Workshop metabolic cardiomyopathies

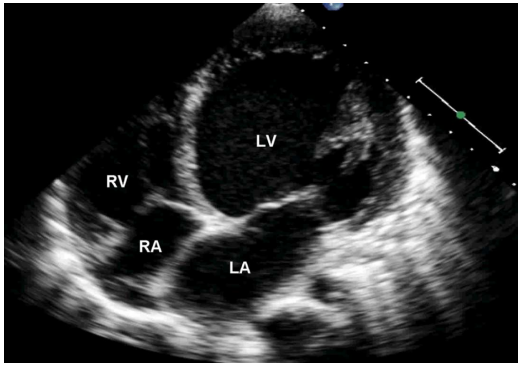
Case 1
Acute exercise intolerance & dyspnoea

Clinical presentation

- 16 year old male adolescent
- consanguineous Hispanic parents, adopted at 2 years
- acute exercise intolerance, fatigue, breathlessness and dyspnoea on exertion
- symptoms lasted for 1 week
- medical history unremarkable except for a nonproductive cough and generalized myopathy for 1 month

Additional information

- attended special school education due to behavioral problems (on methylphenidate)
- recent neurocognitive evaluation: various mild deficits, IQ 83
- Echocardiography: - severe dilated cardiomyopathy



- ejection fraction 25% → ICU

- Electrocardiography: prolonged QTc-time (457 ms; ref. <440)

Further course on ICU

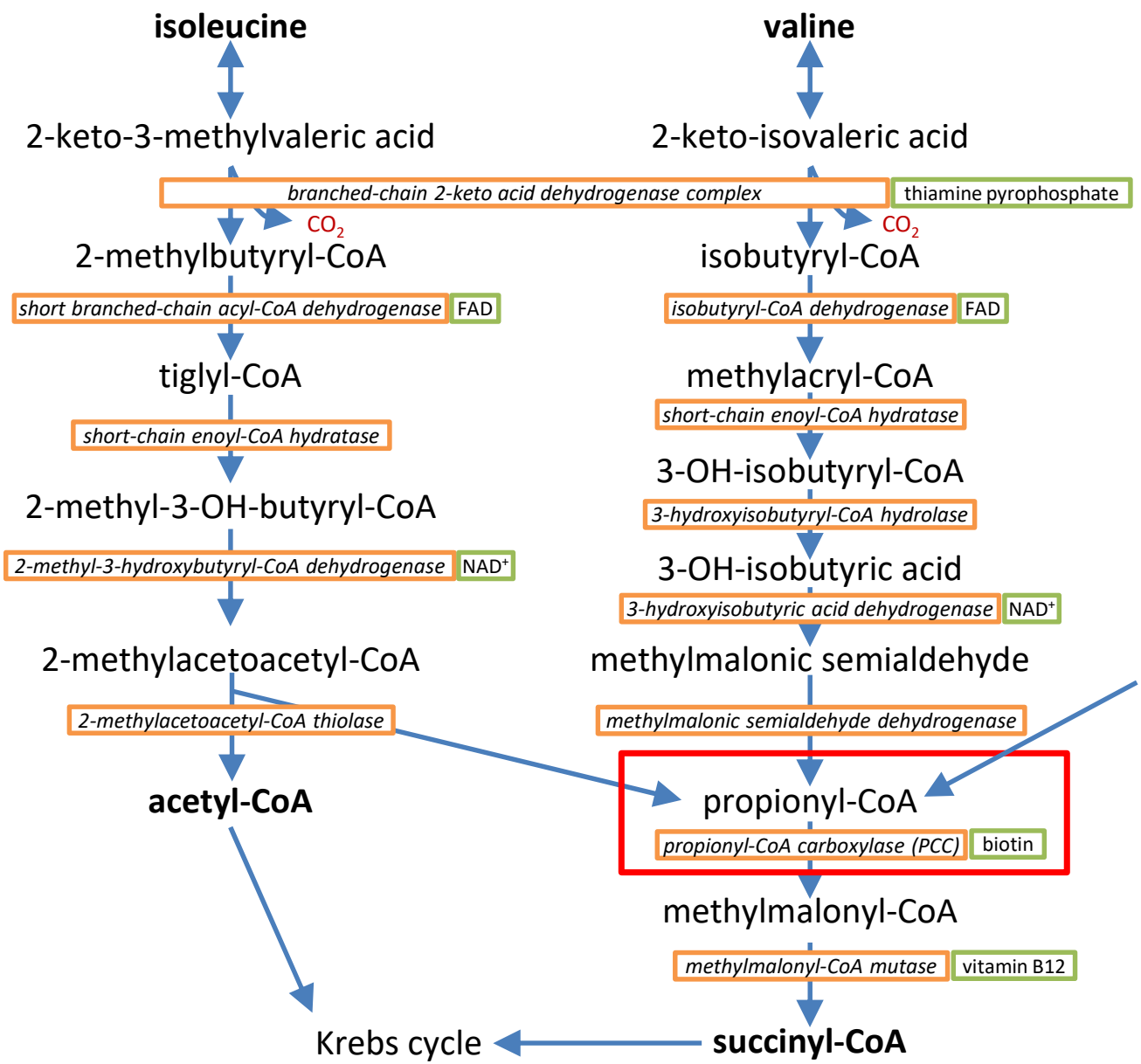
- therapy for congestive heart failure with diuretics furosemide, spironolactone, and hydrochlorothiazide, and continuous milrinone
- later also angiotensin converting enzyme (ACE) inhibitors
- cardiac muscle biopsy: unspecific lymphocyte infiltration
- Multiplex PCR for viral genome in blood & cardiac muscle: negative

What metabolic tests would you want to perform?

Metabolic investigations

- Unremarkable: blood gases, lactate
- Amino acid in plasma: glycine 417 µmol/L (147–299), rest normal (including alanine 360 µmol/L and glutamine 720 µmol/L)
- Acylcarnitines in DBS: propionylcarnitine 13.5 µmol/L (<3.5), free carnitine 32.3 µmol/L (20-54.8)
- Organic acids in urine (in mmol/mol crea): 3-hydroxypropionate 130 (<10), 2-methylcitrate 110 (<20), methylmalonate <10 (<10)

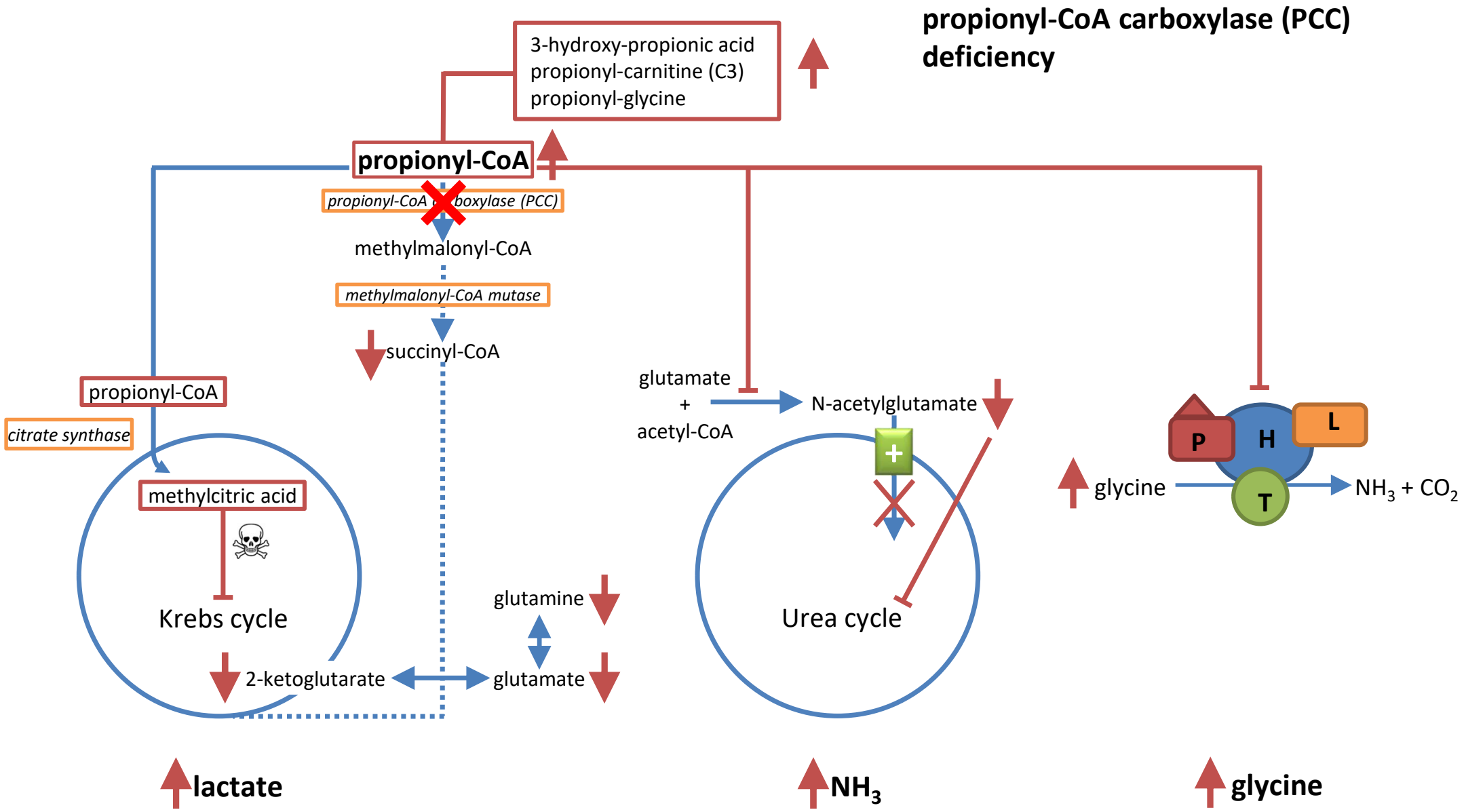
What is your diagnosis?



propionyl-CoA carboxylase (PCC)

(other) sources of propionic acid/propionyl-CoA

- **V**aline
- **O**dd-chain and branched-chain fatty acids
- **M**ethionine
- **I**soleucine
- **T**hreonine
- bacterial metabolism in the gut


propionyl-CoA carboxylase (PCC) deficiency
3-hydroxy-propionic acid
propionyl-carnitine (C3)
propionyl-glycine
propionyl-CoA
propionyl-CoA carboxylase (PCC)
methylmalonyl-CoA
methylmalonyl-CoA mutase
succinyl-CoA
propionyl-CoA
citrate synthase
methylcitric acid
Krebs cycle
2-ketoglutarate
glutamine
glutamate
glutamate
+
acetyl-CoA
N-acetylglutamate
Urea cycle
P
H
L
T
glycine
NH3 + CO2
lactate
NH3
glycine

