehead, large fontanelle, high arched palate, retrognathia, low-set ears
- At 3 days of life
  - Development of convulsive encephalopathy, resistant to anticonvulsant drugs
  - Hepatomegaly
- Biochemical investigation
  - Organic acids: normal profile
- Died at 3 months of age



What test(s) would you like to perform?

Peroxisomal investigation in plasma

Metabolite	Results	Control range
C26:0	**10.35** µmol/L	0.43 – 1.06
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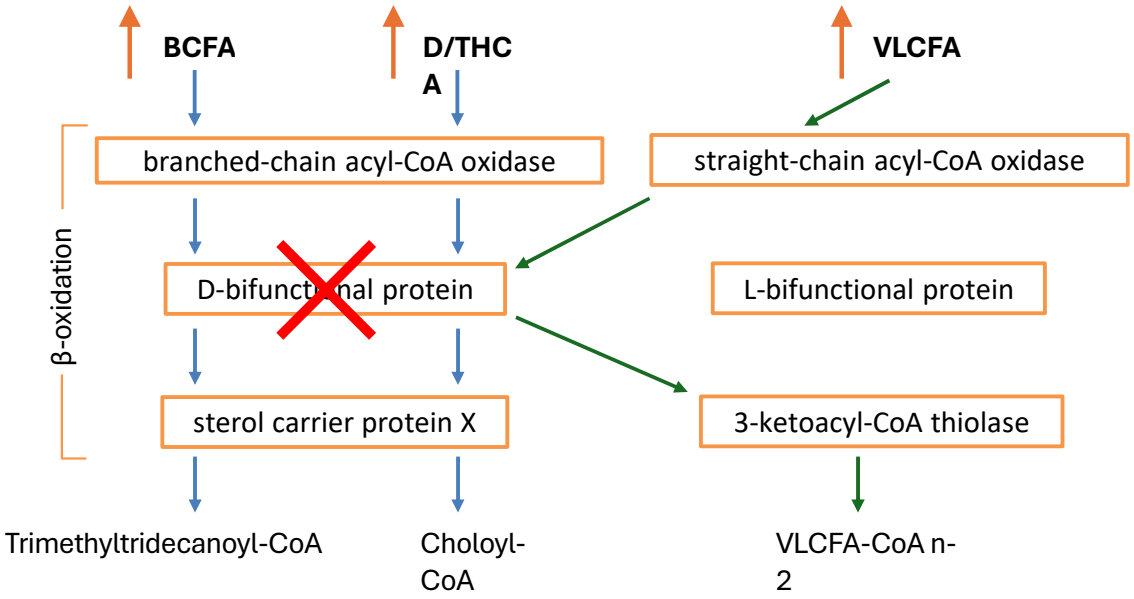
Pristanic acid	**1.58 µmol/L**	< 1.0
Pipecolic acid	4.6 µmol/L	0.5 – 5.0

What is your differential diagnosis?

D-bifunctional protein deficiency



DBP deficiency : Take home message

- # 150 reported patients (Ferdinandusse & al, Ann Neurol 2006;59:92)
- Most patients present with neonatal hypotonia, seizures, failure to thrive
- Death occurs within 2 years of age with severe psychomotor retardation, usually from pneumonia (similar to peroxisomal biogenesis disorders – PBD)
- Some prolonged survival > 7.5 years / milder phenotypes have been described
- VLCFA, bile acids, pristanic & phytanic acids measurement can indicate PBD <u>but</u>
  - Abnormal pristanic / phytanic ratio
  - Normal plasmalogens levels
- Correlation between biochemical parameters and survival of patients, C26:0 β-oxidation activity in fibroblasts being the best predictive marker

Biochemical investigation (1)

Disorder	VLCFA	C26:0-lysoPC	Pristanic acid	Phytanic acid	THCA DHCA	Plasmalogens	Pipecolic acid
PBD	↑	↑	N - ↑	N - ↑	↑	N - ↓	↑
RCDP type I	N	N	↓ - N	N - ↑	N	↓	N
RCDP type 5	N	N	↓ - N	N - ↑	N	↓	N

- Peroxisomal biogenesis disorders (PBD) → all peroxisomal functions are affected

  (PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26)
- Rhizomelic chondrodysplasia punctata (RCDP) type I (*PEX7*) and 5 (*PEX5L*): primary (I) and secondary (5) PEX7 deficiency → all PTS2-related protein functions affected

Biochemical investigation (2)

Disorder	VLCFA	C26:0-lysoPC	Pristanic acid	Phytanic acid	THCA DHCA	Plasma logens	Pipecolic acid
X-ALD	N - ↑	↑	N	N	N	N	N
ACOX1	↑	↑	N	N	N	N	N
ACBD5	↑	↑	N	N	N	N	N
DBP	↑	↑	N - ↑*	N - ↑	N - ↑	N	N - ↑
SCPx	N	N	N - ↑*	N - ↑	N - ↑	N	N
AMACR	N	N	N - ↑*	N - ↑	↑	N	N
RCDP type 2	N	N	N	N	N	↓	N
RCDP type 3	N	N	N	N	N	↓	N
RCDP type 4	N	N	N	N	N	↓	N
Refsum disease	N	N	N	↑↑	N	N	N

* **Pristanic > phytanic**

Biochemical investigation (3)

Disorder	VLCFA	C26-lysoPC	Pristanic acid	Phytanic acid	THCA DHCA	Plasmalogens	Pipecolic acid
ACOX2	N	N	N	N	↑	N	N
PMP70	N	N	N	N	↑	N	N
BAAT	N	N	N	N	N	N	N
Hyperoxaluria type I	N	N	N	N	N	N	N

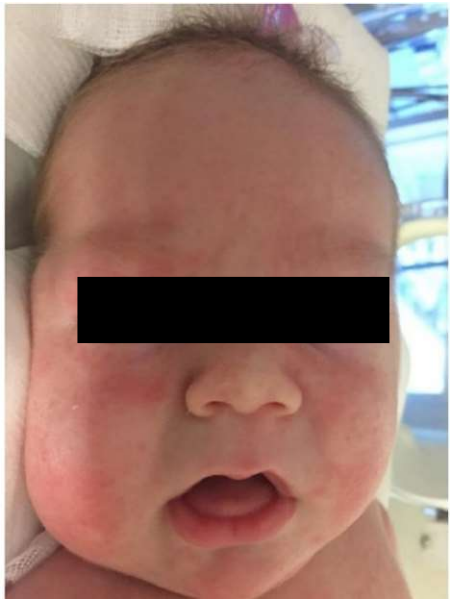
- In BAAT deficiency: bile acid spectrum shows only unconjugated bile acids
- In Hyperoxaluria type I: oxalic and glycolic acid urinary excretion are elevated

Patient 1 (baby boy)

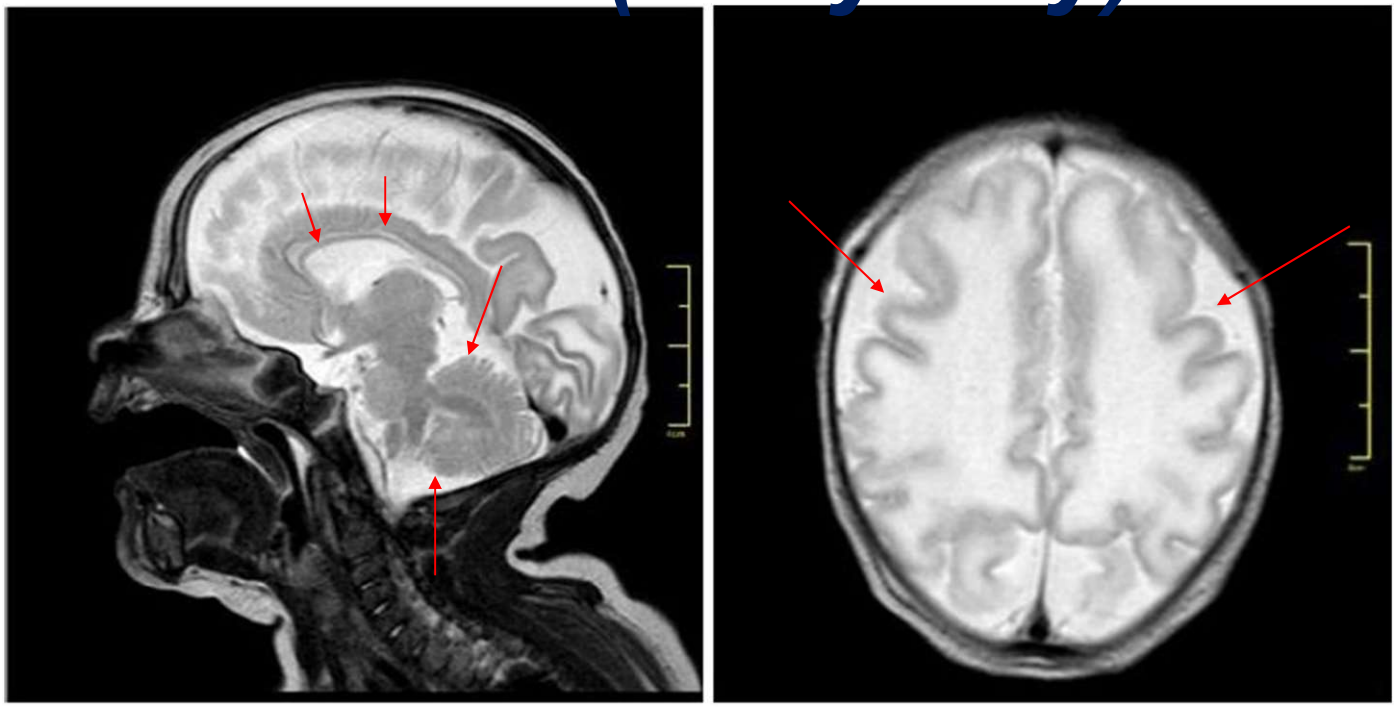
- Pregnancy: polyhydramnios
- Born at 38 weeks, APGAR 4/6/8, normal growth parameters
- generalized hypotonia, respiratory insufficiency leading to intubation
- 1st day: generalized tonic seizures, controlled with phenobarbitone
- Screening eyes and hearing: normal

- Some dysmorphic signs:
  - flat occiput, brachycephaly
  - small nose, long philtrum, thin upper lip

- Died at 10 days from respiratory distress and infection



Patient 1 (baby boy)



corpus callosum hypoplasia, volume loss in the cortex and cerebellar vermis, pachygyria, delayed myelination, enlarged subarachnoid spaces

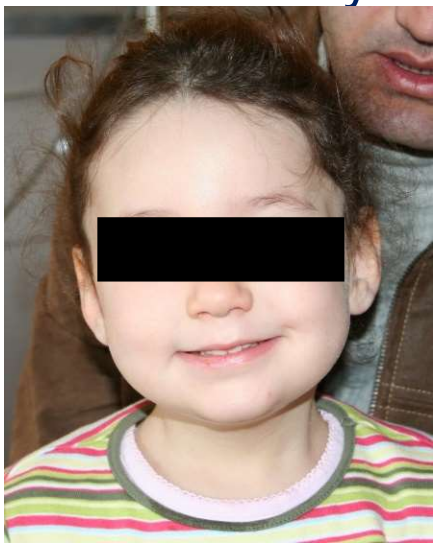
F. Cakmak Celik *et al.*, Clinical Neurology and Neurosurgery 202 (2021) 106506

Differential diagnosis?

