Cambridge University Hospitals NHS Foundation Trust

A tale of two sisters

Nick Flynn

Addenbrooke's Hospital, Cambridge

A tale of two sisters

Presentation and family history

- 2 year old girl
- Seen by community paediatrician due to global developmental delay

- Healthy unrelated parents, uneventful pregnancy, born in good condition
- Older sister (15) diagnosed with autism spectrum disorder
- Father has first cousin with learning disability, and another male cousin with son with learning disability

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Development

- Smiled within 2 months
- Sat: 6-8 months
- Commando crawled: 9-10 months
- Walked: 15-16 months
- First words: 2 years

- Very clumsy and falls over often

Other problems

- **Hearing:** passed newborn screen
- **Vision:** squint detected at 1 year, prescribed glasses at 15 months, myopia
- **Feeding:** Easily chokes, very fussy eater, drinks from a bottle
- **Challenging behaviour:** biting and hair pulling, sometimes self-harms by banging feet on wooden floor
- **Seizures:** several blank episodes, including jerking of limbs and eyes

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Examination

- **Head circumference:** 25-50th centile
- **Height:** 2.5th centile
- **Weight:** 27th centile
- Esotropia in left eye, no nystagmus
- Normal gait
- Non-dysmorphic

Investigations

- **Plasma amino acids**: normal
- **Urine glycosaminoglycans**: 23 mg/mmol (0-26)

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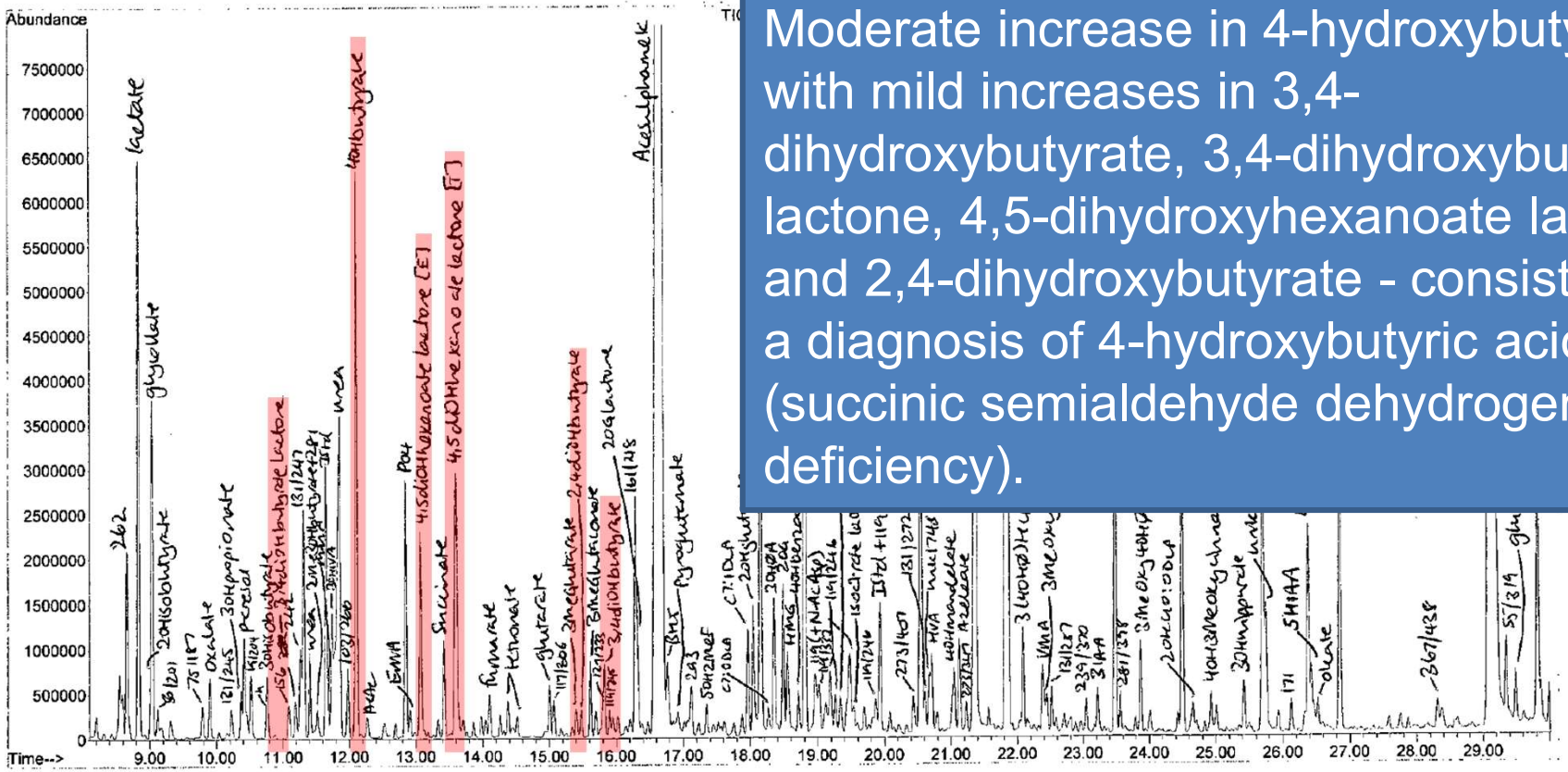
Urine organic acids



Moderate increase in 4-hydroxybutyrate with mild increases in 3,4-dihydroxybutyrate, 3,4-dihydroxybutyrate lactone, 4,5-dihydroxyhexanoate lactone and 2,4-dihydroxybutyrate - consistent with a diagnosis of 4-hydroxybutyric aciduria (succinic semialdehyde dehydrogenase deficiency).

Genetics and follow up

- Homozygous likely pathogenic variant detected in the ALDH5A1 gene: c.803G>A p.(Gly268Glu)

- Referred to paediatric metabolic service and local genetics department

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Older sister

- Older sister – aged 15
- Seen by genetics department due to sister's diagnosis and parental concerns

- Normal pregnancy and delivery
- Normal speech development. Stutter developed in the last year

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History

- Autism diagnosed aged 12 after parents concerns from pre-school age – not socializing, poor eye contact, poor peer interactions
- Attends mainstream school, some extra support for autism
- No seizures

Investigations

Genetics

- Homozygous likely pathogenic variant in ALDH5A1 detected: c.803G>A; p.(Gly268Glu)