Diagnostics for confirmation

- *PCCB* gene: homozygous for novel variant c.1229G>A, p.R410Q
- PCC activity assays in lymphocytes 32.5 pmol/min/mg protein (lower limit of normal 228)
- PCC activity assays in fibroblasts 39.7 pmol/min/mg protein (lower limit of normal 287)

PCC: propionyl-CoA carboxylase

Further course and outcome

- treatment for congestive heart failure continued (later tapered)
- start with L-carnitine 80 mg/kg /day
- reduced dietary protein from 3 g/kg /day to 1.5 g/kg /day
- no additional amino acid mixtures
- methylphenidate stopped due to reported association with DCM
- cardiac function improved but remained impaired (EF 40%)

DCM: dilated cardiomyopathy; EF: ejection fraction

Other reports in literature

Table 1 Patients with cardiomyopathy (CM) leading to diagnosis of late-onset propionic acidaemia (PA)

Patient, sex	Age at onset of CM	Acute therapy	Clinical course/recovery after diagnosis	PCC activity	Genotype (*PCCB* gene)	Reference[a]
1, m	6 years	Diuretics and inotropes	Rapid recovery; asymptomatic 2 months after onset of CM	Unknown	Unknown	[7]
2, f	13 months	Diuretics and bicarbonate	Rapid recovery within 1 week	Unknown	Unknown	
3, m	14 years	Cardiac transplantation	Rapid recovery after cardiac transplantation	19.9 %[b]	IVS7+2 T>G; p.R410Q	[5]
4, m	16 years	Diuretics and inotropes	Rapid recovery within 2 months	14.3[b] and 13.8 %[c]	Homozygous for p.R410Q	Present case

m Male, *f* female

[a] Reference search was restricted to PubMed. Search restrictions were: "propionic acidemia or propionic acidaemia and cardiomyopathy"

[b] PCC activity in lymphocytes and [c] fibroblasts: percentage of normal lower limits

Take home message

- Late-onset cardiomyopathy can be presenting symptom in propionic acidemia in various populations – not only in the Amish
- Propionic acidemia does not necessarily present with very strongly elevated metabolites
- Amino acid findings are of limited use in diagnosing propionic acidemia

Adult stroke

Clinical presentation

56 year-old man with right-sided numbness & weakness

Past history

- Sudden right-sided unilateral hearing loss at 49 years
- Chest pain – left ventricular hypertrophy and hypertension at 46 years
- Mild renal impairment in his 50's

Examination

- Normal, apart from hearing impairment
- Weight 103.4 kg, BMI 32 kg/m^2, BP 109/76 mmHg

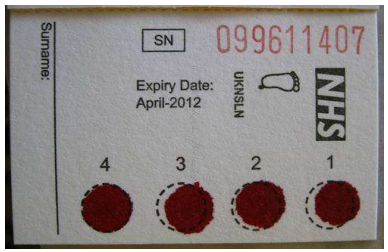
What investigations would you do?

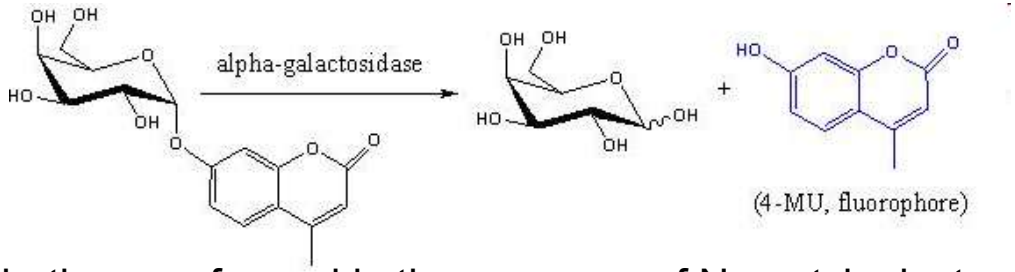
- Lipid profile: cholesterol 6.8 mmol/L (<5), LDL 5.8 mmol/L (<3)
- Fasting glucose normal
- Urine: proteinuria
- MRI brain – white matter hyperintensity, left lacunar cerebral infarction
- Blood spot alpha-galactosidase activity < 1% of control
- *GLA* sequencing c.496-497delinsTC, p.Leu166Pro

What is your differential diagnosis?

Diagnosis: Fabry disease

Fabry disease - diagnosis





Incubations performed in the presence of N-acetylgalactosamine

-Inhibition of α-galactosidase B

What Fabry phenotypes are there?

		Classic	Renal Variant	Cardiac Variant
Age at onset		4-8 yrs	>25 yrs	>40 yrs
Average age of death		41 yrs	>60 yrs	>60 yrs
Manifestation	Angiokeratoma	++	–	–
	Acroparesthesia	++	–/+	–
	Hypohidrosis/ anhidrosis	++	–/+	–
	Corneal/lenticular opacity	+	–	–
	Cardiac disease	LVH/ischemia	LVH	LVH/cardiomyopathy
	Cerebrovascular disease	TIA/stroke	–	–
	Renal disease	ESRD	ESRD	Proteinuria
	Residual α-Gal A enzyme activity	<1%	>1%	>1%

Mehta & Hughes, *GeneReviews*, 2017

How would you manage this patient?

SSIEM ACADEMY 2023

Enzyme replacement therapy (ERT)

- Fabrazyme® (agalsidase beta 1 mg/kg every 2 weeks), or
- Replagal™ (agalsidase alfa 0.2 mg/kg every 2 weeks)
- Both probably don't prevent stroke or hearing loss
- Both expensive & inconvenient
- Slow renal & cardiac complications

General supportive care

- Statins for lipids,
- Weight management,
- Consider aspirin & anti-platelet drugs Stop smoking,
- Blood pressure control,
- ACE inhibitor,
- Pacemaker, implantable defibrillator
- Renal dialysis or transplant
- Carbamazepine or gabapentin for acroparesthesia

Chaperone

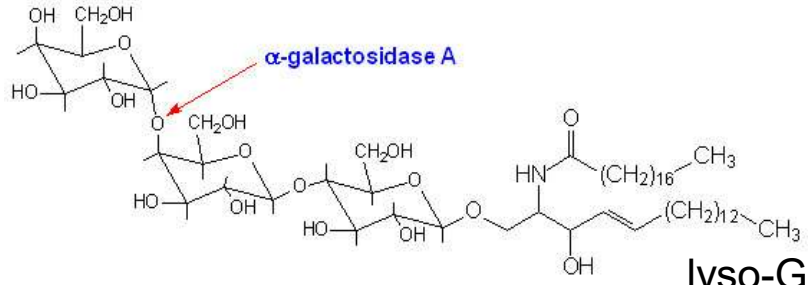
- Oral migalastat (1-deoxygalactonojirimycin, DGJ) enhances trafficking of mutated α-Gal A to lysosomes in specific mutations & may achieve similar benefit as ERT
- www.galafoldamenability.com (sponsored by pharma company)
- L166P is not amenable to migalastat

What monitoring would you do?

- Regular cardiology review
- Renal function, proteinuria annually
- Maybe brain MRI, neurocognitive testing - how often?
- Plasma lyso-GB3

Lyso-GB3

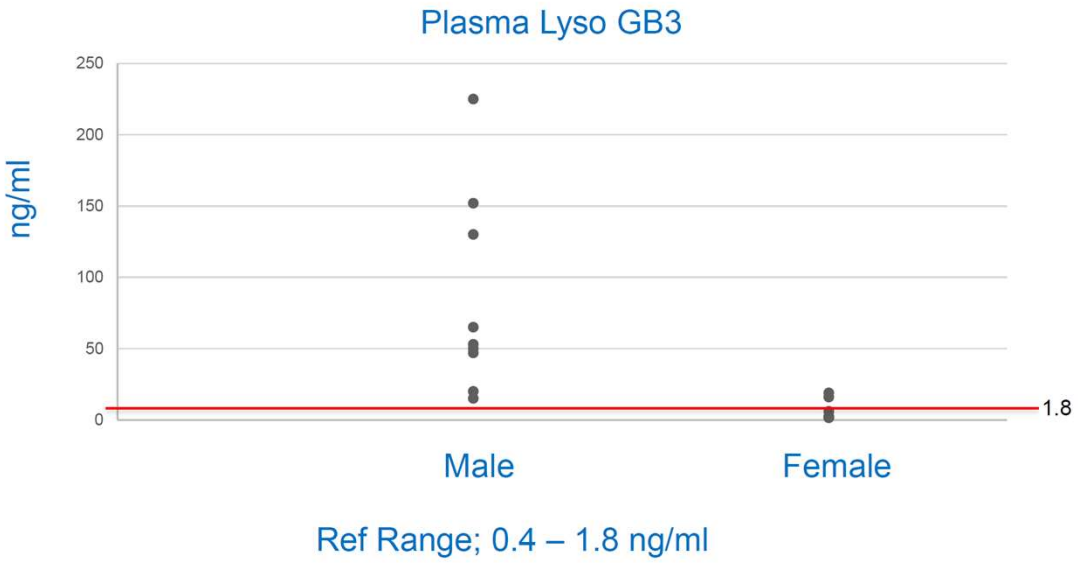
Enzyme deficiency leads to substrate accumulation: Globotriaosylceramide (Gb3)

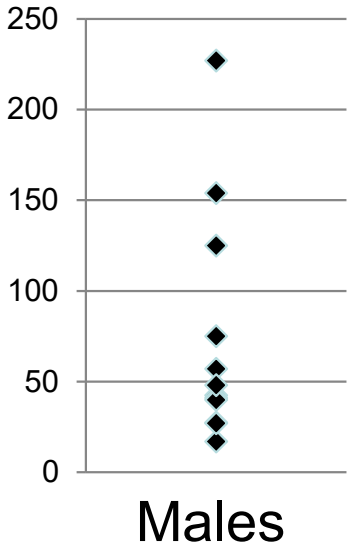


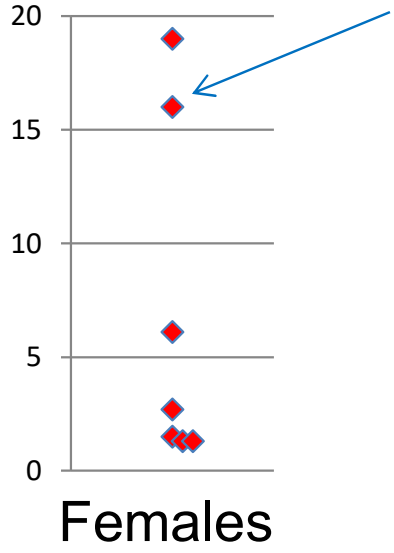
lyso-Gb3 formed by action of Acid Ceramidase