Which investigations would you do?

Patient 2 (3-year-old girl)

- First child of consanguineous Turkish parents
- Addressed to neuropediatric outpatient clinic for psychomotor delay and language regression

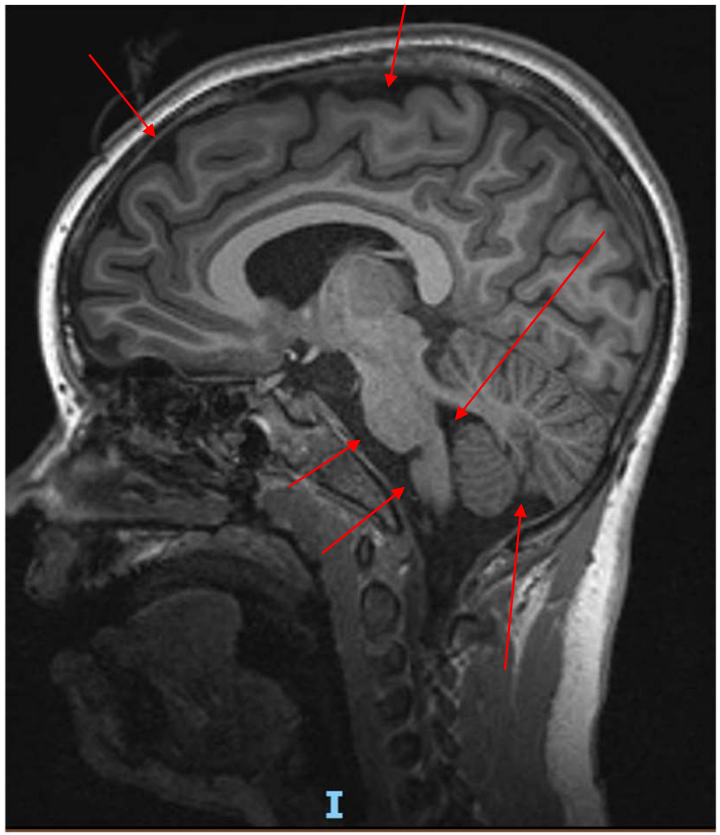


Examination:

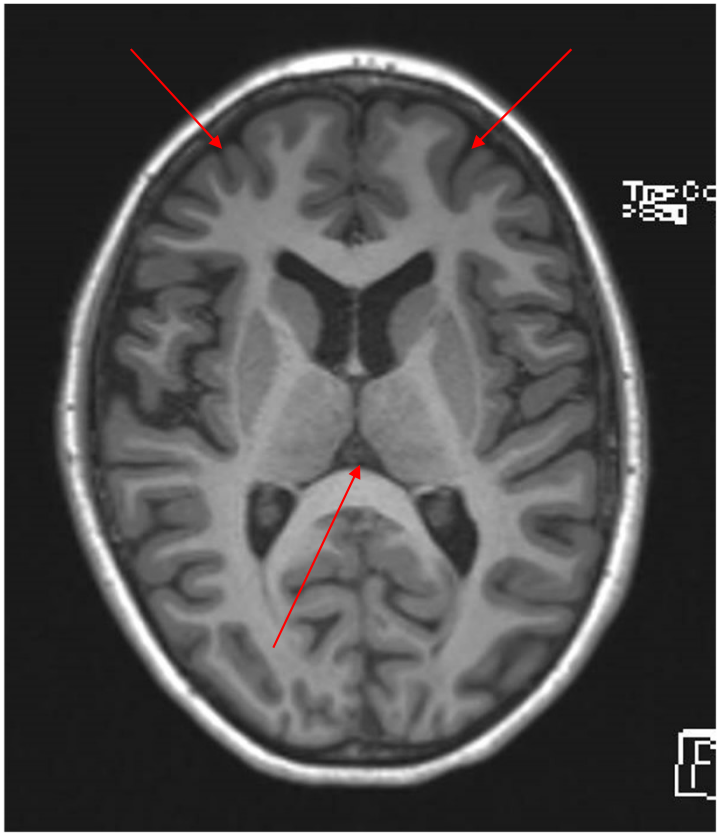
- Strabismus
- Axial hypotonia
- Behaviour: autistic features
- Transient contact interruptions
- small nose
- long philtrum
- thin upper lip

EEG confirms absence seizures, controlled with levetiracetam

Patient 2 (3-year-old girl)



- Large pericerebral spaces
- Small brain stem
- Enlarged 4th ventricle
- Cerebellar vermis atrophy



Differential diagnosis?

Which investigations would you do?

Patient 3 (20-year-old woman)

- Treated for absence epilepsy since the age of 8 years, seizures well-controlled with lamotrigine
- Mild cognitive impairment
- Lives and works in a protected environment

Differential diagnosis?
Which diagnosis have all 3 patients in common?
How can you proceed to establish the diagnosis?

Laboratory results

- Metabolic work-up (intoxication: amino acids, organic acids, acylcarnitines): normal for all 3 patients
- Purines/pyrimidines analysis in urine:
  - Patient 1: not done
  - Patient 2: S-Ado*: 93.8 mmol/mol creat. (n <4.8)
    SAICAR*: 105.2 mmol/mol creat. (n <0.5)
  - Patient 3: S-Ado: 57.2 mmol/mol creat. (n <4.8)
    SAICAR*: 37.3 mmol/mol creat. (n <0.5)

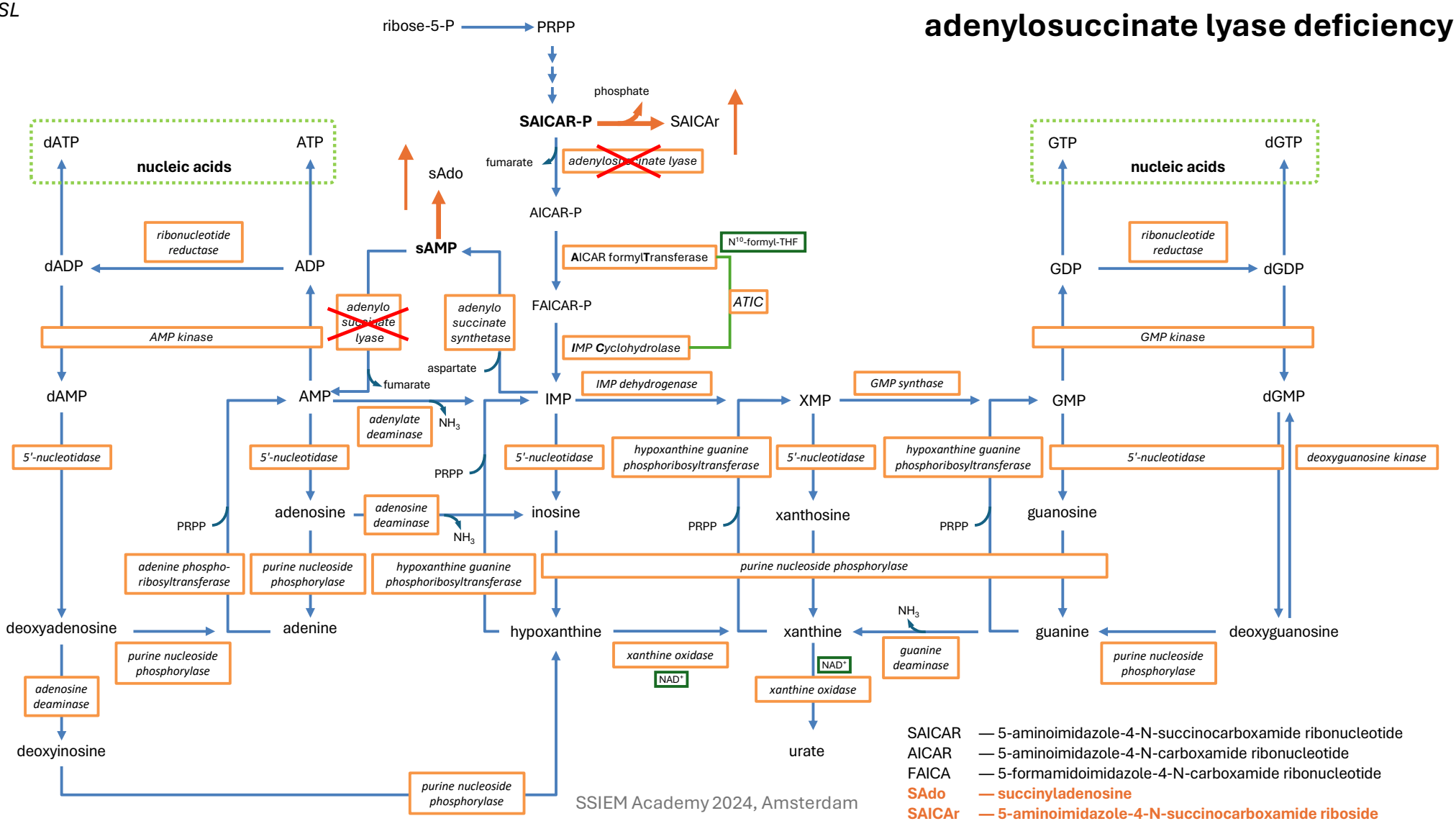
→ **Adenylosuccinate lyase (ADSL) deficiency**

Confirmation by molecular analysis of *ADSL* gene and/or ADSL activity in red blood cells