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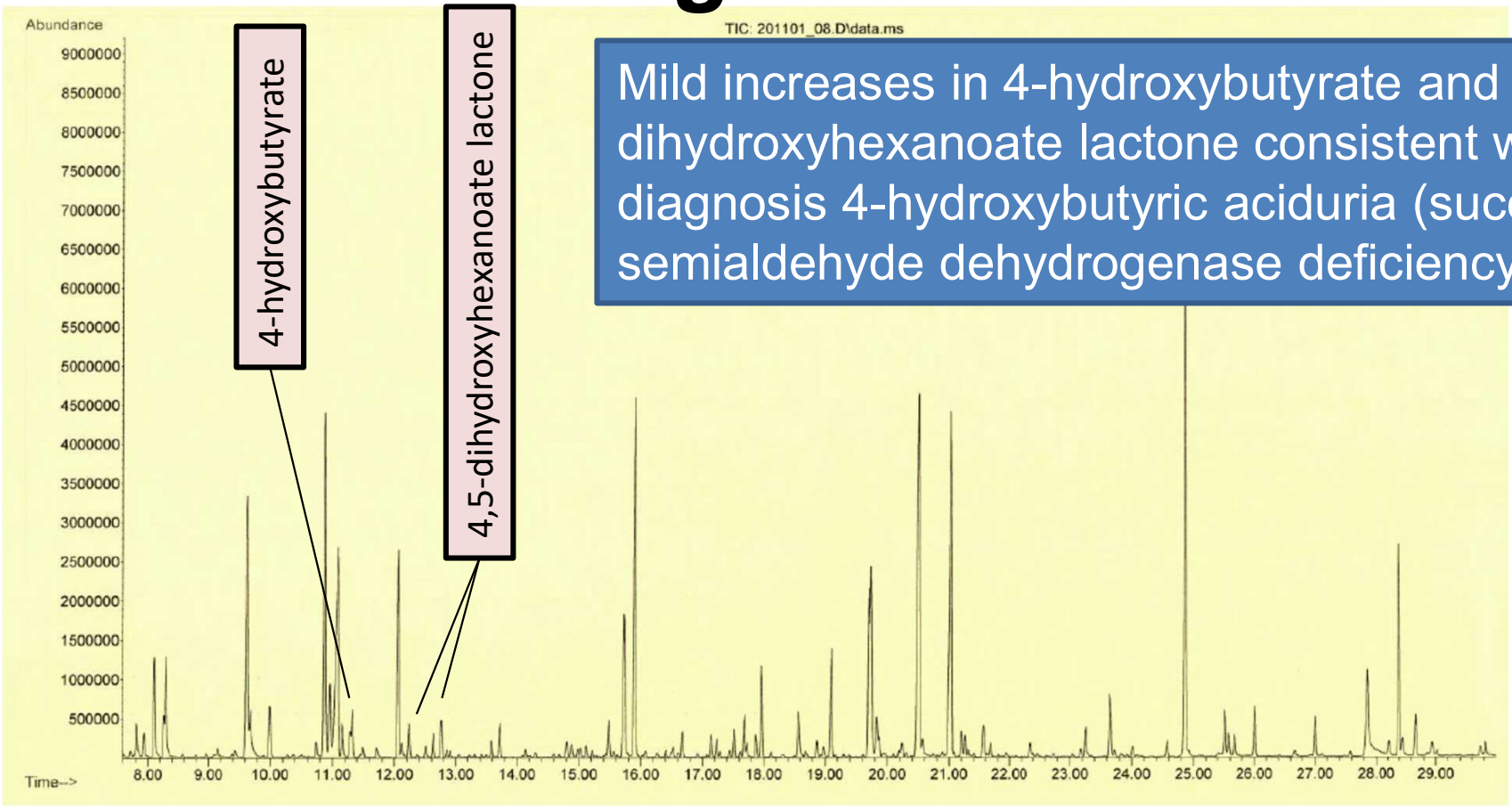
Urine organic acids



Mild increases in 4-hydroxybutyrate and 4,5-dihydroxyhexanoate lactone consistent with a diagnosis 4-hydroxybutyric aciduria (succinic semialdehyde dehydrogenase deficiency)

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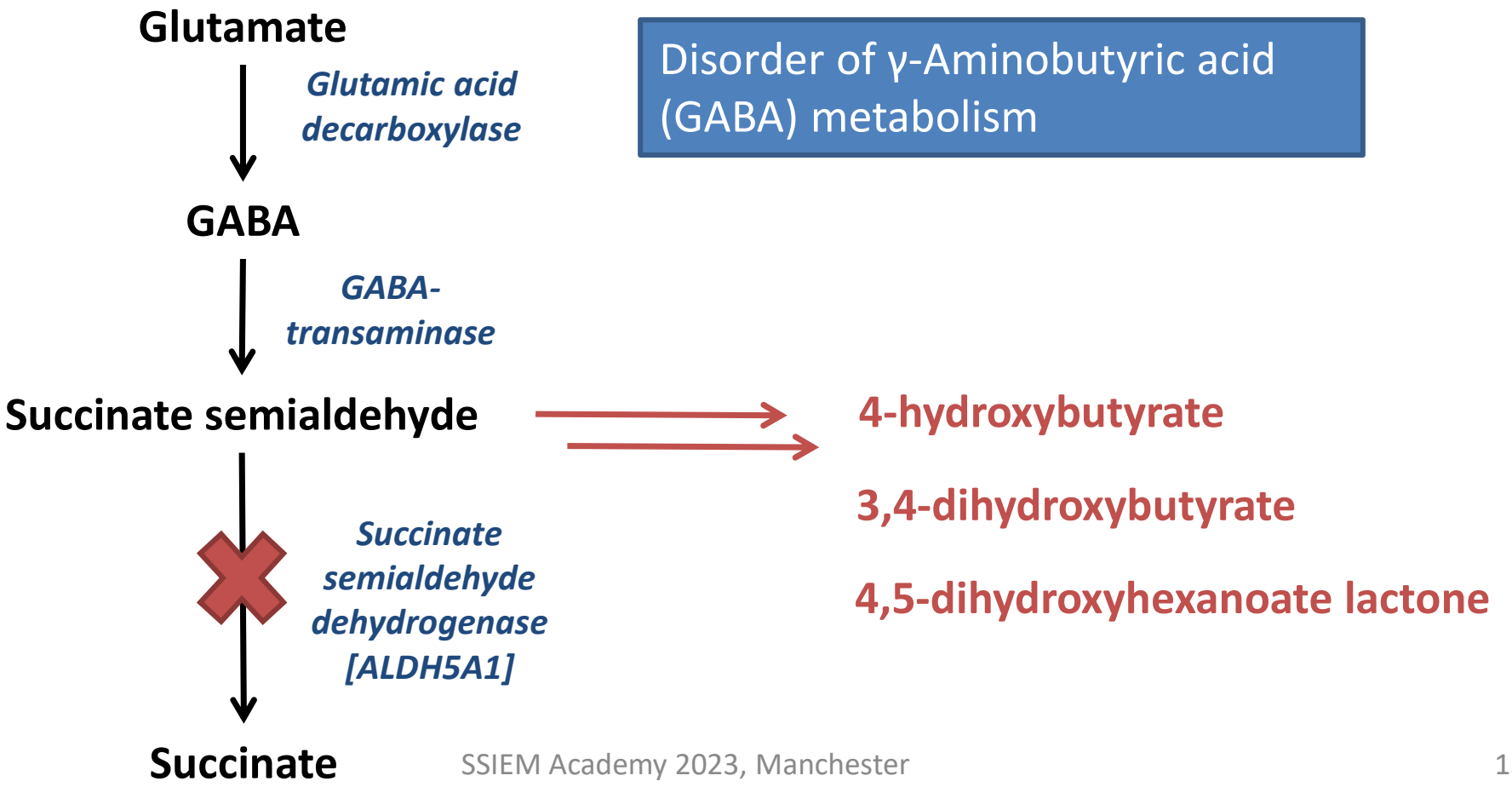
4-hydroxybutyric aciduria



Disorder of γ-Aminobutyric acid (GABA) metabolism

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4-hydroxybutyric aciduria

- Developmental delay
- Hypotonia
- Ataxia
- Seizures
- Aggressive behaviour
- Autistic features
- Microcephaly or macrocephaly

Symptom onset: ≈ 1 year

Mean age at diagnosis: 6.6 years *(Adult diagnoses have been reported)*

Approximately 10% of affected individuals have more severe phenotype with a regressive course

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Autism and IEM

France (PloS ONE. 2011; 6(6): e21932)

- Retrospective metabolic screening of 274 children with nonsyndromic autism spectrum disorder
- Normal for all but 2 (1 x unspecific urine creatine excretion and 1x 3-methylglutaconic aciduria)

Greece (Front Hum Neurosci. 2013 Dec 24;7:858)

- Screened 187 children with autism spectrum disorder
- 5 diagnoses: Lesch Nyhan syndrome (x2), SSADH deficiency (x2), Phenylketonuria (x1)

Conclusions

- 4-hydroxybutyric aciduria (SSADH deficiency) is a disorder of GABA metabolism
- Case of 4-hydroxybutyric aciduria in a child led to diagnosis in a mildly affected teenage sibling with autism spectrum disorder
- Autism spectrum disorder is common (1 in 100 worldwide) but may be the only manifestation of an IEM

Acknowledgements

- Dr Maharasingam
- Dr Soo-Mi Park

Questions?