Date	2014 (Baseline)	2015 (on ERT)	2016 (on ERT)	2017 (on ERT)
Plasma lyso GB3 (<1.6 ng/mL)	32.5	6.0	4.9	4.2
Urine GB3 (<0.03 mg/mmol creatinine)	0.31	0.01	0.01	0.01

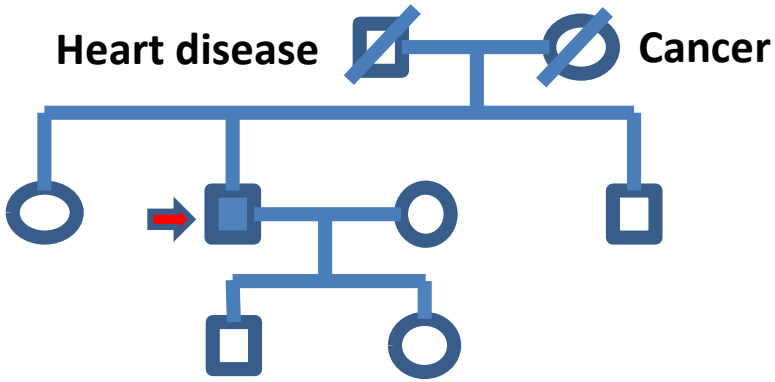
Lyso-GB3


Plasma Lyso GB3
ng/ml
250
200
150
100
50
0
1.8
Male
Female
Ref Range; 0.4 – 1.8 ng/ml


250
200
150
100
50
0
Males


20
15
10
5
0
Females

Genetic counselling



X-linked

- At risk: siblings: do they have symptoms? offer testing
- His daughter – obligate carrier of the variant: does she have symptoms? offer testing for reproductive options
- His son – not at risk

Outcome, 2 years after start of ERT

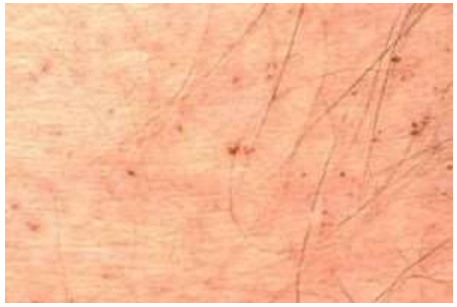
- Second cerebrovascular accident – significant residual right-sided weakness

Take home messages

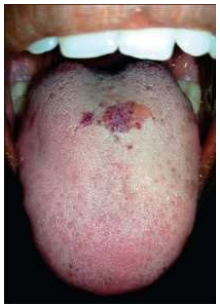
Lyso-GB3 can be a useful screening tool – but is not always elevated in individuals with a Fabry gene variant

The phenotype is variable

Angiokeratomata may be difficult to detect



Source: fabrydisease.org



Source: ijohsjournal.org

Workshop Metabolic cardiomyopathies

Case 3
From the Heart

Clinical presentation

Baby boy, day one of life

- Blood glucose 1.9 mmol/L
- Metabolic acidosis with high lactate in blood

  pH 7.15,
  Base Excess –23.4,
  lactate 14.7 mmol/l (N<2.1 mmol/l)

Test performed

Plasma

- Insulin
- Acylcarnitines
- Amino acids

Urine

- Organic acids

Test performed

Plasma

- Insulin → undetectable
- Acylcarnitines → essentially normal
- Amino acids → high alanine

Urine

- Organic acids → nonspecific mitochondrial pattern with increased excretion of lactic, fumaric, malic and 4-hydroxy-phenyllactic acids

Clinical presentation

One month of age

- *Echocardiography: dilated cardiomyopathy with noncompaction of the ventricular myocardium*
- *Progressive worsening of cardiac symptoms*
- *Persistent hypoglycemia*

What is your differential diagnosis?
Any further investigations?

Test performed

Muscle biopsy for mitochondrial investigations

- A reduction in respiratory chain enzyme activity was detected in the muscle biopsy
- No specific diagnosis made

Clinical presentation

Follow up at 3 years of age

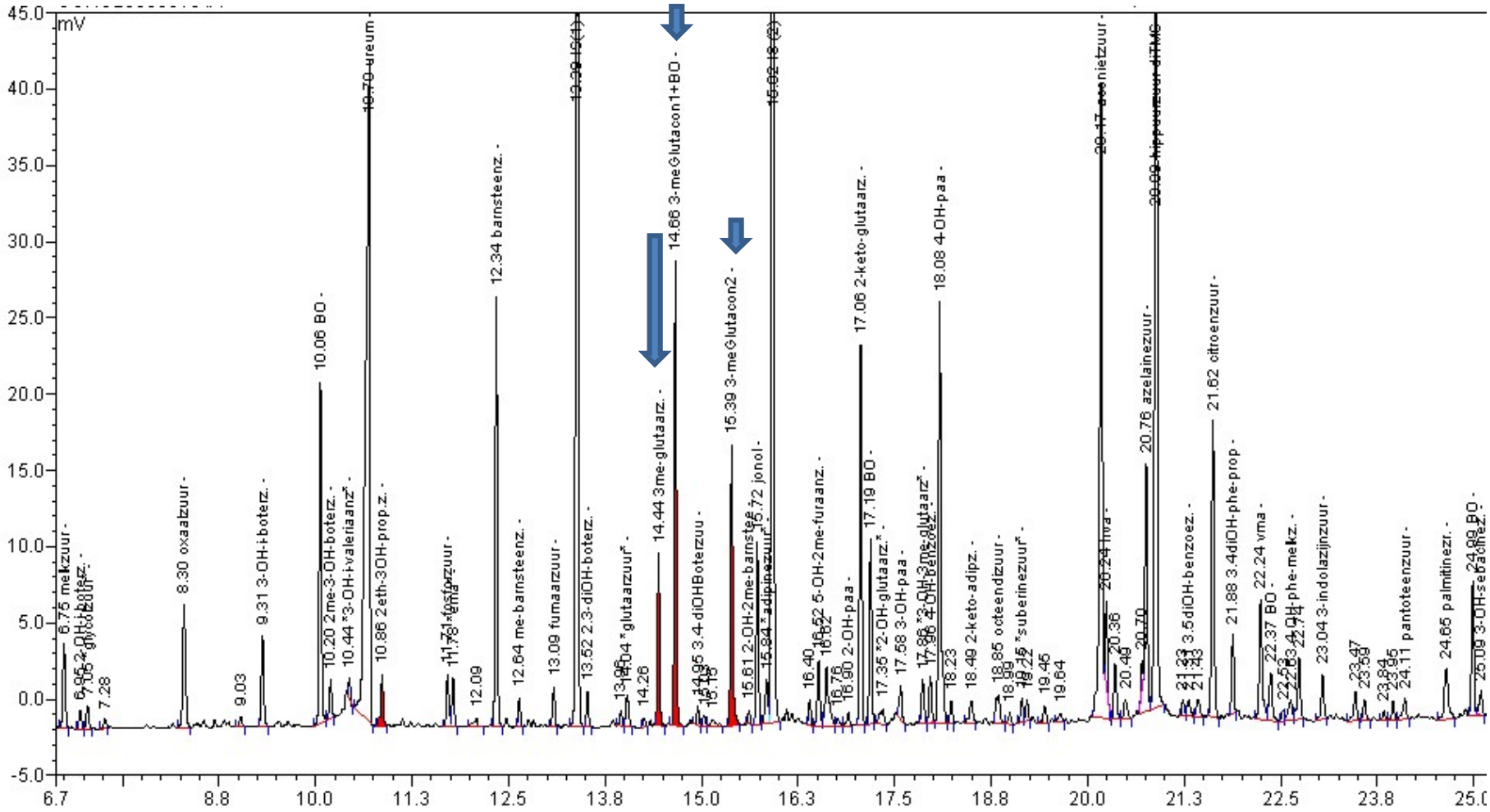
- Growth delay
- Mild intermittent neutropenia
- No more hypoglycemia
- Cardiomyopathy

Follow up testing:

- Cardiomyopathy protocol reviewed and tests repeated

What is your current differential diagnosis?
Are there specific metabolic abnormalities to look for?

Urine Organic Acids → 3-methylglutarate, 3-methylglutaconate



3-methylglutaconate

Primary

- 3 HMG CoA hydratase deficiency → grossly increased 3-MeGCA, also 3-OH isovalerate; neuromuscular presentation

Secondary

- Phospholipid remodelling disorders
  - Barth syndrome
  - MEGDEL syndrome
  - Sengers syndrome
- Other mitochondrial abnormalities
- Unknown

What is the likely diagnosis? What confirmatory tests?