**SAICAR: succinylaminoimidazole carboxamide ribotide*
** S-Ado: succinyladenosine*

ADSL

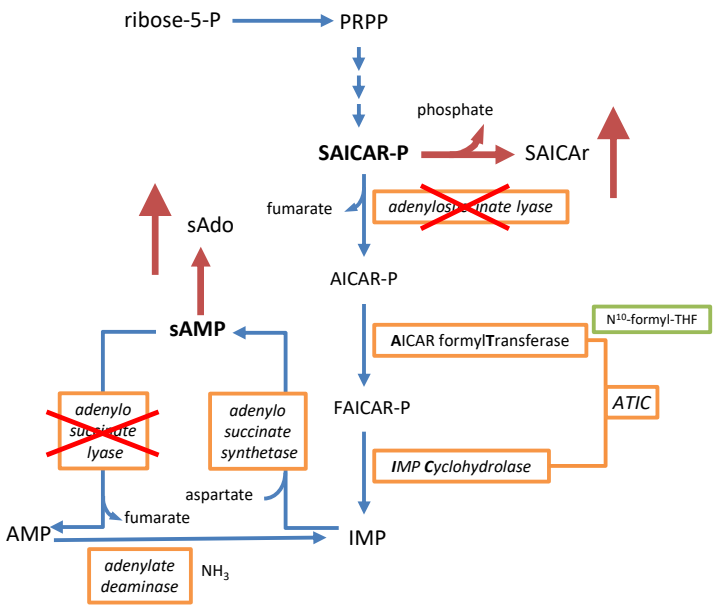
adenylosuccinate lyase deficiency



SAICAR — 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide
AICAR — 5-aminoimidazole-4-N-carboxamide ribonucleotide
FAICA — 5-formamidoimidazole-4-N-carboxamide ribonucleotide
SAdo — succinyladenosine
SAICAr — 5-aminoimidazole-4-N-succinocarboxamide riboside

ADSL

adenylosuccinate lyase deficiency



How can you define the severity of the disease?

S-Ado/SAICAR ratio

Patient 2: 0.89 → <1 severe form

Patient 3: 1.53 →>1 mild form

Correlation residual enzyme activity/genotype/severity

DD: ATIC deficiency (AICA-ribosuria), SAICAR ↑ AND AICAR ↑

Treatment of ADSL deficiency

- **How would you treat these patients?**

- Symptomatic, supportive care
- Search for epilepsy and treat if necessary
- Clinical trial with allopurinol (www.clinicaltrial.gov):

NCT03776656

Evaluation of a Treatment With Allopurinol in **Adenylosuccinate Lyase** Deficiency

Conditions

Adenylosuccinate Lyase Deficiency

Allopurinol (a structural analogue of hypoxanthine) can be a substrate for hypoxanthine phosphoribosyltransferase (HPRT) and thus produce allopurinol ribonucleotides → enter the *de novo* synthesis of purines
Hypothesis: allopurinol in children with ADSL deficiency would reduce the production of the toxic metabolite SAICAr.

Take-home messages

- Wide clinical spectrum from severe neonatal (lethal) to mild
- Epileptic seizures are very frequent, but may take time to appear
- Autistic behaviour is common
- Microcephaly not obligatory
- Frequent structural alterations of brain MRI
- Some (suggestive) dysmorphic signs

- If suspected: perform purines and pyrimidines analysis in urines for S-Ado/SAICAR measurement (CSF also suitable for diagnosis)

- Indications for screening:
  → unexplained delayed, disharmonious development +/- autistic features
  → unexplained (in the neonatal form intractable) seizures
  → hypotonia and acquired microcephaly

Pharmacoresistant Epilepsy

Workshop 1, Case 4.

SSIEM Academy 2024, Amsterdam

History

- *14 year old boy*
- *Non-consanguineous parents*
- *Onset of seizure disorder from 2.5 years of age*
- *Now multi-type seizures with 4 anticonvulsants*
- *Learning difficulties*
- *Progressive spinocerebellar ataxia*
- *Hypermetropic astigmatism*

- *Wheelchair for mobility, two people to assist walking*
- *Tremor*
- *Drooling, dysphagia, gastrostomy fed*

What metabolic biochemical investigations would you want to do?

Previous investigations

- Renal & electrolytes: normal
  Na 135 mmol/L, K 4.3 mmol/L, Urea 3.9 mmol/L, Creatinine 45 µmol/L
- Liver function: Normal (ALT 35U/L, bilirubin 15 µmol/L)
- Full blood count: mild normocytic anaemia
  - Hb 97 g/L (115-155)
  - Red cell count 3.82 $x10^{12}$/L (4-5.2)
  - MCV 77.2 fL (77-94)
  - Mean cell haemoglobin 25.4 pg (25-33)
  - RDW 25.5 % (11.0-16)
  - Platelet count 229 $x10^{9}$/L (150-450)
  - White cell count 4.29 $x10^{9}$/L (4.5-13.5)
- Ammonia: normal
- Lactate: Normal
- Glucose: normal
- Urate: normal
- Urine organic acids: normal
- Plasma amino acids and homocysteine: normal
- Acylcarnitine profiles: normal
- Cholesterol and triglycerides: normal
- Vitamin A & E: normal
- Very long chain fatty acids: normal

Previous investigations

- Vacuolated lymphocytes: not detected
- Lysosomal enzymology (neurodegenerative panel): no specific abnormalities
- White cell ubiquinone: normal
- Purine and pyrimidine analysis (blood and urine): normal
- Riboflavin level: normal
- Urine guanidinoacetate and creatine: normal
- Transferrin isoelectric focussing: normal
- Urine α-aminoadipic semialdehyde: normal
- CSF glucose, lactate, amino acids, neurotransmitters, pyridoxal phosphate, 5methyltetrahydrofolate normal
- Muscle biopsy: histopathology and mitochondrial respiratory chain enzymology normal

What further investigations would you want to do?

Previous investigations

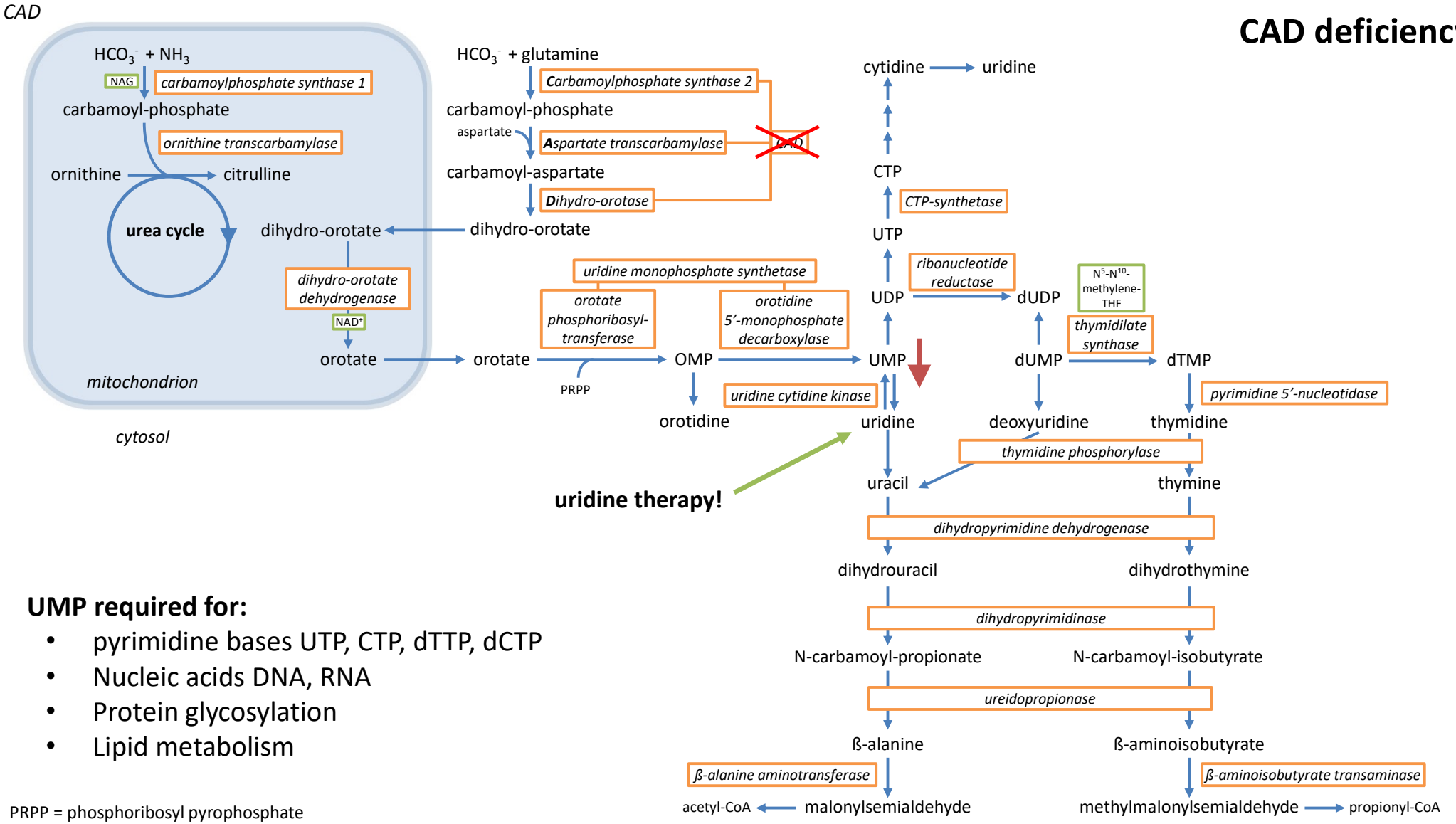
Previous Molecular Genetics

- Ataxia and mitochondrial disease panels: no pathogenic variants
- Epilepsy/Dev Delay 72 gene NGS panel: no pathogenic variants

- MtDNA sequencing and rearrangements: normal
- NGS panel for SCA 1,2,3,6,7 - normal
- Muscle mtDNA quantification normal (74% of mean normal) - no evidence of depletion

What would you do next?