HEPATIC ENCEPHALOPATHY WITH SEVERE HYPOKETOTIC HYPOGLYCAEMIA IN A NEONATE

Dr. med. Susanne Weiß
Department of General Paediatrics,
Neonatology and Paediatric Cardiology
University Children's Hospital Duesseldorf, Germany

Patient history (1)

- Female, first child
- parents not consanguineous, northern European origin
- Uncomplicated pregnancy
- Spontaneous birth at term,
- APGAR 9/10/10, art. pH 7,28, 3380 g, 54 cm
- Good postnatal adaptation
- Newborn screening was done in time
- Discharge from hospital after 48 h, “fully breastfeeding”

Patient history (2)

- At Home: Drowsiness, fasting 8 hours
- Evening: first seizures?, “foaming at the mouth”,
- loss of consciousness, emergency call

- GCS 4, SO_2 80%, HR: 80-120/Min., Resp. rate <15/Min., blood sugar “low”
- Difficulties to manage i.v./i.o. access, transport to ICU
- ICU: Asystolia, SO_2 - , 3 Min. of CPR, ROSC,
  -> volume, dextrose, catecholamines i.o.

Laboratory analysis

BGA	pH 7,28, pCO_2 54 mmHg, lactate 8 mmol/l, Gluc. 1mg/dl (0,05mmol/l) BE -4,3 mmol/l Bicarbonate 21 mmol/l Electrolytes ↔
Ketones (U)	neg.
BC	↔
CRP	19,2 mg/l (< 5 mg/l)
IL-6	51 ng/l (< 8 ng/l)

Missing: C-peptide, FFA, GH, uric acid, triglycerides, CK

GOT, GPT, У-GT, Bili	(↑)
Ammonia	492 µmol/l (< 180 µmol/l)
Coagulation	(↓)
Albumin	↔
Creatinine	0,92 mg/dl (< 0,9 mg/dl)
Urea	↑
Insulin	↓
Cortisol	↑↑
Albumin	↔

Clinical course (1)

- i.v. dextrose/electrolytes, no protein, no lipids
- pharmacological treatment of hyperammonaemia (Sodiumbenzoate, Arginine)

- Supportive intensive care
- Intermittent seizures: levetiracetam

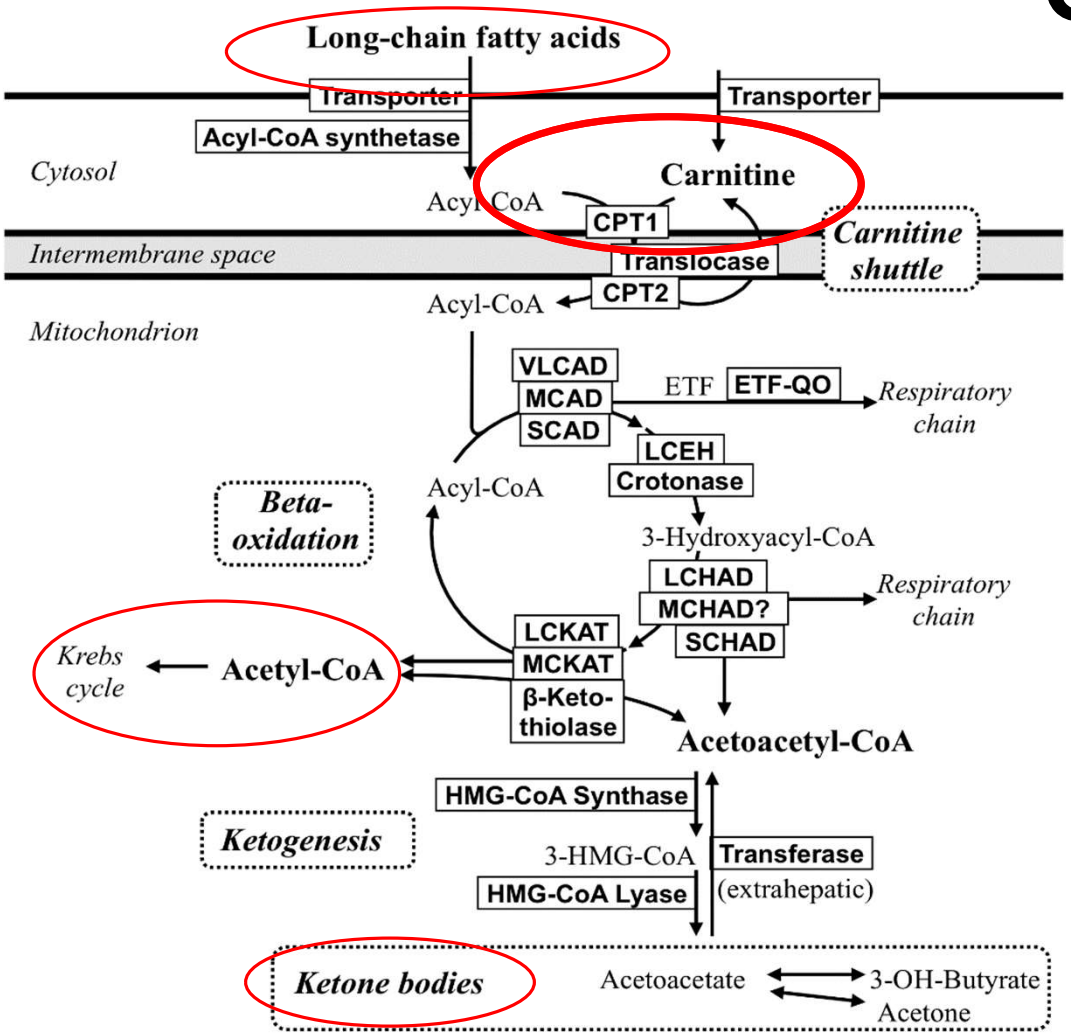
- Echocardiography: (↓) contractility, no structural abnormalities
- Head and abdominal sonography: no pathologies

Metabolic results

- Severe Hypoketotic hypoglycaemia
- Hyperammonaemia, elevated liver enzymes, impaired coagulation

- Acylcarnitine profile:
  - ↑ free carnitine (C0), 119 µmol/l (6-78 µmol/l)
  - ↓ C16, C18, C18:1
  - ↑ ratio C0/(C16+C18), 850 (2,8-47,7)
- Aminoacid profile: (↑) tyrosine, no succinylacetone
- Organic acids (U): (↑) dicarboxylic acids, pyruvate