Diagnosis

Barth syndrome most likely

- Cardiomyopathy, growth retardation, 3-methylglutaconic aciduria, neutropenia

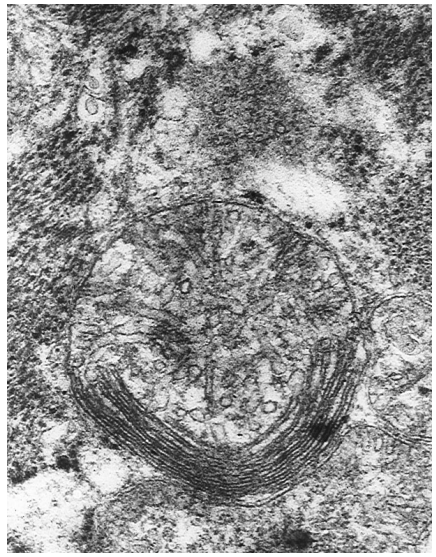
Confirmatory tests

- Bloodspot cardiolipin analysis
  monolysocardiolipin/cardiolipin ratio: 3.90 (N<0.3) 

- Genetic testing
  Hemizygote variant in *TAFAZZIN* gene c.337_339delTTC p.Phe113del

Barth syndrome

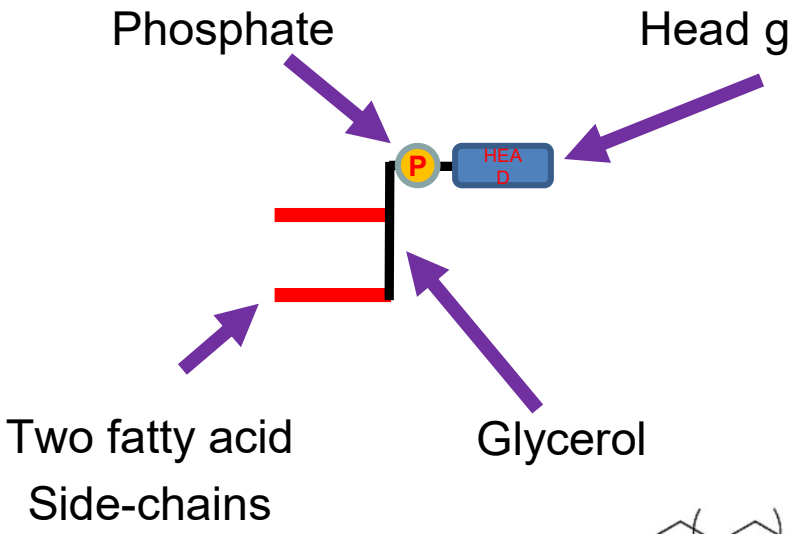
- First described in 1983 by Dr. Barth (AMC Amsterdam)
- Presentation variable, may include
  - Cardiac and skeletal myopathy
  - Neutropenia
  - Growth delay
  - Hypoglycaemia
  - Lactic acidosis
  - 3-methylglutaconic aciduria
- Consistent findings
  - Cardiolipin abnormalities
  - Abnormal mitochondrial structure and function
  - Variants in *TAFAZZIN* gene (Xq28)
- Diagnostic challenge as features are non-specific and inconsistent



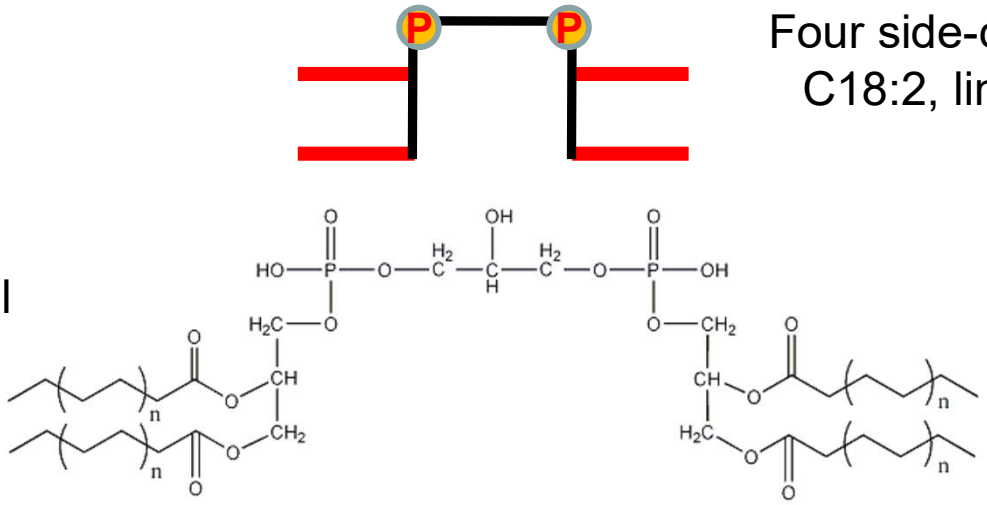
From the heart

Cardiolipin?

"Regular" phospholipid structure

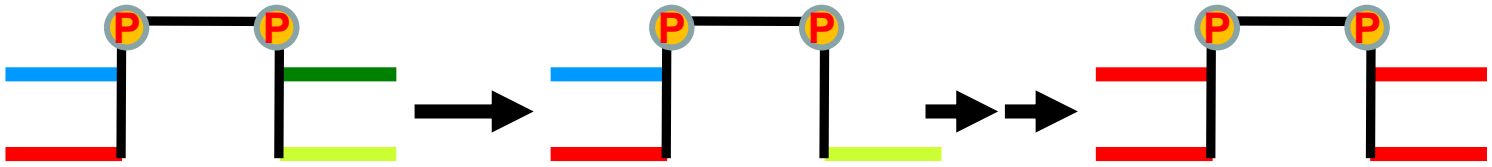


Cardiolipin



Four side-chains of
C18:2, linoleic acid

Cardiolipin synthesis and remodeling



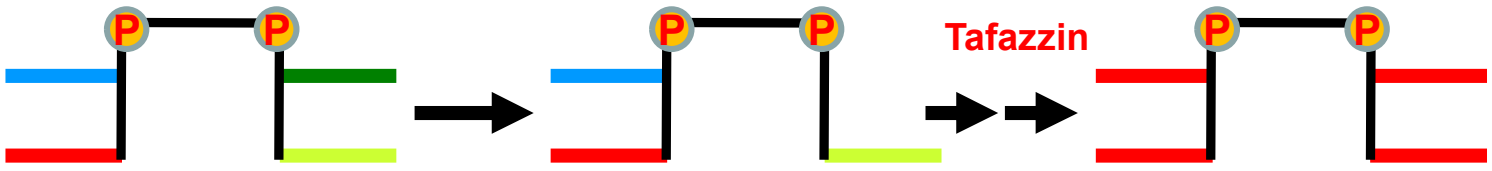
Immature cardiolipin

Monolysocardiolipin (MLCL)

Mature cardiolipin

- Cardiolipin is actively remodelled to achieve the mature acyl composition
- This is affected in Barth syndrome

Cardiolipin remodeling



Immature cardiolipin

Monolysocardiolipin (MLCL)

Mature cardiolipin

For Barth syndrome patients you then expect:

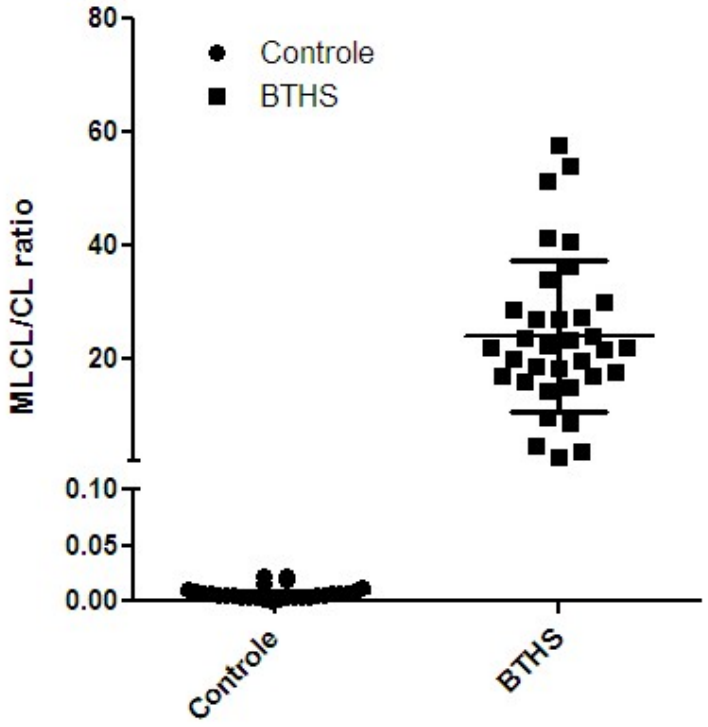
- Lower mature CL levels
- Higher MLCL levels
- Altered CL and MLCL composition

Barth syndrome diagnostics

For Barth syndrome:

Monolysocardiolipins (MLCL)	↑
Cardiolipins (CL)	↓
MLCL/CL ratio	↑↑

- MLCL/CL ratio is a powerful biomarker for Barth syndrome



Take home messages

- Cardiomyopathy is a key feature in most cases
- Barth syndrome should be considered when urine organic acids show increased 3-methylglutarate and 3-methylglutaconate
- 3-methylglutaconic acid is non-specific for Barth syndrome and is not a consistent finding
- MLCL/CL ratio in blood spot is a powerful, fast screen for Barth syndrome and a useful addition to genetic testing

Workshop Metabolic cardiomyopathies

Case 4
Neonatal cardiomyopathy

Case background

- Baby girl born at 37^{+1} weeks, 2.4 kg, small for gestation age
- Second child of consanguineous parents, sibling is well
- Well at birth, transferred to postnatal ward
- 18 hours of age, unresponsive, bradycardic, complete heart block
- Transferred to neonatal intensive care
- Blood glucose 0.9 mmol/L, samples taken for hypoglycaemia screen
- Cardiac rhythm changed to ventricular tachycardia
- Transferred to cardiac intensive care ward
- Cardiomyopathy screen requested
- *What is your differential diagnosis? What further tests?*