- **Whole genome sequencing: Compound heterozygous VUS in *CAD***
- **C**arbamoyl phosphtate synthetase/ **A**spartate transcarbamylase/ **D**iydroorotase



UMP required for:

- pyrimidine bases UTP, CTP, dTTP, dCTP
- Nucleic acids DNA, RNA
- Protein glycosylation
- Lipid metabolism

PRPP = phosphoribosyl pyrophosphate

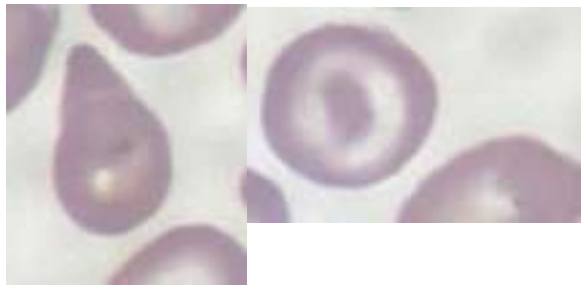
Full blood count

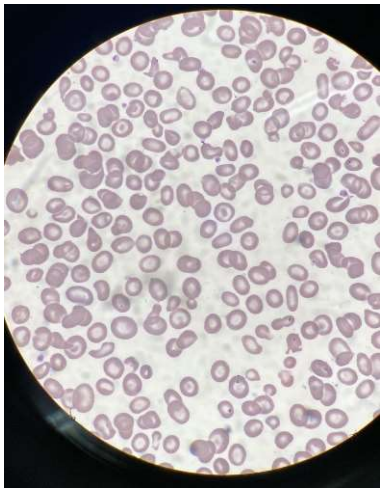
Haemoglobin 97 (115 – 155) g/L
RED BLOOD CELL COUNT 3.82 (4.00 - 5.20) $x10^{12}$/L
HAEMATOCRIT 0.295 (0.350 - 0.450) L/L
MCV 77.2 (77.0 - 94.0) fL
MEAN CELL HAEMAGLOBIN 25.4 (25.0 - 33.0) pg
MEAN CELL HB CONC 329 (315 – 355) g/L
RDW 25.5 (11.0 - 16.0) %

NEUTROPHILS 2.63 (1.5 - 8.0) $x10^9$/L
LYMPHOCYTES 1.16 (1.5 - 7.0) $x10^9$/L
MONOCYTES 0.33 (0.1 - 0.8) $x10^9$/L
EOSINOPHILS 0.16 (0.1 - 0.8) $x10^9$/L
BASOPHILS 0.01 (0.00 - 0.2) $x10^9$/L

RBCs show marked **anisopoikilocytosis** with hypochromasia, and many microcytes, teardrop cells and target cells present on blood film

PLATELET COUNT 229 (150 – 450) $x10^9$/L
WHITE BLOOD COUNT 4.29 (4.5 - 13.5) $x10^9$/L



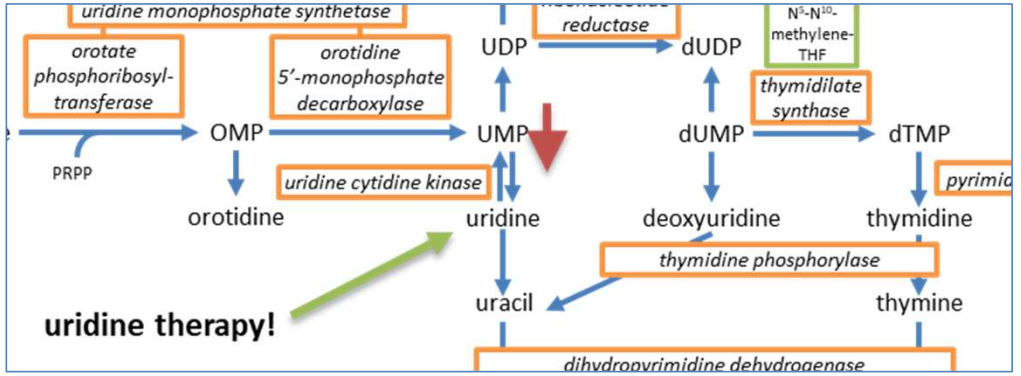


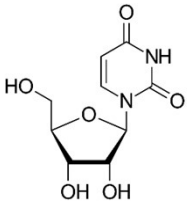
Uridine replacement therapy

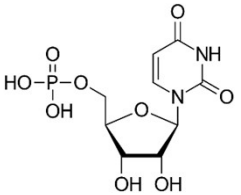
- Replenish UMP pool via salvage pathway

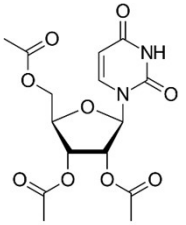
- Uridine
- Uridine monophosphate
  - Dietary supplement (eg Tulip Biopharma/Sigma)
    - ~£4200 per year
- Uridine triacetate
  - Prodrug, activated by non-specific esterases
  - “Vistogard” – 5FU toxicity (~£530,000 per year)
  - “Xuridine” – FDA-approved for UMPS deficiency

- 70-150mg/kg/day uridine equivalent

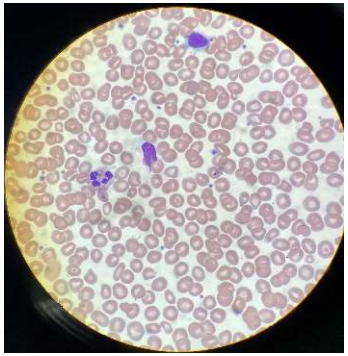








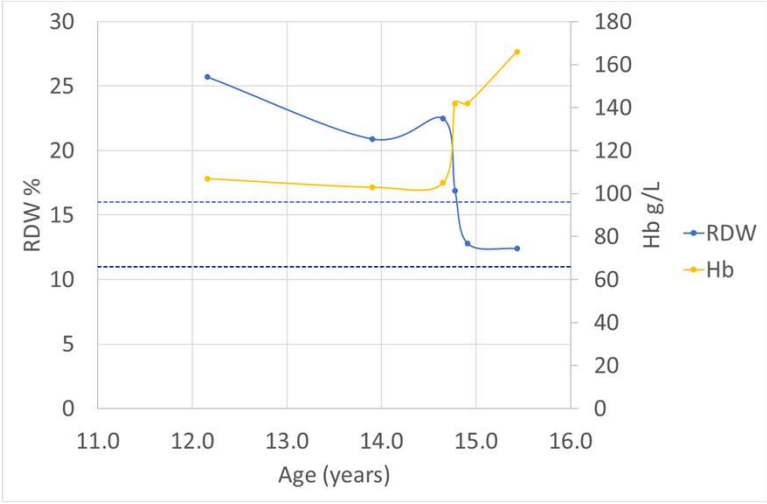
Treatment Response



- *Uridine monophosphate 120mg/kg/day*

- *Resolution of anisopoikilocytosis/ RDW*
- *Rapid cessation of seizures*
- *Regained motor function and able to walk independently*
- *Reduced dysphagia – fully orally fed*

- *Ongoing behavioural issues*



Phenotype spectrum of CAD

Early infancy: severe epileptic encephalopathy

↓

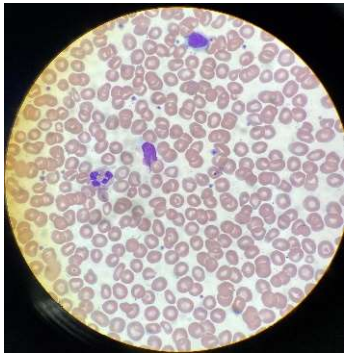
Childhood

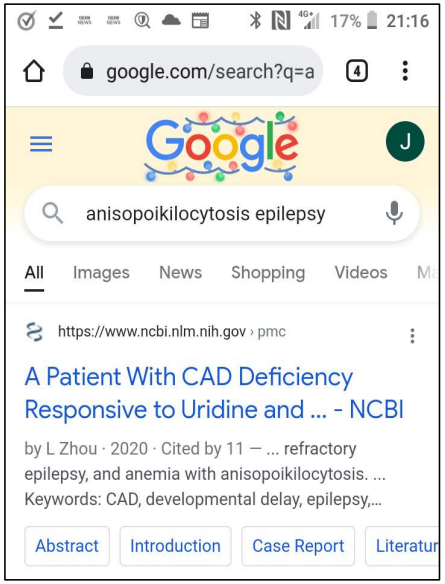
↓

Adult: e.g. presentation of seizures during pregnancy (fetal consumption of maternal UMP)

Key Take home

- *CAD-related disorder has broad phenotype from neonatal to adult-onset presentation*
- *Anisopoikilocytosis/ increased RDW as key diagnostic clue*
- *Treatable with good effect with Uridine replacement*





- Johannes Koch et al. CAD mutations and uridine-responsive epileptic encephalopathy. Brain 2017 Feb;140(2):279-286
- Daisy Rymen et al. Expanding the clinical and genetic spectrum of CAD deficiency: an epileptic encephalopathy treatable with uridine supplementation. Genet Med 2020 Oct;22(10):1589-1597

Encephalopathy in a 47-year-old woman

SSIEM Academy 2024, Amsterdam

Clinical presentation

- 47 year old woman
- Referred to the adult inherited metabolic disease clinic with a possible IMD
- Before the appointment flew to Pakistan
- Whilst in Pakistan she developed
  - Impaired vision – couldn't see anything from one side of her eyes
  - Headache
  - Some confusion - loss of passport and money
  - Intermittent incontinence of bladder and bowel
- Her brother flew to Pakistan to bring her back to the UK

Question

- What are the possible (metabolic) causes in an adult of
  - Encephalopathy?
  - Stroke-like event?