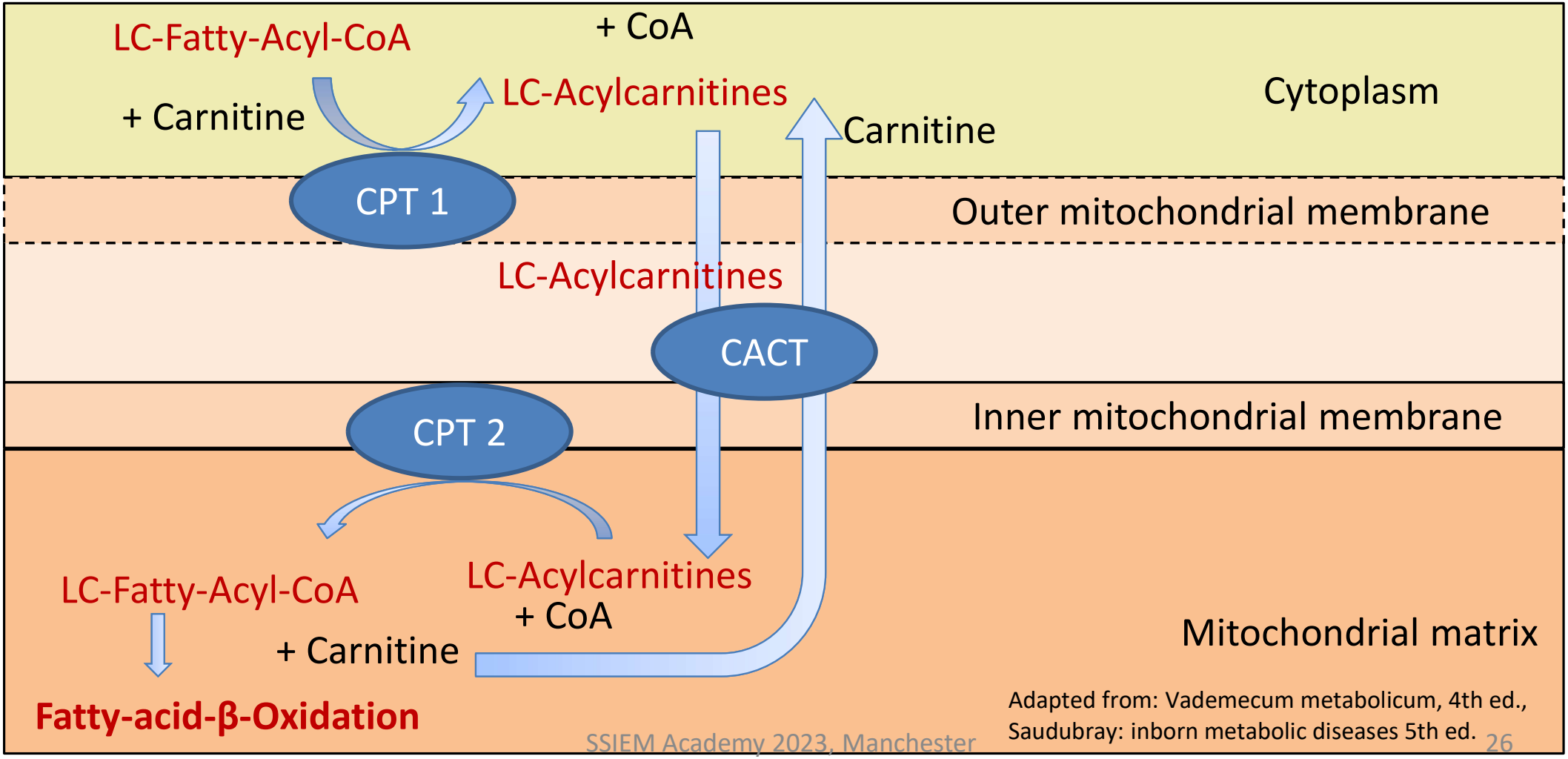
CPT 1



- Carnitine-Palmitoyltransferase 1 (CPT 1)
  - A: liver/kidney, leucocytes, fibroblasts, (others ?)
  - B: muscle
  - C: brain
- Key role in FAO

Vademecum metabolicum, 4th ed.

Carnitine-Shuttle



Adapted from: Vademecum metabolicum, 4th ed., Saudubray: inborn metabolic diseases 5th ed.

CPT 1A Deficiency

- First metabolic decompensation: mostly early childhood
- Triggers: Infections and/or prolonged fasting
- **Hepatic dysfunction, hypoketotic hypoglycaemia, hyperammonaemia, encephalopathy (seizures, sudden death in infancy),** renal tubular acidosis, (asymptomatic cases!)

- Heterozygous women with fetus affected by biallelic variant: acute fatty liver of pregnancy

- (Rare: cardiac problems?, CK-Elevation?)
- development prognosis normal (?), no long term liver damage (?)

Diagnostics

- Inheritance: autosomal recessive
- Prevalence: 1/500.000-1/1.000.000 (non inuit), ↑ Canada (Alaska), Greenland (first nations, inuit) (1,3/1000),

- **Single gene (CPT1A) analysis**
- Multigene panel (unclear phenotypes)
- Exome analysis

- **CPT 1A enzyme activity on skin fibroblasts**
- Residual activity mostly 1%-5% (symptomatic cases)

Clinical course (2)

- Metabolic stabilization -> enteral feeding (breast milk and MCT-rich formula, e.g. Lipistart®)
- cMRT: signs of (mild) hypoxic ischaemic encephalopathy
- EEG slightly abnormal

- **Confirmation of diagnosis:**
  - Genetics:
    - CPT1A: Exon 15: c. 1792C>T, p.R598* (**het.**)
      (new mutation, class 5 by in silico analysis)
  - Enzymatics:
    - CPT1 activity ↓ 0,05 nmol/(min.mg protein) (Ref. 1,16-2,2)

Clinical course (3)

- 3 years later: acceptable development, rarely seizures (anticonvulsive therapy)
- dietary therapy (avoid fasting, carbohydrate rich diet, reduced fat, additional MCT fat)
- rare decompensations during common infections
- Family:
  - Genetic counselling,
  - intensive pregnancy monitoring (Risk: Fatty liver of pregnancy)

Conclusions

- Signs of encephalopathy: check glucose and ammonia levels
- Emergency treatment as soon as possible
- Check potential differential diagnoses
- Don´t forget to do key metabolic screening tests
- Disorders of fatty acid metabolism can be a cause for hypoketotic hypoglycaemia and hepatic failure with encephalopathy

Thank you for your attention!

Questions?