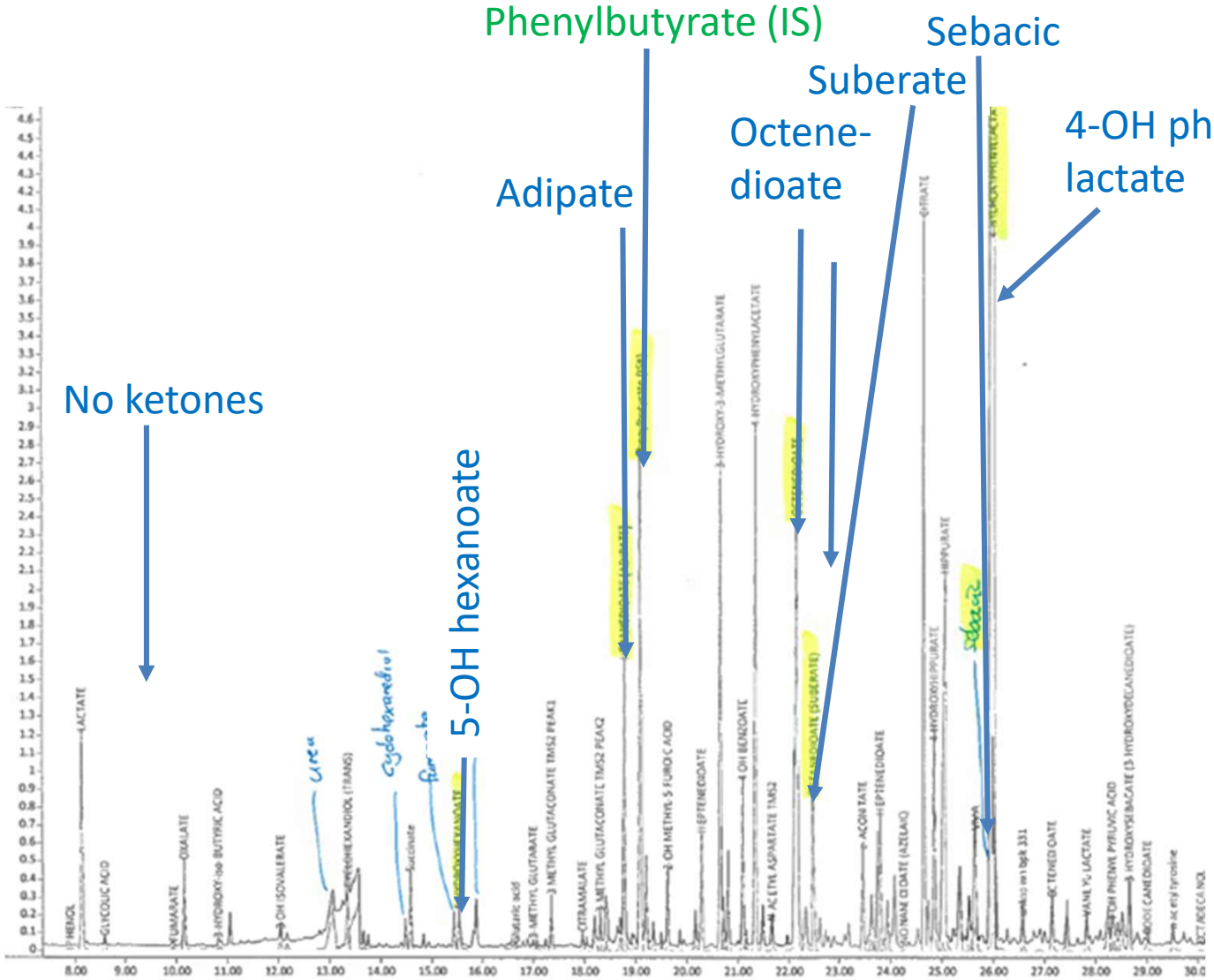
Hypoglycaemia screen

Test (plasma, serum)	Result (reference range)
Lab glucose	2.2 mmol/L
Lactate	2.2 mmol/L (0.6 – 2.5)
Cortisol	3412 nmol/L (< 410)
Insulin	< 6 pmol/L (12 – 150)
3-OH butyrate	<0.1 mmol/L (<0.9 non-fasting)
Free fatty acids	2.43 mmol/L (<0.2 non-fasting)
Ammonia	259 µmol/L (<100)

How do you interpret these results?
What further tests would you prioritise?

Hypoglycaemia screen

Test (plasma, serum)	Result (reference range)	Interpretation
Lab glucose	2.2 mmol/L	Hypoglycaemia
Lactate	2.2 mmol/L (0.6 – 2.5)	Normal
Cortisol	3412 nmol/L (< 410)	Stress response
Insulin	< 6 pmol/L (12 – 150)	Appropriately suppressed
3-OH butyrate	<0.1 mmol/L (<0.9 non-fasting)	FFA/3-OHB ratio > 2 indicates a fatty acid oxidation defect
Free fatty acids	2.43 mmol/L (<0.2 non-fasting)	
Ammonia	259 µmol/L (<100)	Hyperammonaemia



What is the abnormality? What further investigations are required?

Carnitine, acylcarnitines

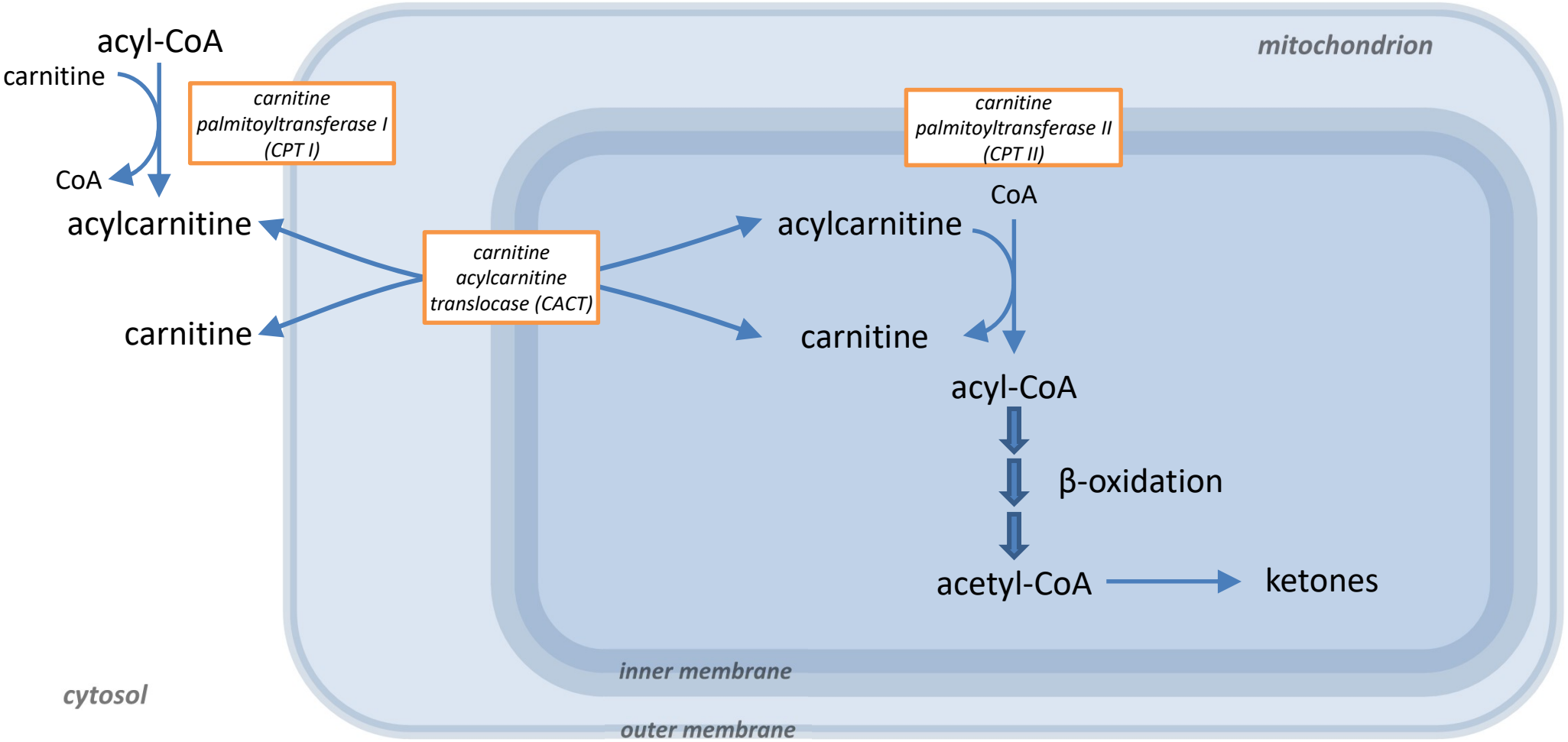
Analyte (µmol/L) (MSMS, derivatised)	DBS	Ref range	Plasma	Ref range
C2	6.9	8.8 - 24	5.3	3.0 – 14.0
C16	15.8	< 5.0	11.3	< 0.32
C18	2.2	< 1.6	1.3	< 0.13
C18:1	4.4	< 2.6	5.1	< 0.54
(C16+C18+C18:1)/C2	3.2	0.2 – 0.5	3.4	0.02 – 0.16

Plasma free carnitine (underivatised MSMS method) 2.3 µmol/L (11 – 18)

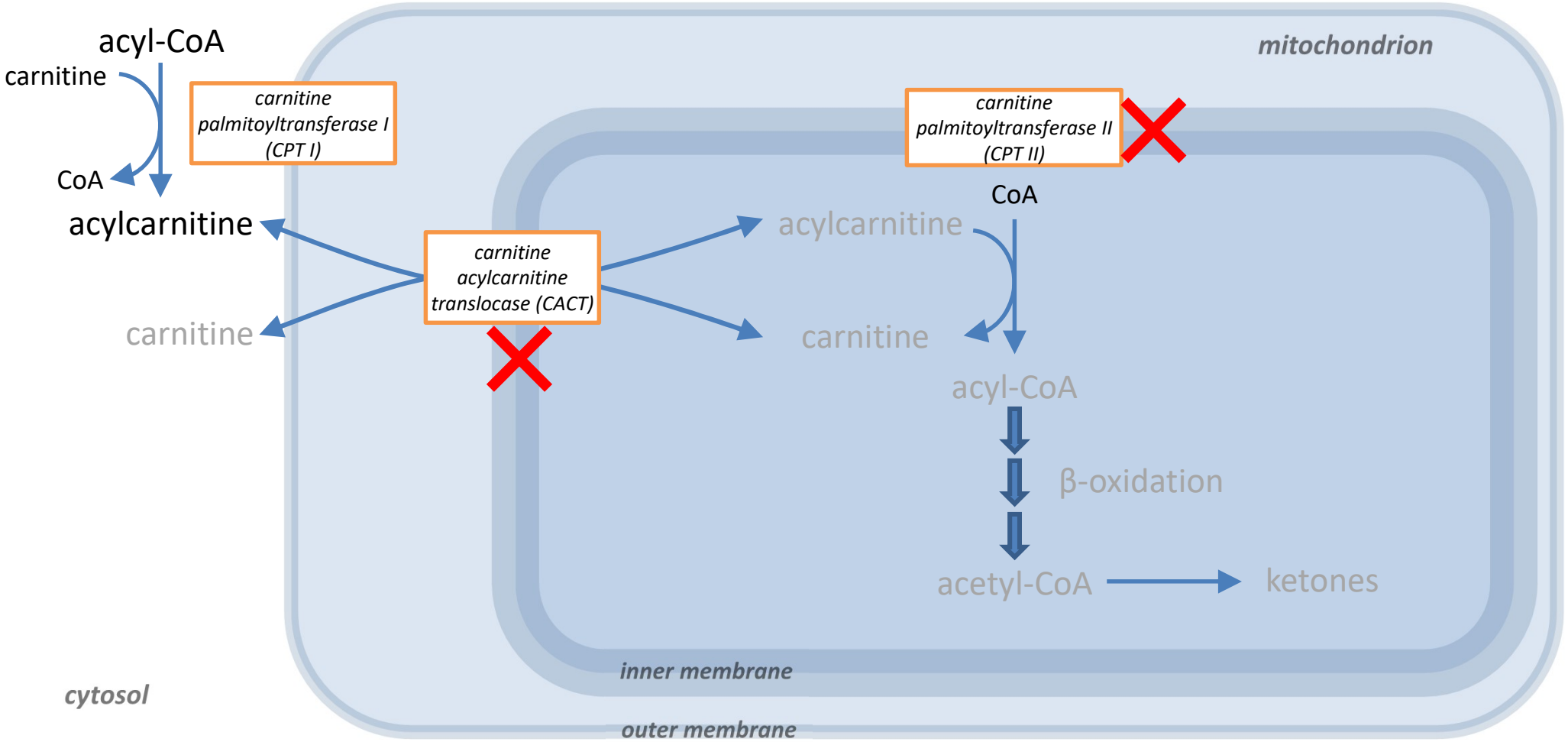
What is the diagnosis? How do you confirm it?