Urea cycle defects
Homocystinuria (pyridoxine responsive)
Fabry disease
Mitochondrial disorders - MELAS
Organic acidemias – Propionic, methylmalonic acidemia

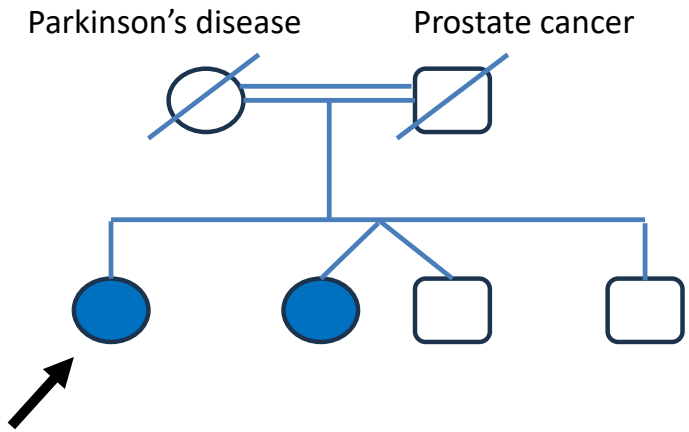
Question

- **What other information would you ask?**

Normal birth and development
Normal schooling – no education support
Works in airport security

Has worn glasses from 12 years
Noticed a deterioration in handwriting over recent years, due to tremor in hands (sister too)

No medications



Examination

- Unsteady ataxic gait - unable to tandem walk
- Tremulous dystonia of the upper limbs
- No limb weakness

- Montreal Cognitive Assessment (MoCA) score 9/30

Question

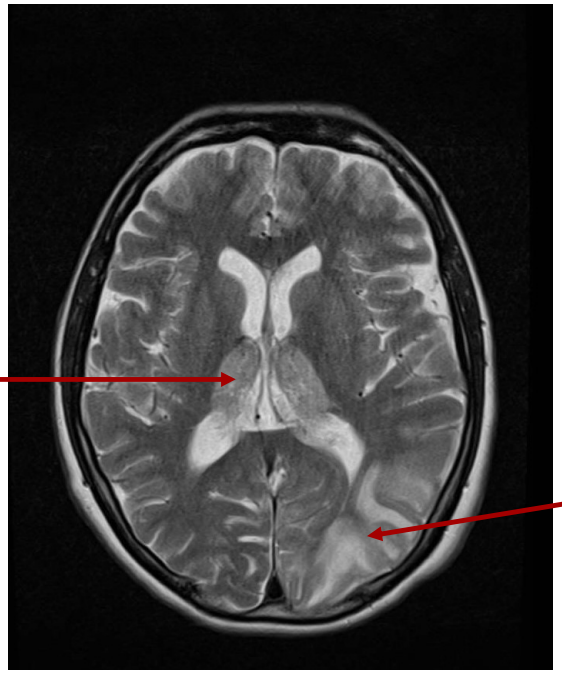
- **What investigations you would request?**

CT / MR imaging of the brain
Ophthalmology review

Total homocysteine
Ammonia
CK
Lactate
Plasma amino acid profile
Acylcarnitine profile
Methylmalonic acid
Urine organic acid profile
Very long chain fatty acid profile

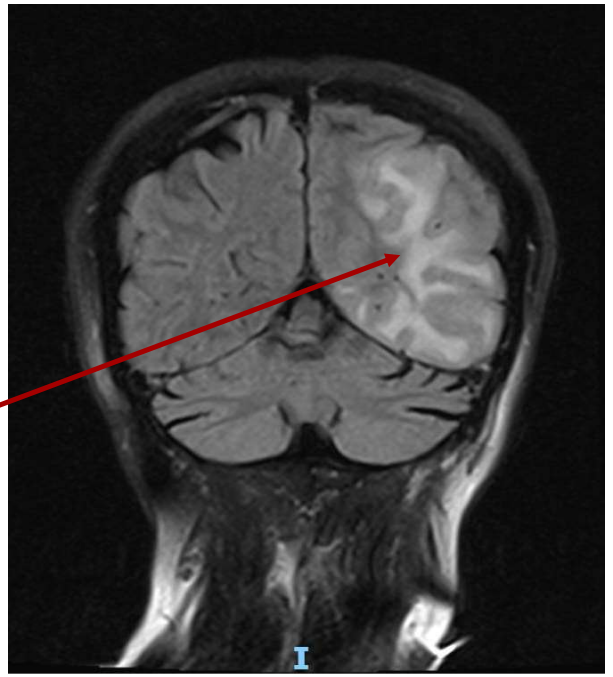
MR imaging

Chronic changes: bilateral signal abnormality affecting the thalamus, midbrain and pons.



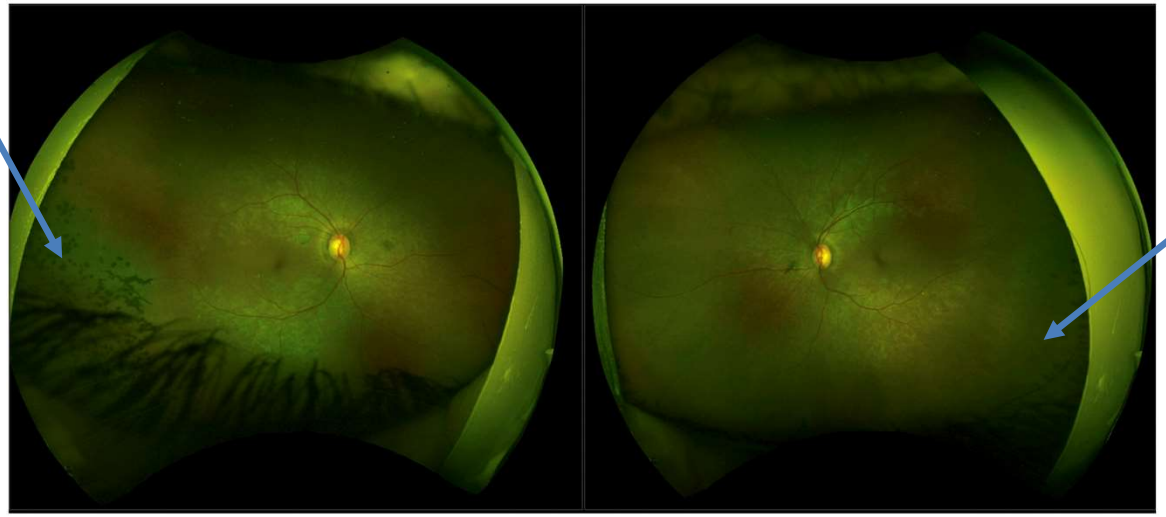
T2-weighted sequence

Acute changes: affecting the parieto-occipital-temporal lobes with parenchymal swelling and cortical enhancement.



Flair sequence

Ophthalmology



Retinitis pigmentosa

- Ophthalmology
  - Visual acuities of 6/12 in both eyes
  - Long-standing retinal dystrophy and associated peripheral visual loss
  - New sudden onset right homonymous hemianopia

Peroxisomal investigations in plasma

Metabolite	Results	Reference range
C26:0	0.68 µmol/L	0.15 – 0.91
C22:0	52.9 µmol/L	30.5 - 97.7
C24:0	34.4 µmol/L	24.4 - 65.9
C26:0 / C22:0	0.013	< 0.022
C24:0 / C22:0	0.65	< 0.96
Phytanic acid	**8.93 µmol/L**	< 5.0
Pristanic acid	**133 µmol/L**	< 1.0

Further investigations

- In plasma: bile acids (C_{27} bile acid intermediates):

Metabolite	Results	Control range
T-trihydroxycholestanoic acid (THCA)	**4.0** µmol/L	< 0.05
D-dihydroxycholestanoic acid (DHCA)	**6.9** µmol/L	< 0.05

Catalase immunofluorescence microscopy – showed import-competent peroxisomes.

Peroxisomal beta-oxidation measurements:
Using C26 as substrate 1390 pmol/(hour.mg protein) Normal: 826-2110
Using Pristanic acid as substrate 478 pmol/(hour.mg protein) Normal: 790-1690

Sterol carrier X protein activity 26 pmol/(min.mg) Normal: 25 ± 14

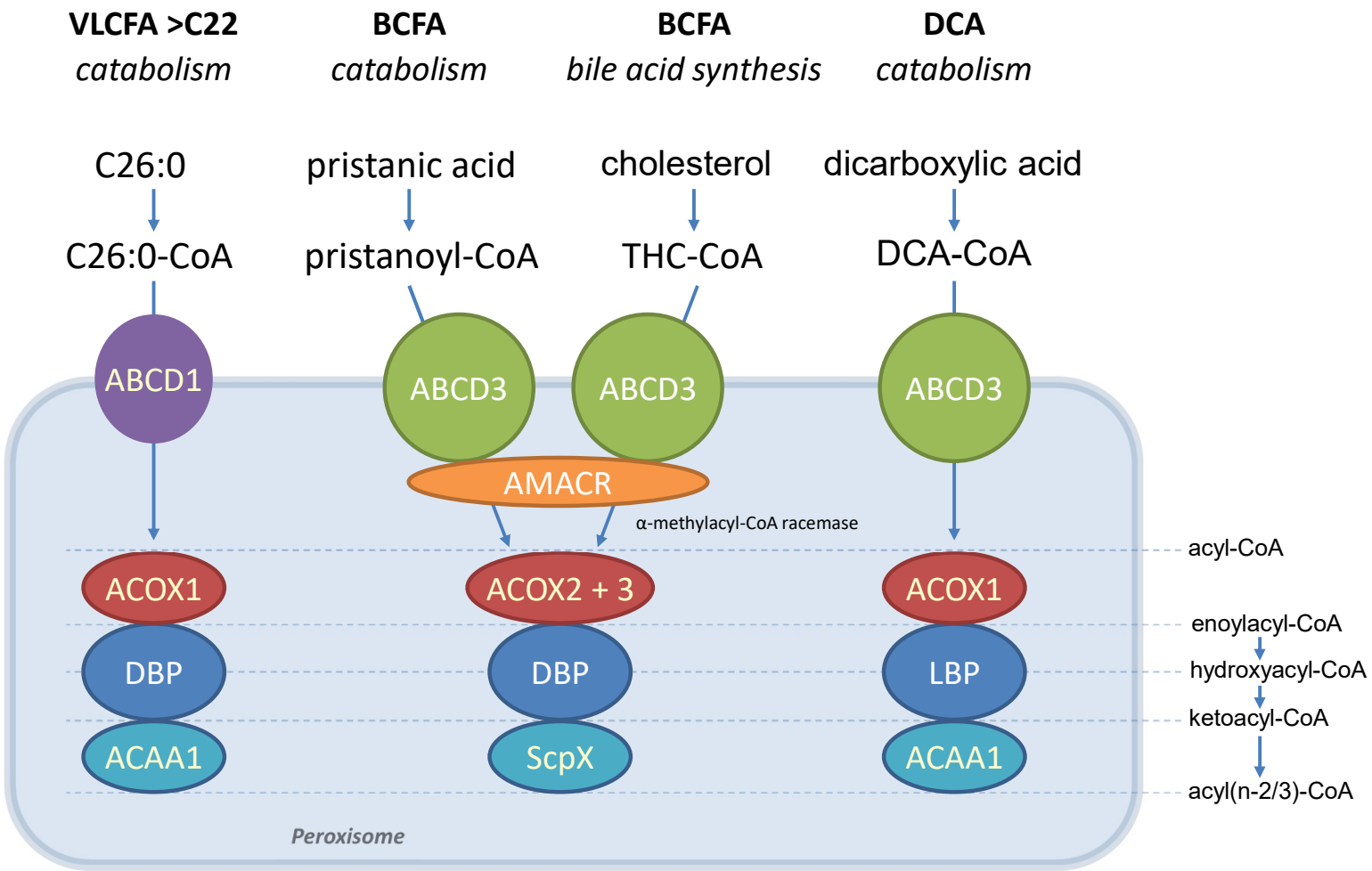
Separation of R and S isomers indicative of **alpha-methylacyl-CoA racemase deficiency**
(Dr Sacha Ferdinandusse, Amsterdam)