FATAL HYPOGLYCEMIA AND HYPERLACTATEMIA IN NEONATAL PERIOD

M Gilleron
Institute of Biochemistry
University Hospital of Lille

Eli

- Single spontaneous pregnancy, G2P2
- Healthy 11-years-old brother
- Birth induced at 39 weeks and 1 day /heart rhythm abnormalities
- Normal birth parameters
- 24H :
  - ✓ Pale, hypotonic and whining
  - ✓ Recurrent hypoglycemia and hyperlactatemia
- Infusion of G10% + calcium gluconate

Hospitalisation in neonatal ICU

- ✓ Moderate jaundice
- ✓ Respiratory distress
- ✓ Axial hypotonia with peripheral hypertonia
- ✓ Complicated gastroesophageal reflux
- ✓ Hepatosplenomegaly
- ✓ Suspicion of early neonatal bacterial infection (bacteriology negative)
- ✓ Toxoplasmosis test negative
- ✓ Viral tests negative

Biological analysis

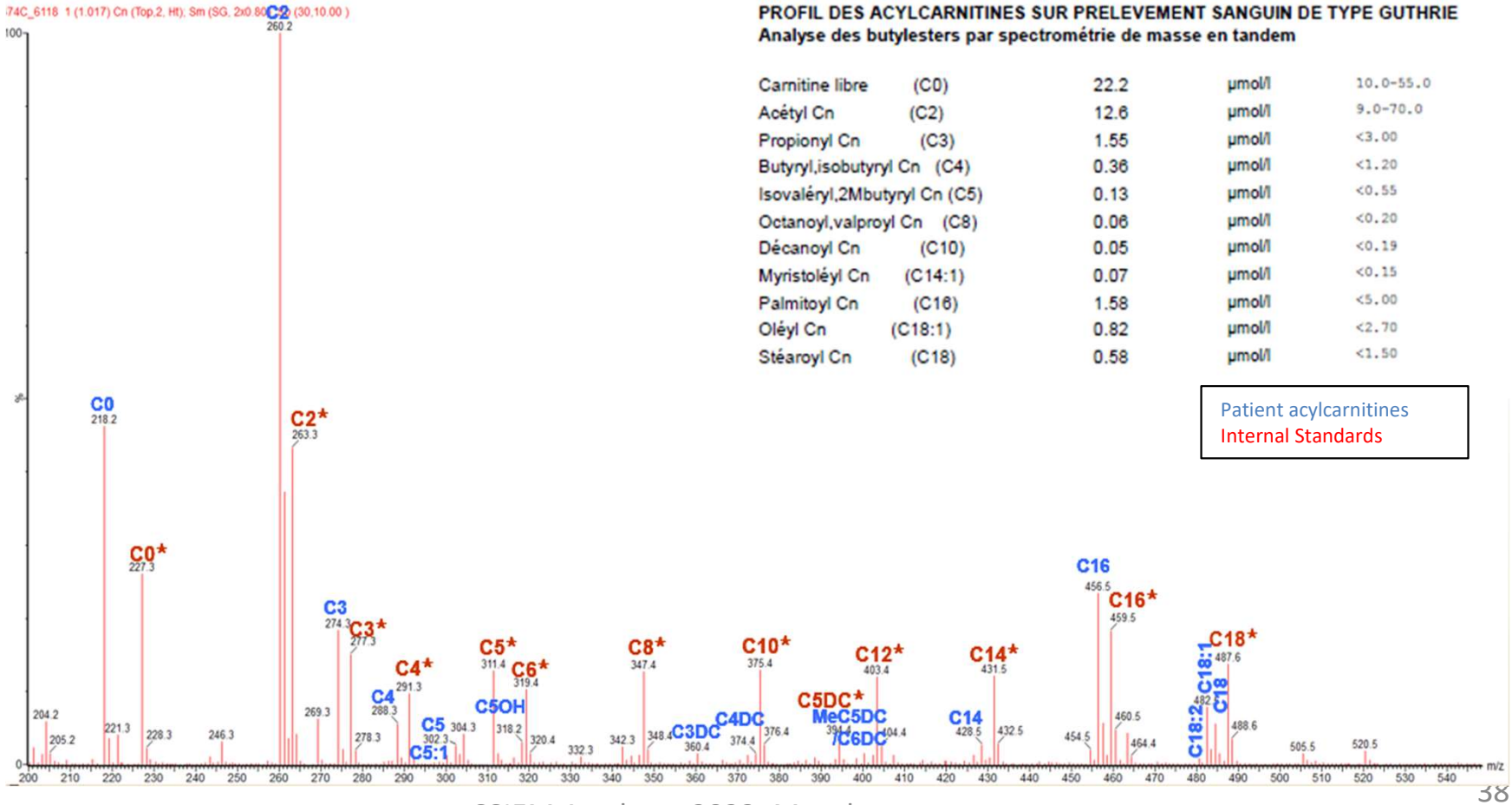
	Normal values	D1 arrival in ICU	D2	D3	D6	D23	D35	D72	D93	D96	D97	D99	D106
Glycemia	0,3-0,75 g/L	**0,25** (0,59 in ICU)	-	0,88	0,65	0,86	-	0,72	0,53	-	0,73	0,93	1,3
Lactatemia	0,50-2,86 mmol/L	**15,93**	**6,97**	2,94	2,78	**3,72**	-	-	-	**3,03**	-	**7,49**	1,1
NH3	<100 µmol/L	92	-	-	-	-	-	-	-	-	-	-	48
TGO	10-50 UI/L	**245**	**277**	-	-	27	34	-	-	-	-	-	65
TGP	10-50 UI/L	**223**	**326**	-	-	18	23	30	-	-	-	-	60
LDH	225-600 U/L	**2180**	**2593**	**1640**	-	-	-	-	-	-	-	-	-
GGT	5-50 U/L	**136**	**173**	-	-	**166**	61	17	-	-	-	-	-
CPK	60-600 U/L	**4170**	**3364**	**1349**	-	-	-	-	-	-	-	-	317
Total bilirubine	<90 mg/L	-	**101**	**138**	-	6	-	6	-	-	-	-	-

Biological analysis (2)

- No other specific abnormalities on the basic biochemical check-up
- Normal whole blood count and haemostasis
- Endocrinological workup D1 :
  - ✓ insulinemia 9.5 mU/l (2,7-10,4)
  - ✓ blood C-peptide 2.59 ng/ml (0,95-2,30)
- Routine biological exploration of CSF without particularity except for an hyperlactatorachy

Metabolic screening (1)