Is there a difference between DBS and plasma acylcarnitines?

The carnitine shuttle / cycle



CPT II or CACT deficiency



Further investigations

- Muscle biopsy for mitochondrial studies
  Mild deficiency of complex I and complex IV
- Fibroblast fatty acid oxidation flux

Assay	Myristate (C14)	Palmitate (C16)	Oleate (C18:1)
% controls	1 %	2 %	6 %

Consistent with severe defect of long chain fatty acid oxidation e.g. CPT2, CACT, MADD

- Genetic testing
  Homozygous *SLC25A20* variants c.713A>G, p.Gln238Arg
  Carnitine-acylcarnitine translocase (CACT) deficiency

CPT2 or CACT?

- Biochemistry the same
- CPT2
  - Typically presents with rhabdomyolysis following exercise or fasting
  - Severe neonatal disease is a rare presentation
  - Intermediate form with hypoglycaemia and liver dysfunction
- CACT
  - Most cases have neonatal presentation with early death
  - Features: ↓glucose, ↑NH_3, cardiomyopathy, atrioventricular block
  - Mild presentation is very rare

Patient Outcome

- Supportive therapy
- Died day 5

Is it necessary to test the older sibling for CACT deficiency? If so, which test would you recommend?

Three further pregnancies, no prenatal testing.
Infants managed in specialist centre and plasma acylcarnitines analysed on day 2. Genetic testing also performed.
None has CACT deficiency

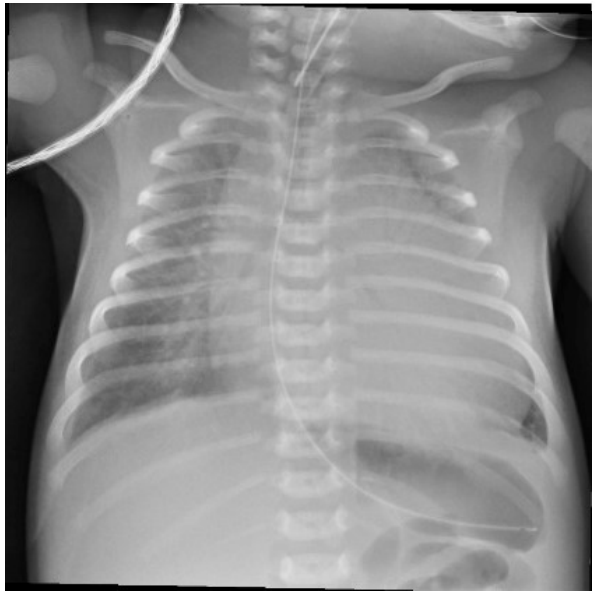
Take Home Messages

- CACT deficiency can present with neonatal non-ketotic hypoglycaemia
- It is not possible to distinguish CACT and CPT2 with acylcarnitines
- Genetic testing is required to confirm a diagnosis
- Plasma acylcarnitines is more sensitive than DBS for detection of abnormalities of long-chain acylcarnitines
- Appropriate investigations requested during acute illness and liaison between the clinical and laboratory teams can provide quick diagnosis of fatty acid oxidation disorder

A neonate with respiratory distress

J Davison

Clinical presentation



- 3 day old male child

- 1st child born to consanguineous parents
- Pregnancy: normal (no gestational diabetes)
- Term normal delivery

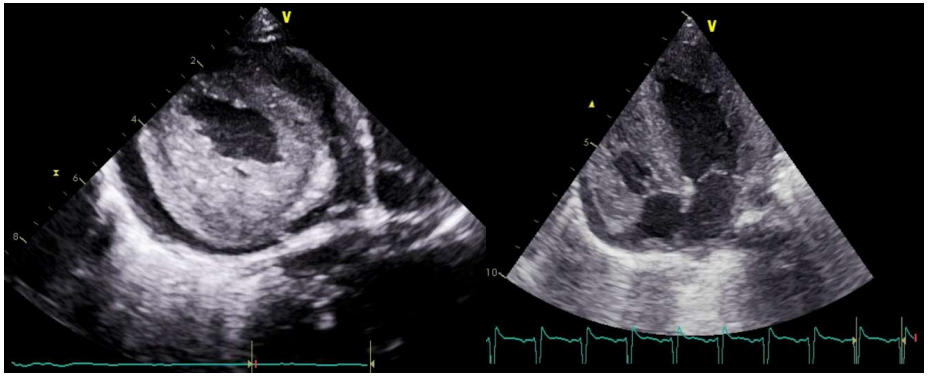
- Noted to have tachypnoea, mild increased work of breathing
- Oxygen saturation 99% pre- and post-ductal
- Parasternal heave, loud second heart sound.
- Femoral pulses palpable.

- pH 7.34, pCO2 3.9 kPa, HCO3 19 mmol/L, Base excess –3 mmol/L, Lactate 3.9 mmol/L, Glucose 4.1mmol/L

- What is your differential diagnosis?

What is your differential diagnosis?

- **Echocardiogram**:
  - Severe biventricular hypertrophy
  - Dyskinetic septum
  - Appearance of “non-compaction”
  - No aortic coarctation



- **What is your differential diagnosis now?**

Infantile Cardiomyopathy
- Gestational diabetes
- Neuromuscular
- Syndromic (eg Noonan’s)
- Primary hereditary cardiomyopathies
- Metabolic cardiomyopathy

Metabolic Cardiomyopathy
- Lysosomal/glycogen storage disorders
- Fatty acid oxidation defects
- Mitochondrial disorders
- Secondary nutritional deficiencies

What metabolic investigations would you do?

- CK 211 U/L
- Ammonia <9 µmol/L
- Glucose 4.2 mmol/L
- Lactate 6-11 mmol/L (higher when on IV 10% dextrose)
- Renal function normal
- Liver function: ALT 230 U/L
- PT 25 sec (8.2-14.1), aPTT 86 sec (28-55)

- Free carnitine + Acylcarnitine profile
- Urine organic acids
- Plasma amino acids, total homocysteine
- DNA sample

- Thiamine, Selenium, Vitamin D

- Lysosomal
  - Urine GAG: 37 mg/mmol (5-40)
  - Alpha-glucosidase: normal activity
  - Vacuolated lymphocytes: not seen
  - Urine tetrasaccharide: normal

- Transferrin isoforms: normal
  - Clotting, Protein C/S

- Cardiolipin

What metabolic investigations would you do?

Urine organic acids:

- ↑↑↑ Pyruvate 308 µmol/mmol creatinine
- ↑↑ Lactate 258 µmol/mmol creatinine
- ↑↑ 2-oxo-glutarate
- ↑ ethylmalonate 24 µmol/mmol creatinine

Plasma amino acids

- Unremarkable
- Alanine normal range (sample obtained when lactate normal)

Bloodspot acylcarnitine

- Free carnitine 34 µmol/L (10-85)
- ↑ C4-OH (hydroxybutyryl)carnitine 0.35 µmol/L

Urine carnitine

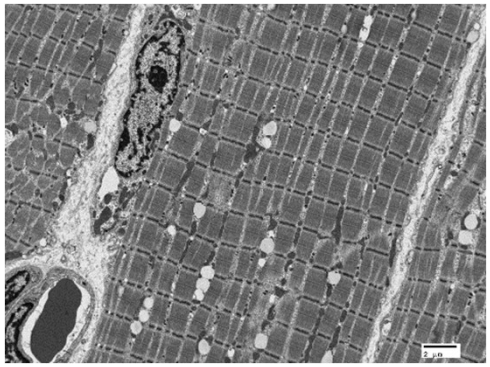
- Normal free carnitine excretion

Biotinidase: normal activity

White cell ubiquinone: 169 pmol/mg (37-133)

What metabolic investigations would you do?