Genetic confirmation: Homozygous c.146G>T; p.Gly49Val variant in the *AMACR* gene

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

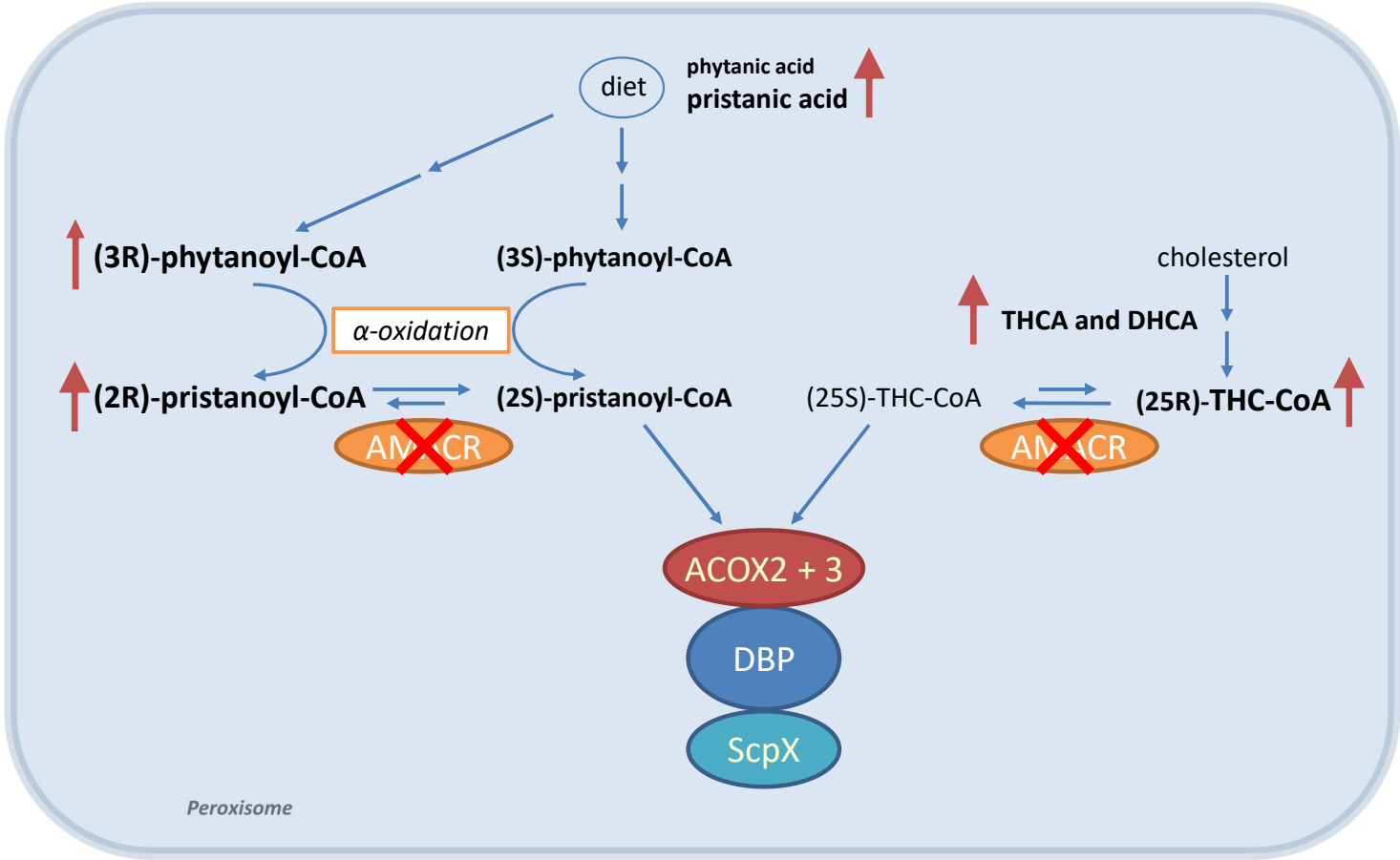
peroxisomal β-oxidation

import and degradation

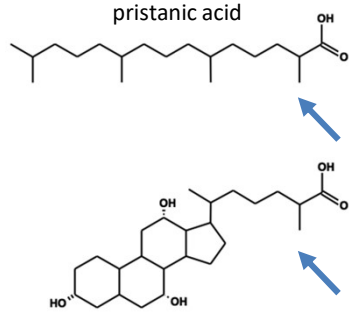


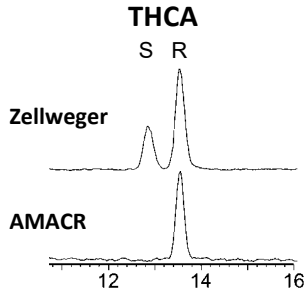
α-methylacyl-CoA racemase deficiency

chirality matters



2-methyl branched-chain





Question

- **What treatment would you suggest?**

Low phytanate diet

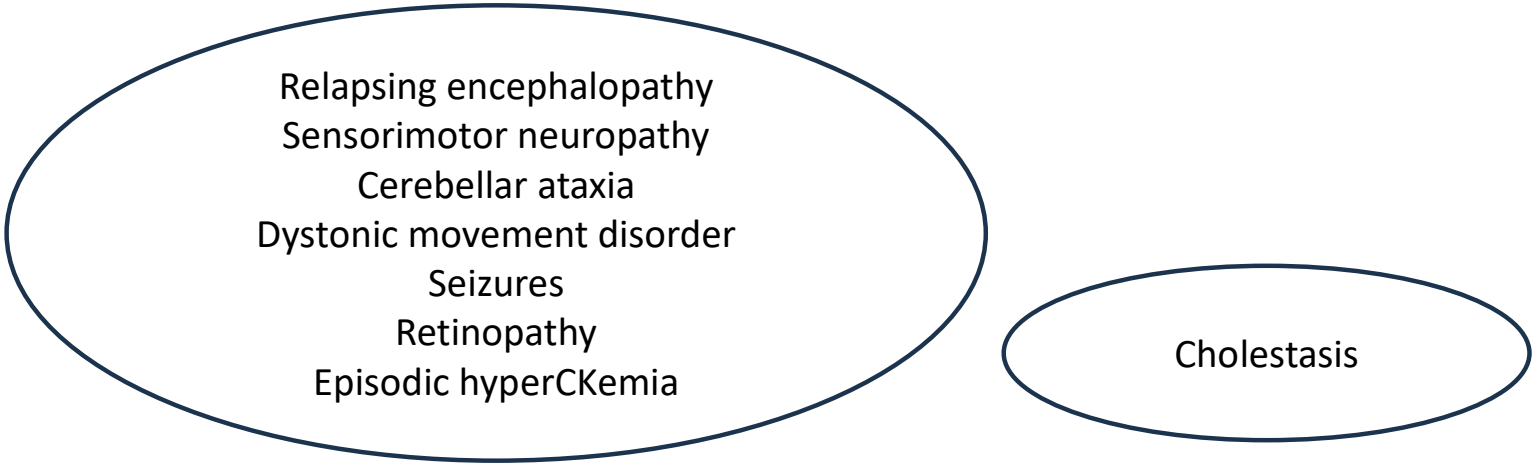
Avoid rapid weight loss (risk of acute stroke/vision loss)

(bile acid therapy)

Outcome
No further encephalopathic episodes
Improvement in hemianopia; vision stable
Variable pristanic acid concentrations: 73-166 µmol/L (N<1.0)

AMACR deficiency: Take home messages

- More commonly presents in adults than children
- A cause of relapsing encephalopathy
- Heterogenous clinical presentation
- Biochemistry can be very similar to sterol carrier protein X deficiency



Biochemical investigation (1)

Disorder	VLCFA	C26:0-lysoPC	Pristanic acid	Phytanic acid	THCA DHCA	Plasma logens	Pipecolic acid
PBD	↑	↑	N - ↑	N - ↑	↑	N - ↓	↑
RCDP type I	N	N	↓ - N	N - ↑	N	↓	N
RCDP type 5	N	N	↓ - N	N - ↑	N	↓	N

- Peroxisomal biogenesis disorders (PBD) → all peroxisomal functions are affected *(PEX1, PEX2, PEX3, PEX5, PEX6, PEX10, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26)*
- Rhizomelic chondrodysplasia punctata (RCDP) type I (*PEX7*) and 5 (*PEX5L*): primary (I) and secondary (5) PEX7 deficiency → all PTS2-related protein functions affected

Biochemical investigation (2)

Disorder	VLCFA	C26:0-lysoPC	Pristanic acid	Phytanic acid	THCA DHCA	Plasma logens	Pipecolic acid
X-ALD	N - ↑	↑	N	N	N	N	N
ACOX1	↑	↑	N	N	N	N	N
ACBD5	↑	↑	N	N	N	N	N
DBP	↑	↑	N - ↑*	N - ↑	N - ↑	N	N - ↑
SCPx	N	N	N - ↑*	N - ↑	N - ↑	N	N
AMACR	N	N	N - ↑*	N - ↑	↑	N	N
RCDP type 2	N	N	N	N	N	↓	N
RCDP type 3	N	N	N	N	N	↓	N
RCDP type 4	N	N	N	N	N	↓	N
Refsum disease	N	N	N	↑↑	N	N	N

* **Pristanic > phytanic**

Biochemical investigation (3)

Disorder	VLCFA	C26-lysoPC	Pristanic acid	Phytanic acid	THCA DHCA	Plasmalogens	Pipecolic acid
ACOX2	N	N	N	N	↑	N	N
PMP70	N	N	N	N	↑	N	N
BAAT	N	N	N	N	N	N	N
Hyperoxaluria type I	N	N	N	N	N	N	N

- In BAAT deficiency: bile acid spectrum shows only unconjugated bile acids
- In Hyperoxaluria type I: oxalic and glycolic acid urinary excretion are elevated