Hypothesis = Beta-oxidation disorder ➔ Acylcarnitine profile



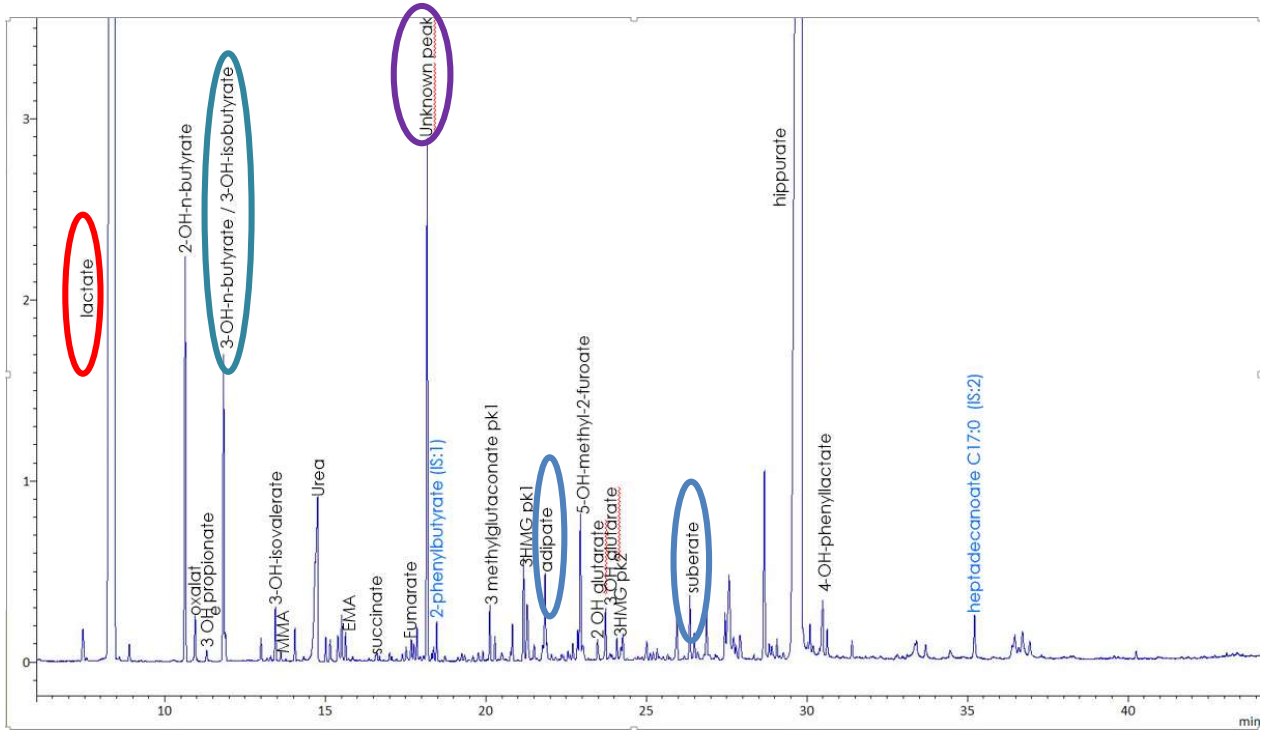
PROFIL DES ACYLCARNITINES SUR PRELEVEMENT SANGUIN DE TYPE GUTHRIE
Analyse des butylesters par spectrométrie de masse en tandem

Carnitine libre	(C0)	22.2	µmol/l	10.0-55.0
Acétyl Cn	(C2)	12.6	µmol/l	9.0-70.0
Propionyl Cn	(C3)	1.55	µmol/l	<3.00
Butyryl,isobutyryl Cn	(C4)	0.36	µmol/l	<1.20
Isovaléryl,2Mbutyryl Cn	(C5)	0.13	µmol/l	<0.55
Octanoyl,valproyl Cn	(C8)	0.06	µmol/l	<0.20
Décanoyl Cn	(C10)	0.05	µmol/l	<0.19
Myristoléyl Cn	(C14:1)	0.07	µmol/l	<0.15
Palmitoyl Cn	(C16)	1.58	µmol/l	<5.00
Oléyl Cn	(C18:1)	0.82	µmol/l	<2.70
Stéaroyl Cn	(C18)	0.58	µmol/l	<1.50

Patient acylcarnitines
Internal Standards

Metabolic screening (2) : urinary organic acids chromatography

Acide lactique		Très augmenté		
Ac glycolique		88.8	mmol/mol créa	inf à 150
Ac 3 OH propionique		Présence		
Ac 3 OH n butyrique	⇑	**95.1**	mmol/mol créa	inf à 50
Ac 3 OH isovalérique		40.9	mmol/mol créa	inf à 50
Ac méthylmalonique		2.6	mmol/mol créa	inf à 5
Ac éthyl malonique		7.5	mmol/mol créa	inf à 10
Ac succinique		1.4	mmol/mol créa	inf à 76
Ac fumarique		4.6	mmol/mol créa	inf à 14
Ac malique		Présence		
Ac glutarique		3.0	mmol/mol créa	inf à 12
Ac adipique	⇑	**19.9**	mmol/mol créa	inf à 12
Ac subérique	↑	**13.9**	mmol/mol créa	inf à 6
Ac sébacique		Absence		
Ac 4 OH phényllactique	⇑	**6.8**	mmol/mol créa	inf à 2
Ac 2 OH glutarique		11.2	mmol/mol créa	inf à 20
Métabolites du paracétamol		Présence		
Rapport 3OHBUT/Adipique		4.8		



Metabolic screening (3)

- Redox balance

	Lactate	Pyruvate	L/P
Blood	5,62	0,48	11,8
CSF	3,54	0,25	14

Hyperlactacidemia with normal L/P ratio ➔ main hypothesis = pyruvate dehydrogenase deficiency ➔ *PDHA1* gene sequencing

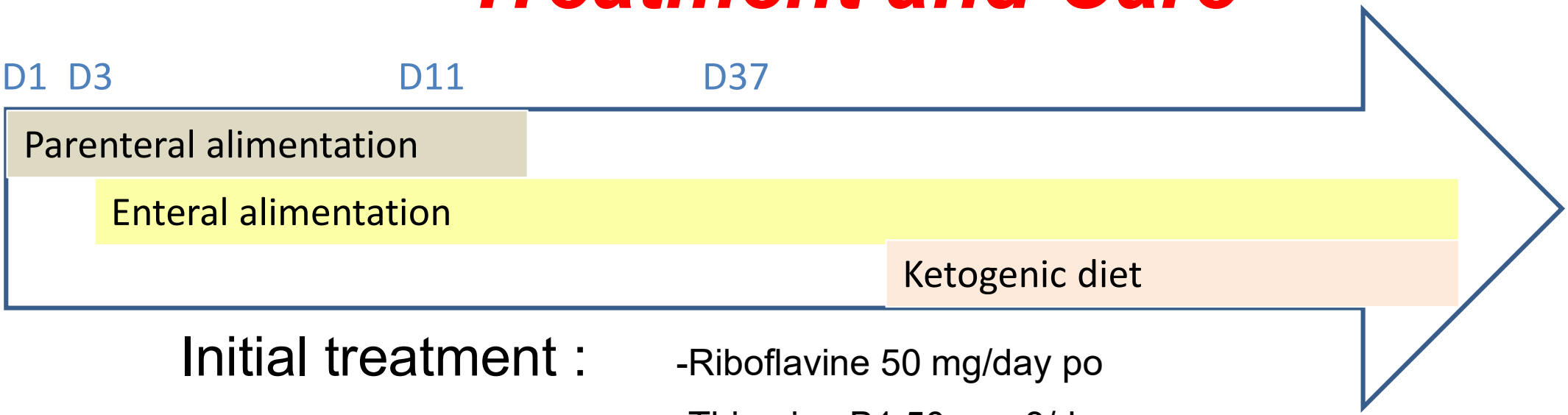
Metabolic screening (4)

- Chromatography of blood and urine amino acids without specific variations
- CSF amino acid chromatography not in favour of hyperglycinemia without ketosis
- Normal peroxisomal and lysosomal balances
- Normal creatine kinase isoenzymes

Additionnal tests

- **D1 Cardiac ultrasound :** Moderate left ventricular hypertrophy
- **D2**
  - ✓ **EEG :** disturbed tracing on the right, discontinuous and periodic with several pauci symptomatic discharges ➔**Gardenal**
  - ✓ **Transfontaneous ultrasound** : thalamo-striatal and periventricular calcification, hyperechogenic parenchyma
- **D16**
  - ✓ **MRI :** Diffuse T2 hypersignal of the supratentorial white matter associated with subependymal cystic formations ➔ underlying leukomalacia?
  - ✓ **Electroneuromyography** : no peripheral demyelination

Treatment and Care



Initial treatment :

-Riboflavine 50 mg/day po

-Thiamine B1 50mg x3/day po

-Biotine 5mg/day po

-Decorenone 10mg/day po

-Ezomeprazole 3,5mg/day po

-Vitamin D 10000 UI/mL 3 drops/day po

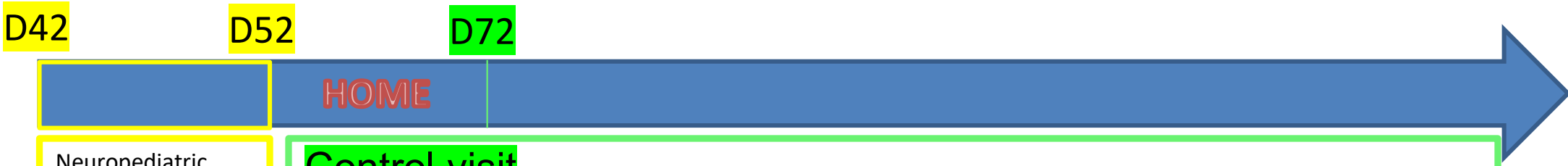
-Polysilane gel 1 nut before each feeding.