- **Muscle biopsy:**
  - Excess lipid droplets
  - No other ultrastructural abnormalities

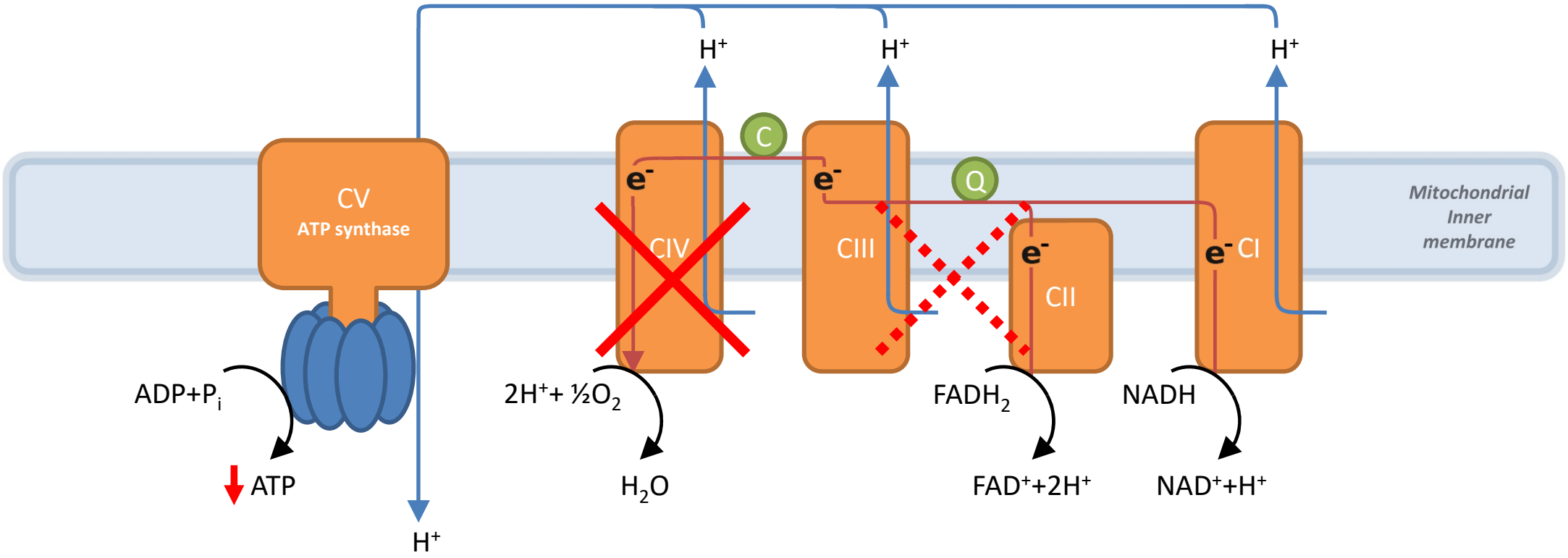


- **Muscle Respiratory Chain Enzymology**

RC enzyme	Results / Citrate Synthase	Reference
Complex I	0.112	0.118-0.332
Complex II+III	0.005 ↓	0.072-0.335
Complex IV	0.001 ↓↓↓	0.013-0.039
Ubiquinone	50 pmol/mg protein	140-580

What further diagnostic investigations would you do?

the mitochondrial oxidative phosphorylation system (OXPHOS)

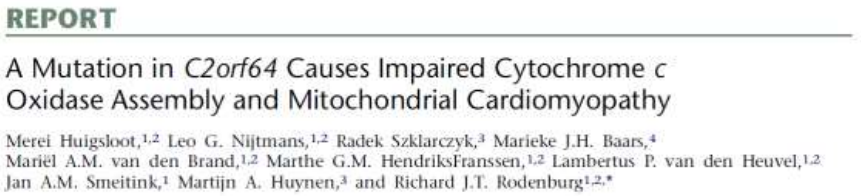


What further diagnostic investigations would you do?

- **Mitochondrial DNA**
  - MT-TE m.14674T>C not detected (associated with “benign reversible COX deficiency”)
  - mtDNA sequencing normal

- **Nuclear DNA**
  - Untargeted Whole Exome Sequencing
  - *COA5 (C2orf64)* homozygous variant of uncertain significance [c.157G>C, p.Ala53Pro]
    - *Complex IV assembly factor*

- **What would you do now?**



REPORT

A Mutation in *C2orf64* Causes Impaired Cytochrome *c* Oxidase Assembly and Mitochondrial Cardiomyopathy

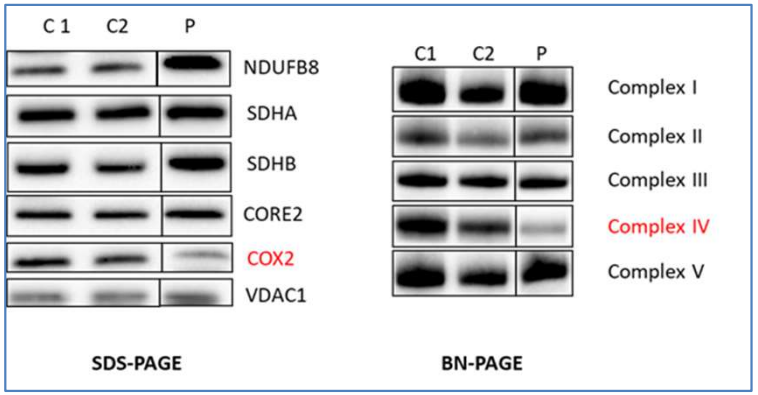
Merei Huigsloot,[1,2] Leo G. Nijtmans,[1,2] Radek Szklarczyk,[3] Marieke J.H. Baars,[4] Mariël A.M. van den Brand,[1,2] Marthe G.M. HendriksFranssen,[1,2] Lambertus P. van den Heuvel,[1,2] Jan A.M. Smeitink,[1] Martijn A. Huynen,[3] and Richard J.T. Rodenburg[1,2,*]

How would you confirm the diagnosis?

- Confirm Isolated C IV (COX) deficiency
  - Muscle OXPHOS immunohistochemistry
    - Significantly decreased complex IV
  - Fibroblast enzymology: significant complex IV deficiency

- Muscle Western Blot and BN-PAGE
  - Steady state levels of OXPHOS protein and assembly into functioning complexes
    - COX2 levels (C IV subunit) low
    - C IV assembly impaired

RC enzyme (Fibroblasts)	Results / Citrate Synthase	Reference
Complex I	0.190	0.197±0.034
Complex II	0.216	0.219±0.067
Complex III	1.229	0.646±0.192
Complex IV	**0.124**	1.083±0.186



Data Courtesy of Prof Rob Taylor, Newcastle

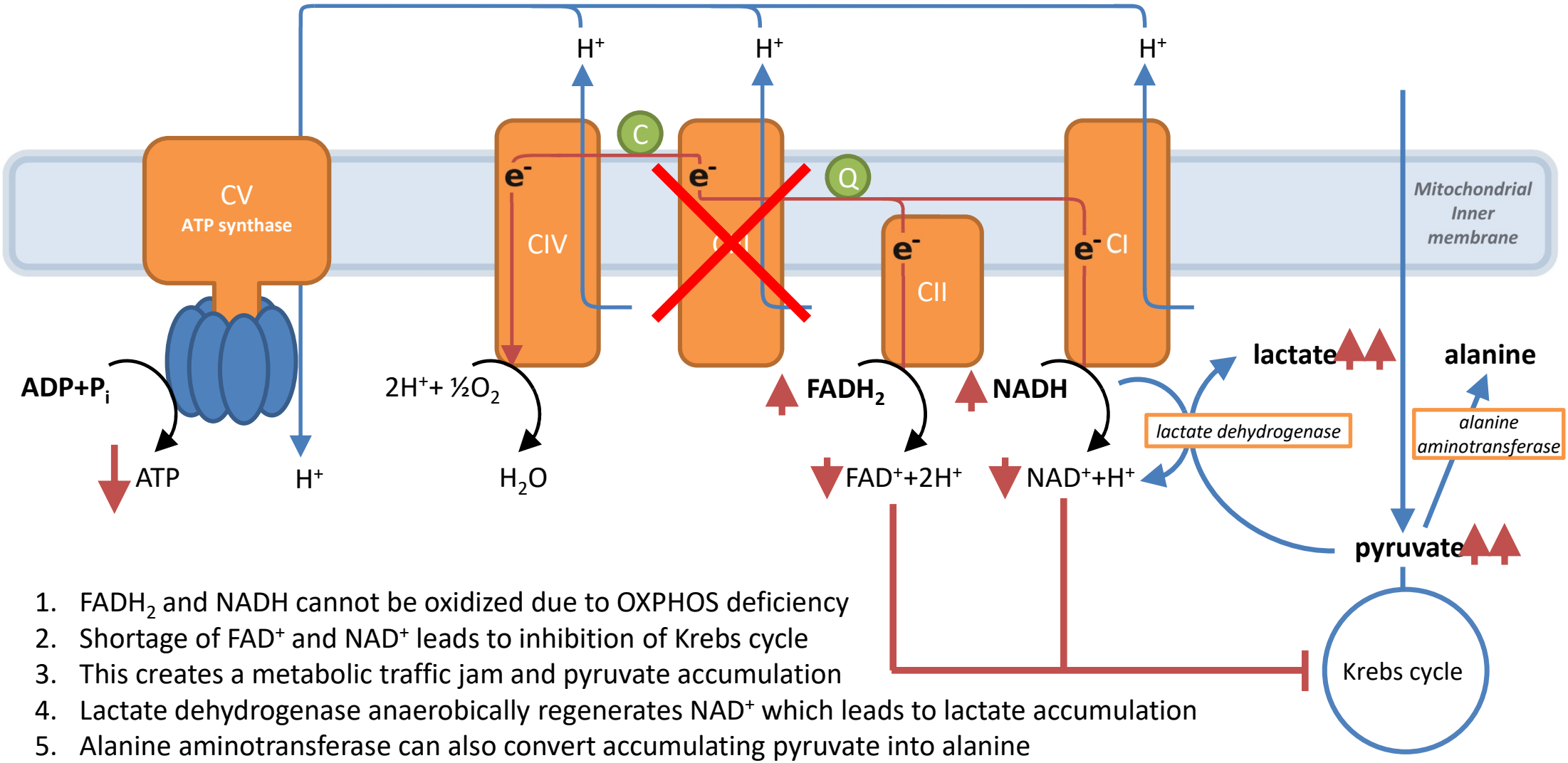
How would you manage the patient?