A 21-year-old woman diagnosed with treatment-resistant anorexia nervosa

SSIEM Academy 2024, Amsterdam

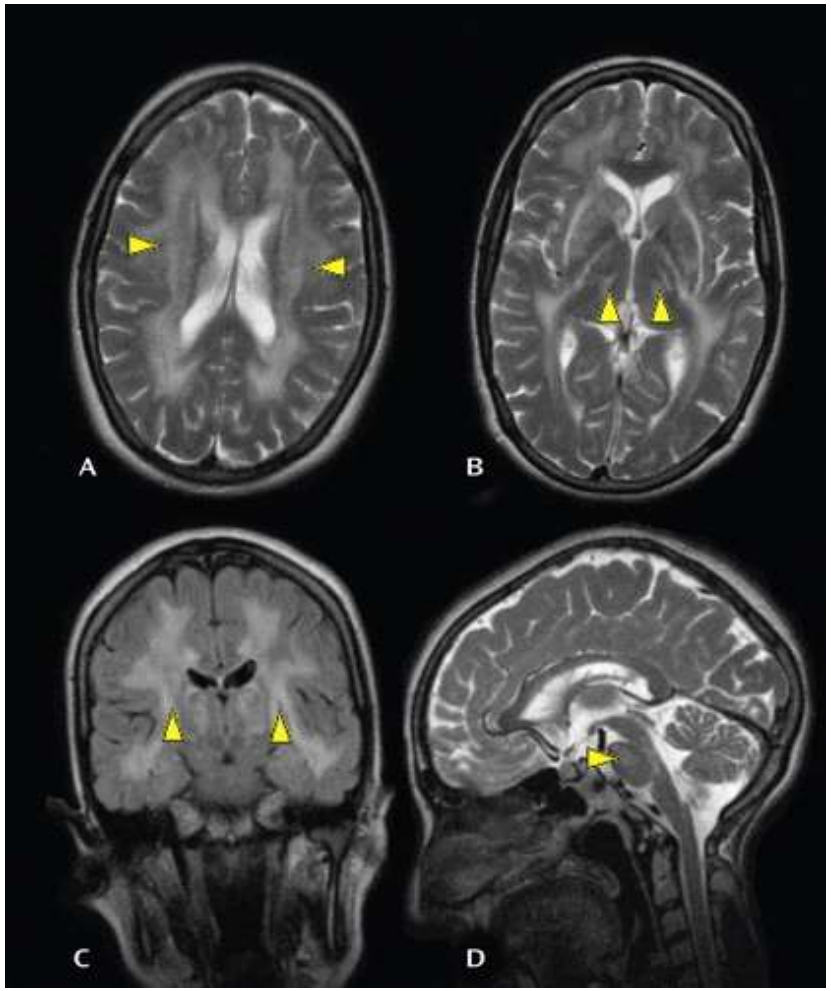
Patient

- Thin patient, considered to be growth retarded
- Flat mood, infantile behavior
- Anorexic symptoms since puberty
- Diffuse abdominal complaints and peculiar, restrictive eating habits
- A distorted body image could not be clearly elicited
  → "atypical anorexia nervosa"
- Weight loss despite receiving specific treatment for anorexia nervosa in a hospital

Patient

- Body mass index of 11.6 kg/m^2 when referred to psychiatry
- Rapid deterioration of the patient's state after placement of a gastric tube to provide high-caloric nutrition: vomiting and loud borborygmi, abdominal pain, fever, physical exhaustion
- Neurological exam: bilateral ptosis, infranuclear ophthalmoparesis, and generalized areflexia
- Electromyography: marked demyelinating sensorimotor polyneuropathy

Brain MRI



Diffuse Leukoencephalopathy

- Increased signal intensity (arrowheads)
- Diffuse leukoencephalopathy with involvement of the A) centrum semiovale (axial T2), B) parts of the thalamus (axial T2), C) internal capsule (coronal fluid-attenuated inversion recovery images), and D) pons (sagittal T2)
- Characteristic sparing of putamina, claustrum, caudate, corpus callosum, and most of the internal capsules

Which tests would you consider?

Laboratory Findings

- Lactate 2-fold above the upper limit of normal in plasma and CSF
- Urine organic acids: Thymine ↑
- Purines & pyrimidines in urine: Thymidine ↑, Deoxyuridine ↑

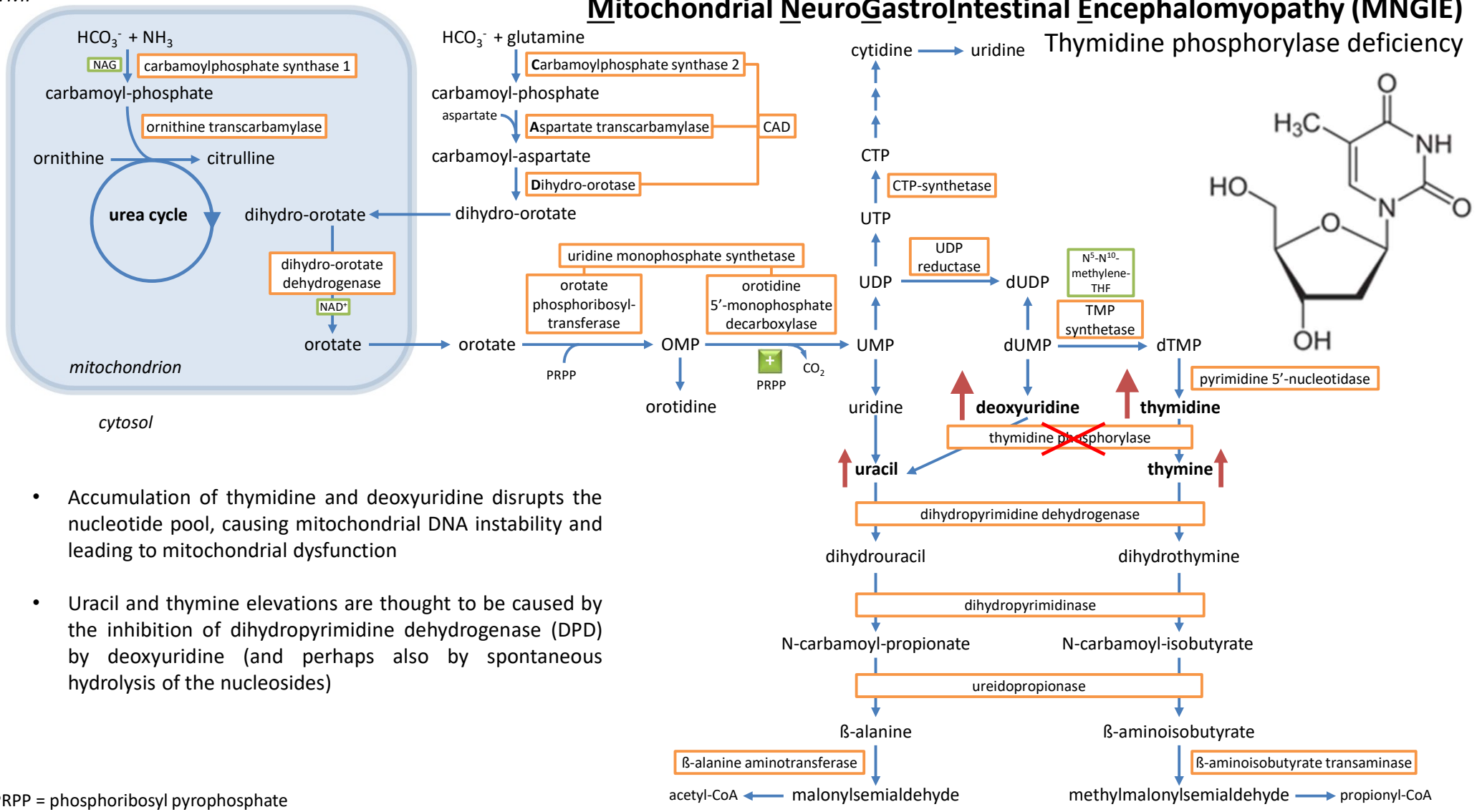
Diagnosis ?

- **M**itochondrial **N**euro**G**astro**I**ntestinal **E**ncephalomyopathy
- Confirmed by a homozygous mutation c.605G>A, p.(Arg202Lys) in *TYMP* gene

TYMP

Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE)

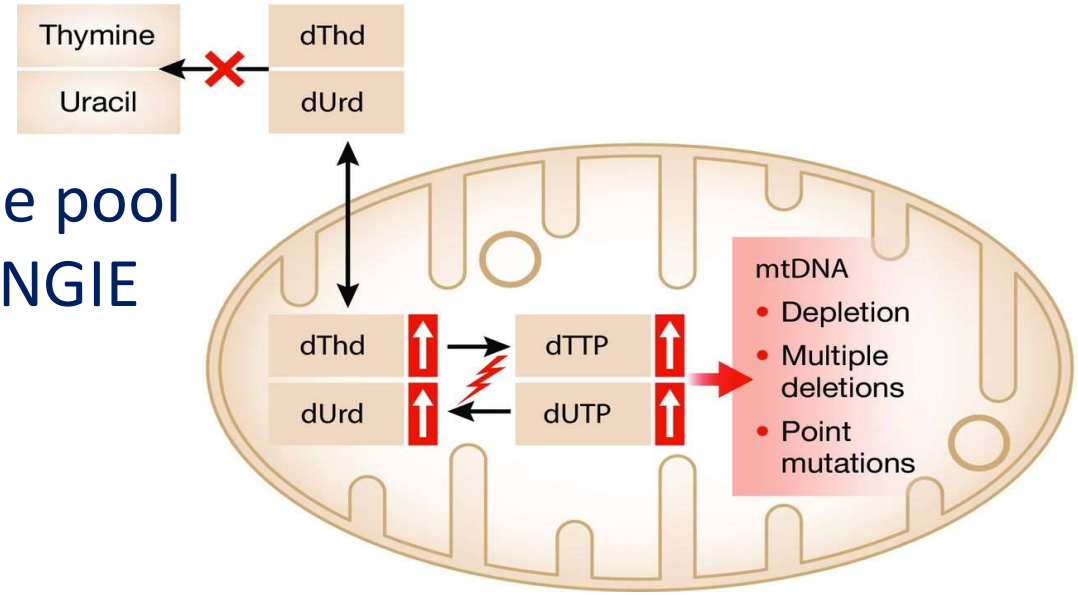
Thymidine phosphorylase deficiency



- Accumulation of thymidine and deoxyuridine disrupts the nucleotide pool, causing mitochondrial DNA instability and leading to mitochondrial dysfunction
- Uracil and thymine elevations are thought to be caused by the inhibition of dihydropyrimidine dehydrogenase (DPD) by deoxyuridine (and perhaps also by spontaneous hydrolysis of the nucleosides)