Evolution and Follow up

D42 D52

Neuropediatric Department :

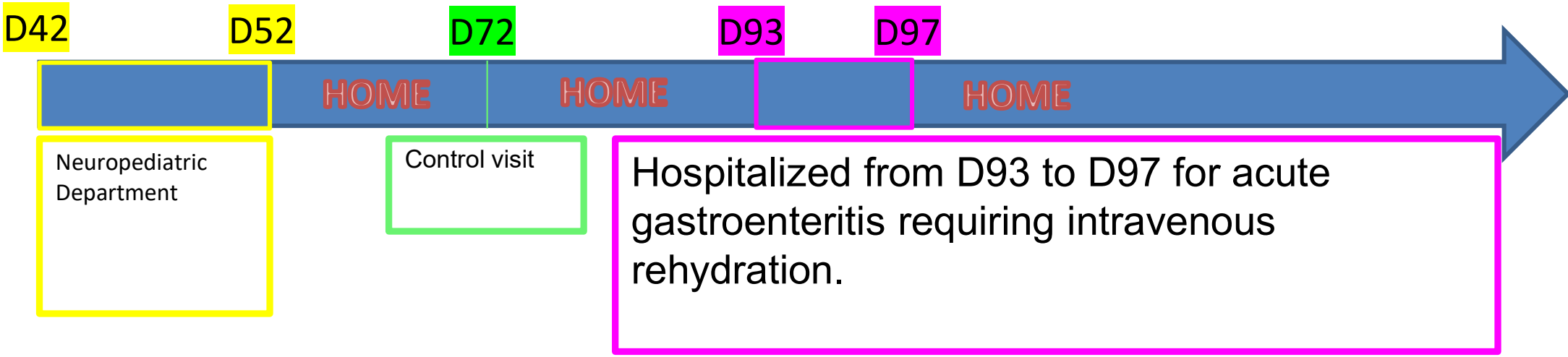
- Neurologically:
  - ✓ Poor motor skills
  - ✓ no eye contact with intermittent eye wandering
  - ✓ central malaise with apneas without bradycardia and cyanosis
- Digestive level:
  - ✓ Poor oral intake
  - ✓ KETOCAL feeding well tolerated without transit disorders

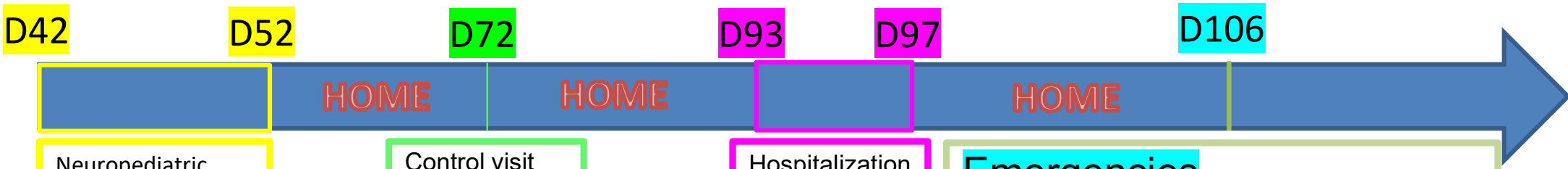


Neuropediatric Department

Control visit

- Difficult contact with almost no eye tracking.
- Reactivity to loud noises
- Kinesitherapy
- Re-evaluation of the ketogenic diet : no abdominal pain, rare vomiting.
- Biological check-up normal
- Weight gain limited :10 to 15g/d
- Oto-neurological consultation: Absence of response to auditory evoked potentials and absence of response to induced acoustic otoemissions


D42
D52
D72
D93
D97
HOME
HOME
HOME
Neuropediatric Department
Control visit
Hospitalized from D93 to D97 for acute gastroenteritis requiring intravenous rehydration.



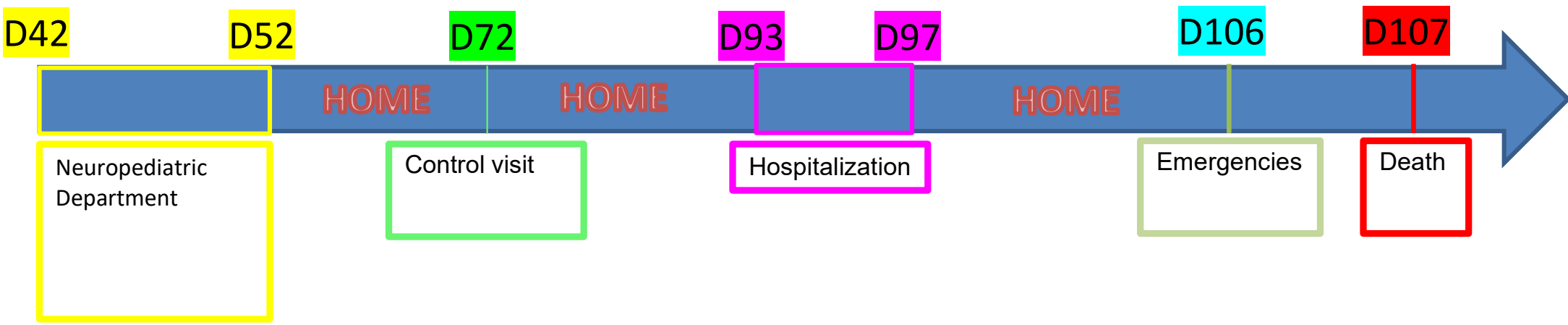
Neuropediatric Department

Control visit

Hospitalization

Emergencies

- Unusual behaviour and hypotonia at home
- Arrival in hospital :
  - ✓ Drowsy, unresponsive to stimuli
  - ✓ No eye contact
  - ✓ Normal cardiopulmonary auscultation
  - ✓ pH 6,96


D42
D52
D72
D93
D97
D106
D107
HOME
HOME
HOME
Neuropediatric Department
Control visit
Hospitalization
Emergencies
Death