- **Symptomatic/ supportive care**
- **Genetic counselling**
- **Ubiquinone supplementation**
  - No sustained biochemical/clinical response
- Patient deceased 2 months – progressive cardiac failure

Take home message

- 20% of hypertrophic cardiomyopathy in infants is due to inherited metabolic cause
- Targeted metabolic and mitochondrial investigations
  - Metabolic differential includes FAOD, storage disorders, mitochondrial…
- Use of rapid genetic testing helpful
  - Ensure DNA samples collected and stored
- Biochemistry and tissue samples (muscle biopsy, skin biopsy) still helpful in substantiating novel genetic findings

Mitochondriopathy with OXPHOS deficiency



1. $FADH_2$ and NADH cannot be oxidized due to OXPHOS deficiency
2. Shortage of FAD^+ and NAD^+ leads to inhibition of Krebs cycle
3. This creates a metabolic traffic jam and pyruvate accumulation
4. Lactate dehydrogenase anaerobically regenerates NAD^+ which leads to lactate accumulation
5. Alanine aminotransferase can also convert accumulating pyruvate into alanine

Workshop Metabolic cardiomyopathies

Case 6
Sisters with cardiomyopathy

Case background

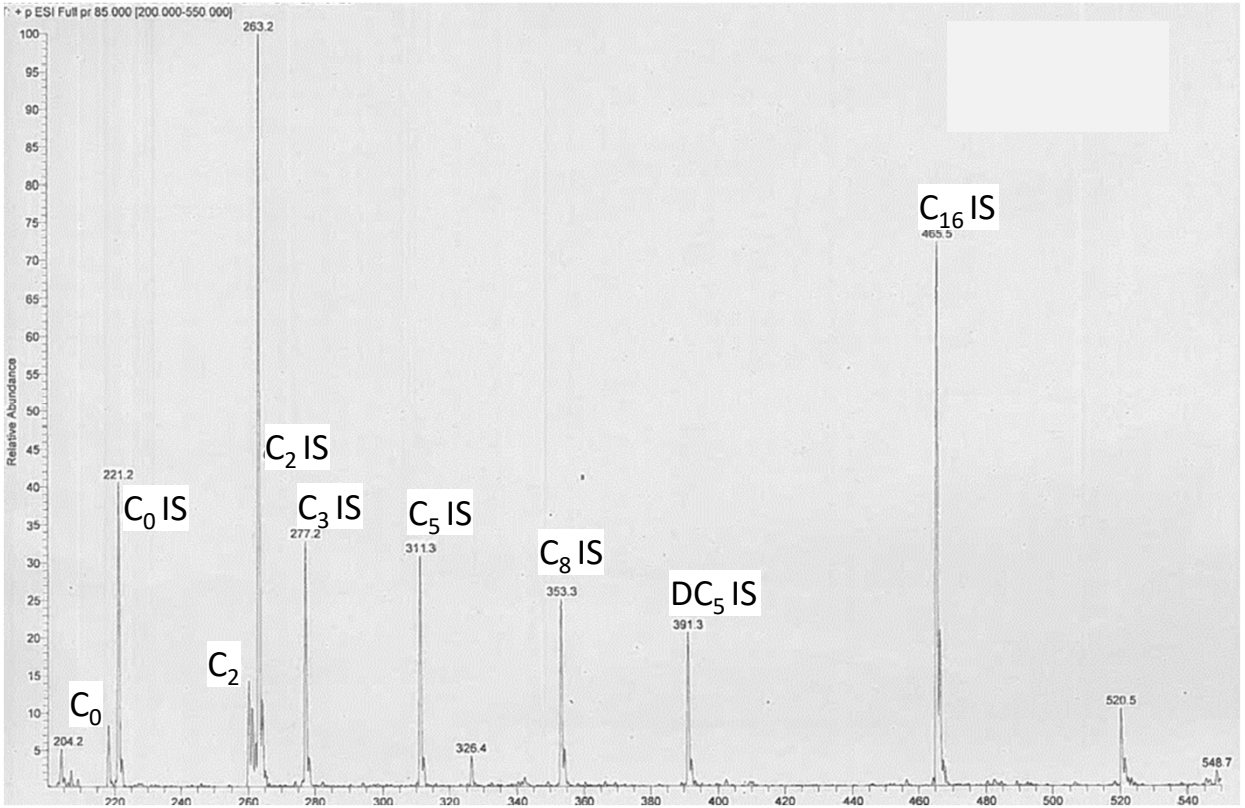
- 11-year-old girl, family recently moved to the area
- The second of four children of consanguineous parents
- Referred to cardiologist with history of dilated cardiomyopathy diagnosed at 7 years, ? viral cause
- Clinically well
- No family history of cardiac problems
- On enalapril, furosemide, spironolactone
- Underlying cause of cardiomyopathy unknown

What is your differential diagnosis? What further tests?

Cardiomyopathy Screen

- Plasma ammonia
  94 µmol/L (< 50)
- Blood spot acylcarnitines →
- Plasma free carnitine
  1.3 µmol/L (23 – 52)

How do you interpret the acylcarnitine profile?
What further tests are required?



Further investigations

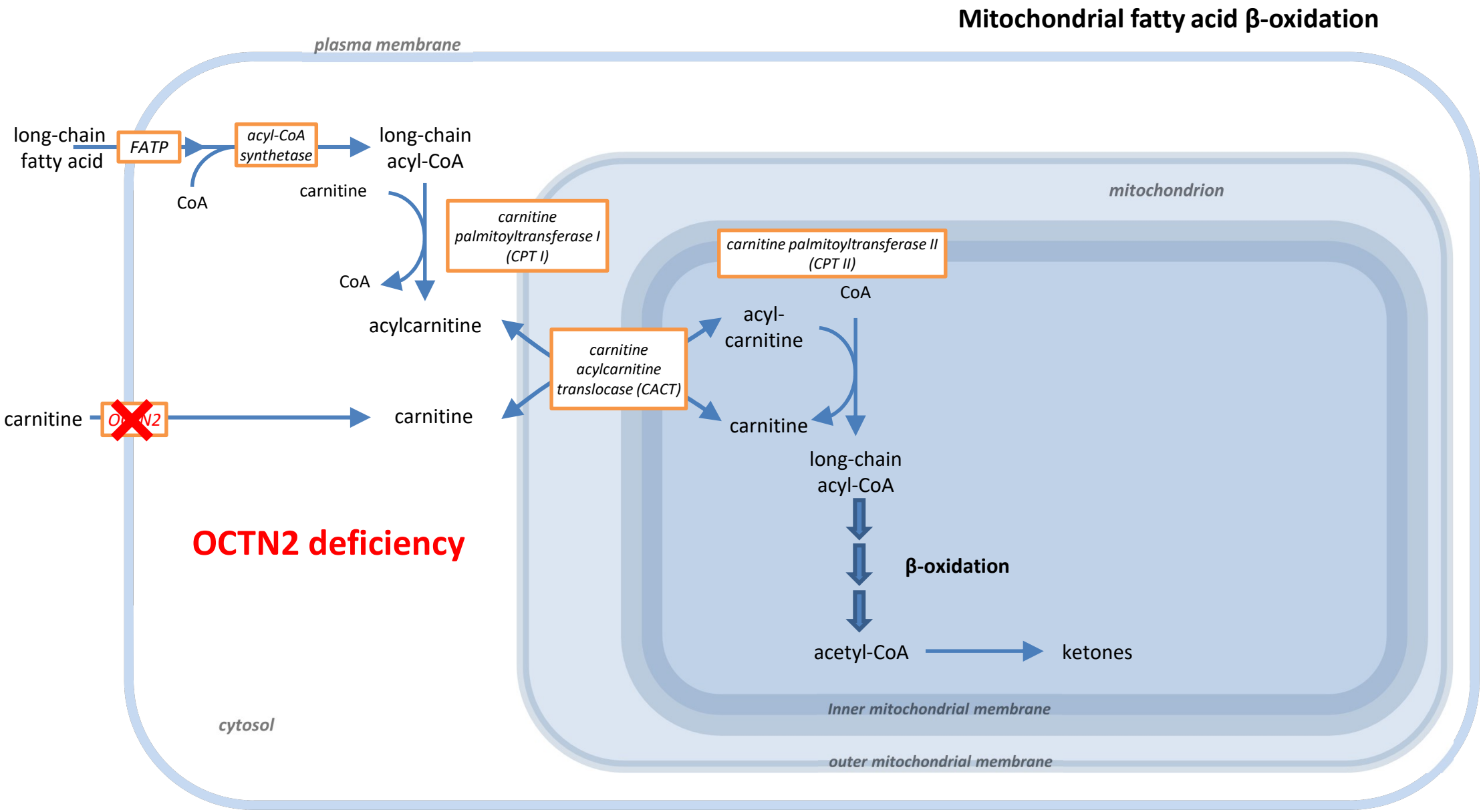
- All acylcarnitines undetectable or well below the reference range
- Calculation of fractional excretion of carnitine

$$\text{FEcarn} = \frac{(\text{urine carnitine} \times \text{plasma creatinine})}{(\text{plasma carnitine} \times \text{urine creatinine})} \times 100$$

Fractional excretion of free carnitine 49% (normal < 2%)

What is the diagnosis?

Any further tests to confirm or exclude other causes?


Mitochondrial fatty acid β-oxidation
plasma membrane
long-chain fatty acid
FATP
acyl-CoA synthetase
CoA
long-chain acyl-CoA
carnitine
carnitine palmitoyltransferase I (CPT I)
CoA
acylcarnitine
carnitine
OCTN2
carnitine
carnitine acylcarnitine translocase (CACT)
mitochondrion
carnitine palmitoyltransferase II (CPT II)
CoA
acyl-carnitine
carnitine
long-chain acyl-CoA
β-oxidation
acetyl-CoA
ketones
OCTN2 deficiency
cytosol
Inner mitochondrial membrane
outer mitochondrial membrane

Diagnosis

- Urine amino acids show no evidence of impaired renal tubular function
- Results consistent with primary carnitine deficiency

  Defect in organic cation transporter 2 (OCTN2)
- Genetic testing

  Homozygous variant of uncertain significance

  SLC22A5 c.503G>C, p.(Gly168Ala)

Is the diagnosis confirmed?

Is there sufficient evidence to upgrade variant?

Are there further investigations to do?

Family studies

- Parents both heterozygous for the variant
- Three siblings, all apparently asymptomatic

Age	Plasma free carnitine (ref range 23 – 52)	FEcarn (ref range < 2%)	Genetics
13 years	0.8 µmol/L	78%	Homozygous *SLC22A5*
8 years	22.5 µmol/L	Not done	Heterozygous
6 years	28 µmol/L	Not done	Heterozygous

- Eldest sibling reviewed
  - Low weight, poor appetite, tired
  - Echocardiogram – left ventricular cardiomyopathy

Is there evidence to upgrade the VUS to pathogenic?

Follow-up

- Patient and elder sister followed up by cardiology and metabolic services
- On carnitine 30% oral solution 900 mg four times daily
    - Uncertain compliance
    - Challenges of taking carnitine (oral solution or tablet?)
    - Plasma carnitine monitored
- VUS has been upgraded to likely pathogenic according to Association for Clinical Genomic Science guideline

https://www.acgs.uk.com/quality/best-practice-guidelines/#VariantGuidelines

Take home messages

- Defect in carnitine transporter OCTN2 causes primary carnitine deficiency and may result in cardiomyopathy
- Analysis of paired urine and plasma can be used to calculate fractional excretion of carnitine
- Patients may be asymptomatic or have mild, non-specific symptoms
- Family studies are important to identify cases
- Biochemistry results can help with classification of genetic variants