PRPP = phosphoribosyl pyrophosphate

Deoxynucleotide pool imbalance in MNGIE



- In thymidine phosphorylase (TYMP) deficiency:
  thymidine (dThd) and deoxyuridine (dUrd) ↑
- (mt) thymidine kinase TK2 constitutively expressed, (ct)TK1 tightly regulated
- Imbalance in mitochondrial dNTP pools

Ivano Di Meo et al. EMBO Mol Med. 2015;7:1257-1266

MNGIE: Clinical Features

- **Gastrointestinal**:
  - progressive dysmotility, abdominal pain, borborygmi
  - Weight loss --> Cachexia
  - (cirrhotic) Liver disease, hypertriglyceridaemia, pancreatitis
- **Neurological:**
  - Demyelinating peripheral neuropathy --> progressive gait disturbance and neuropathic pain
  - Eye movement disorder
  - Leukoencephalopathy on MRI brain scan
- **Other features:**
  - Sensorineural hearing impairment
  - Endocrine (type 1 diabetes, gonadotrophic hormone disorders)

- **Onset** 1st-5th decades (60% before 20years)
- **Mean age of death:** 36 years

Therapy

Received: 20 April 2020 | Revised: 3 August 2020 | Accepted: 5 August 2020
DOI: 10.1002/jimd.12300

REVIEW ARTICLE

JIMD SSIEM WILEY

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): Position paper on diagnosis, prognosis, and treatment by the MNGIE International Network

- **In the reported case:** intravenous feeding in addition to better absorbable high-caloric nutrition via gastric tube resulted in weight gain to a BMI of 12.7 and improvement of the physical state, which remained stable 6 months

- **Temporary improvement of the biochemical imbalance:**
  - Hemodialysis/ continuous ambulatory peritoneal dialysis
  - Platelet transfusions
  - Erythrocyte encapsulated thymidine phosphorylase (experimental)
- **Permanent restoration of the biochemical imbalance**
  - hematopoietic stem cell transplantation (HSCT) in selected cases
    - (10/10 match donor, no liver disease, early gastrointestinal disease)
  - orthotopic liver transplantation
  - Both achieve biochemical correction of purines with variable clinical outcome

Discussion/ Take home messages

- Thorough clinical and biochemical examination together with MRI are essential to clearly differentiate between psychiatric and somatic origins within the spectrum of cachexia inducing disorders

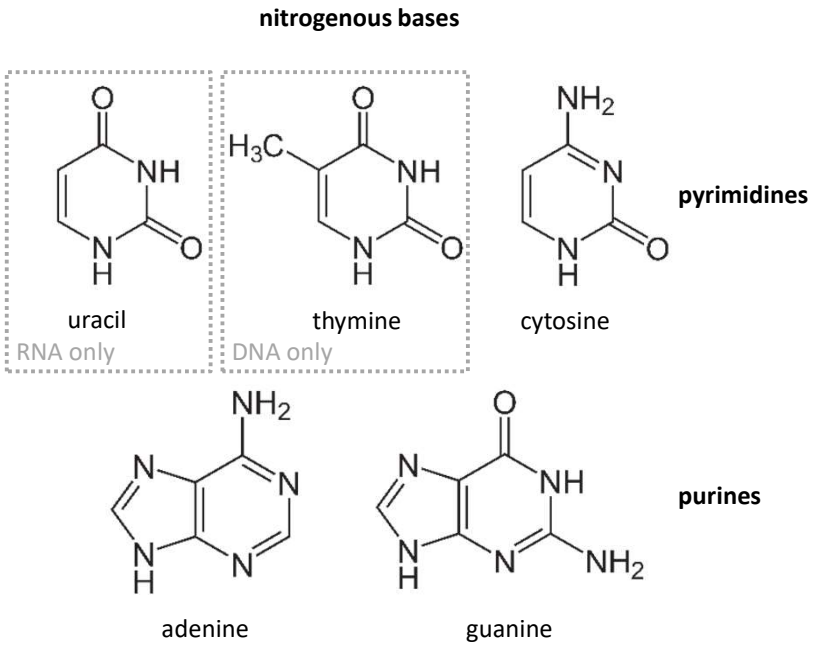
- MNGIE may be diagnosed by purine/pyrimidine screen, although urine analysis alone is not preferred for this and plasma screen is required

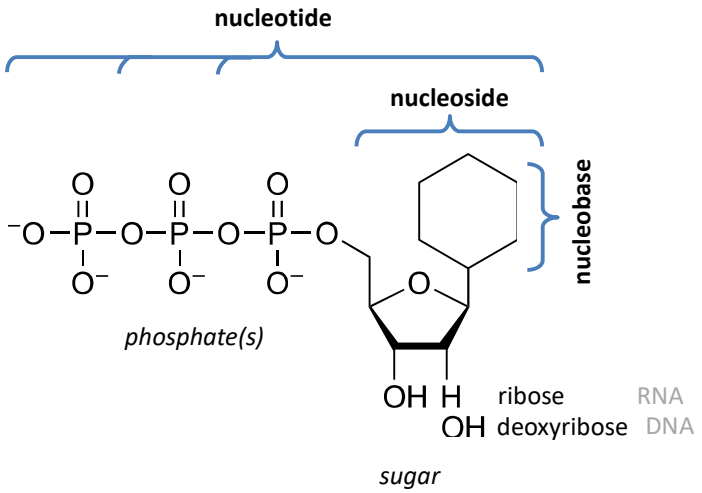
- Early diagnosis of MNGIE allows consideration of disease-modifying intervention with HSCT or liver transplant

Case description based on

Feddersen et al. Am J Psychiatry. 2009;166(4):494-5.
doi: 10.1176/appi.ajp.2008.08101525

purines and pyrimidines





What's in the name?

Purines and pyrimidines
–ine are nitrogenous bases*
*[except uracil/uridine]

Purines:
–osine are nucleosides/tides
*[except cytosine]

Pyrimidines:
–idine are nucleosides/tides

Naming of bases, nucleosides and nucleotides

Category	**Nitrogeous Base (Nucleobase)**	**Nucleoside (Ribose)**	**Deoxynucleoside (Deoxyribose)**	**Nucleotide (Ribose)**	**Deoxynucleotide (Deoxyribose)** *[only monophosphate]*
Purines	Adenine	Adenosine	Deoxyadenosine	Adenosine Monophosphate (AMP)	Deoxyadenosine Monophosphate (dAMP)
	Guanine	Guanosine	Deoxyguanosine	Guanosine Monophosphate (GMP)	Deoxyguanosine Monophosphate (dGMP)
	Hypoxanthine	Inosine	Deoxyinosine	Inosine Monophosphate (IMP)	-
Pyrimidines	Cytosine	Cytidine	Deoxycytidine	Cytidine Monophosphate (CMP)	Deoxycytidine Monophosphate (dCMP)
	Thymine	-	Thymidine (Deoxythymidine)	-	Thymidine Monophosphate (dTMP)
	Uracil	Uridine	Deoxyuridine	Uridine Monophosphate (UMP)	-

Adult patient with neuropathy

SSIEM Academy 2024, Amsterdam

Patient presentation

- 76-year-old patient, consanguineous parents
- Obesity from 30 years of age
- Diabetes from 50 years of age
- Poor observance of dietetic advices
- Development of walking difficulties and neuropathy since few years
- Peroxisomal investigation was requested because of consanguinity and neuropathy

Peroxisomal investigation

Metabolite	Results	Control range
C26:0	3.07 µmol/L	0.43 – 1.06
C22:0	117 µmol/L	40 - 119
C24:0	78 µmol/L	33 - 84
C26:0 / C22:0	0.026	0.006 – 0.019
C24:0 / C22:0	0.67	0.69 – 0.99
Phytanic acid	7.4 µmol/L	< 10.0
Pristanic acid	0.70 µmol/L	< 3.0
Pipecolic acid	1.2 µmol/L	< 3.0

Peroxisomal investigation

Metabolite	Results	Control range
C26:0	**3.07** µmol/L	0.43 – 1.06
C22:0	117 µmol/L	40 - 119
C24:0	78 µmol/L	33 - 84
C26:0 / C22:0	**0.026**	0.006 – 0.019
C24:0 / C22:0	0.67	0.69 – 0.99
Phytanic acid	7.4 µmol/L	< 10.0
Pristanic acid	0.70 µmol/L	< 3.0
Pipecolic acid	1.2 µmol/L	< 3.0

Further investigation

- Appearance of plasma : milky (cloudy) and haemolysis

Metabolite	Results	Control range
Triglycerides	**10.64** mmol/L	0.40 – 1.70
Cholesterol	5.72 mmol/L	3.80 – 6.00
HDL cholesterol	0.61 mmol/L	> 1.00

- Electrophoresis of lipoproteins : high amount of chylomicrons
- *ABCD1* gene (X-ALD) : no mutation

☞ *Lesson to learn from this patient*

- Non-specific increase of C26:0 in patients with hypertriglyceridemia
- Normal C24:0 / C22:0 ratio
- C26:0 / C22:0 ratio only slightly elevated

False positive VLCFA

Other causes

- Severe haemolysis, non-fasting specimens : mild increase
- Ketogenic diet (Theda et al, J Pediatr. 1993;122:724)
- Acute ingestion of peanut butter (Lam et al, Mol Genet Metab. 2012;107:620)
- Liver insufficiency (Stradomska et al, Lipids. 2013;48:405)
- Autoimmune adrenal insufficiency (Zhu & Breault, J Pediatr Endocrinol Metab. 2020;34:517)

Exact mechanism unknown

False negative peroxisomal biomarkers

Normal biomarkers in peroxisomal biogenesis disorders (PBD)

- Phytanic acid
  - Normal levels in the neonate (+/- pristanic acid)
  - Levels depend on diet : phytol
- Plasmalogen levels can be normal in milder PBD phenotypes

Normal VLCFA in X-ALD

- False negative results in 15-25% of women with X-ALD (Moser et al, Ann Neurol. 1999;45:100)