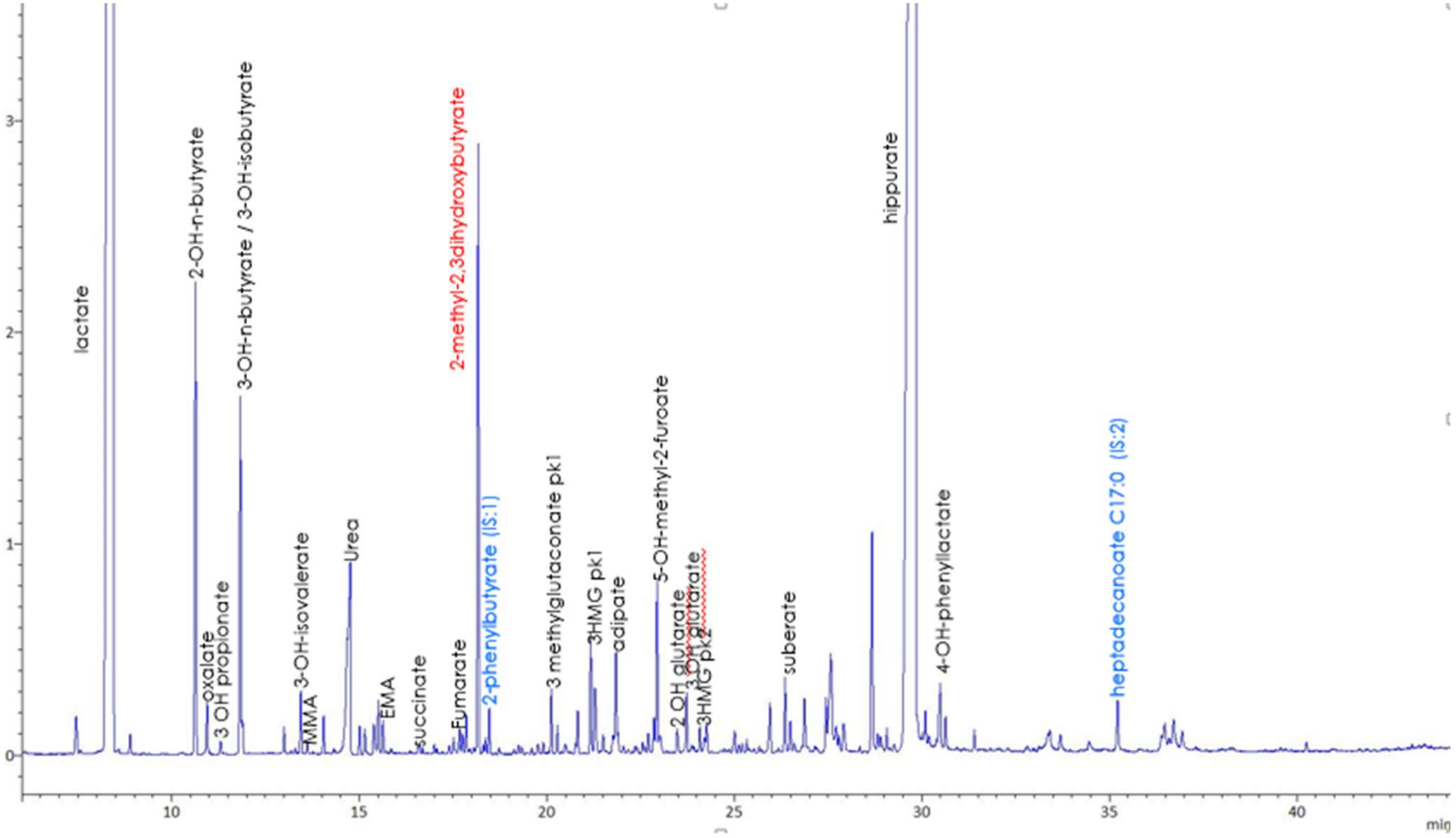
Case conclusion

PDHA1 gene sequencing negative

Diagnosis?

- Sequencing of a mitochondrial cytopathies/PDH genes panel ➔ two variants in the *ECHS1* gene :
  - ✓ c.2T>G already reported in the literature
  - ✓ c.108C>G of unknown pathogenicity.
- Collapsed crotonase activity measured on cultured skin fibroblast

Urinary organic acids chromatography



ECHS1 : Short-Chain Enoyl-CoA Hydratase

Multifonctionnal mitochondrial matrix enzyme involved

- ✓ in the oxidation of fatty acids
- ✓ in the metabolism of essential amino acids such as Valine

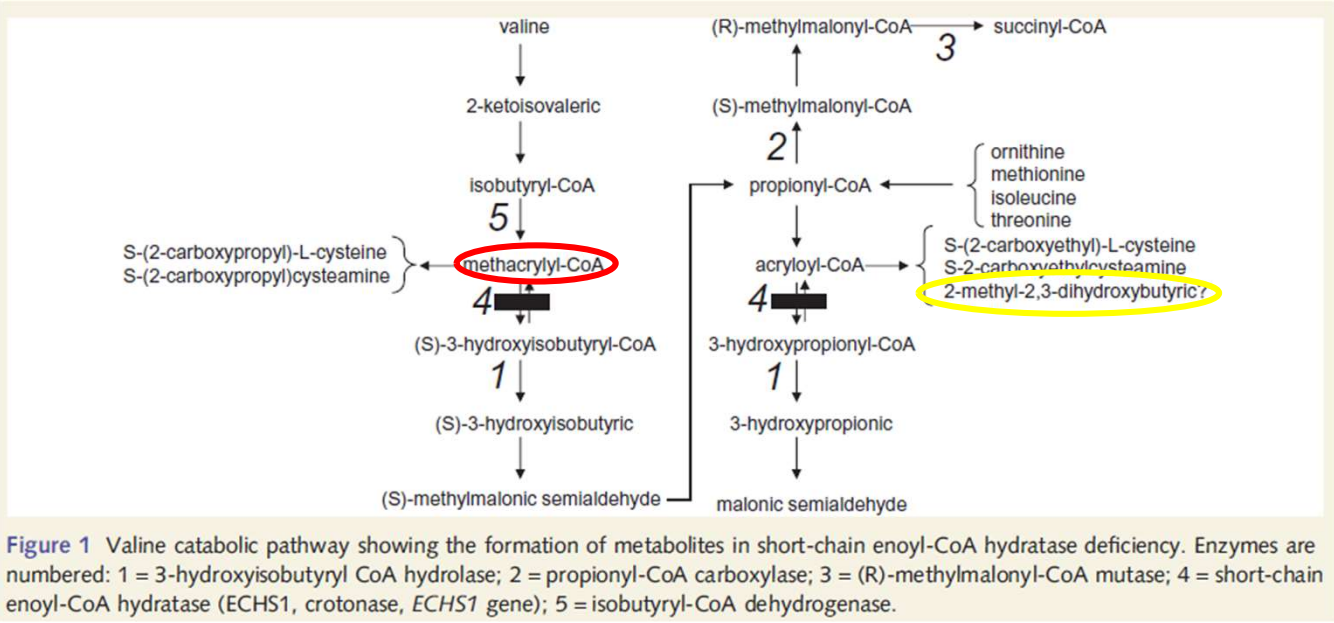


Figure 1 Valine catabolic pathway showing the formation of metabolites in short-chain enoyl-CoA hydratase deficiency. Enzymes are numbered: 1 = 3-hydroxyisobutyryl CoA hydrolase; 2 = propionyl-CoA carboxylase; 3 = (R)-methylmalonyl-CoA mutase; 4 = short-chain enoyl-CoA hydratase (ECHS1, crotonase, *ECHS1* gene); 5 = isobutyryl-CoA dehydrogenase.

Brain 2014: 137; 2903–2908 H. Peters et al.

Acknowledgements

- *Dr Dries Dobbelaere, Pediatrician*

Reference centre for Inherited Metabolic Diseases

- *Dr Elise Lebigot, Biologist*

Biochemistry department, Bicêtre hospital

Questions?

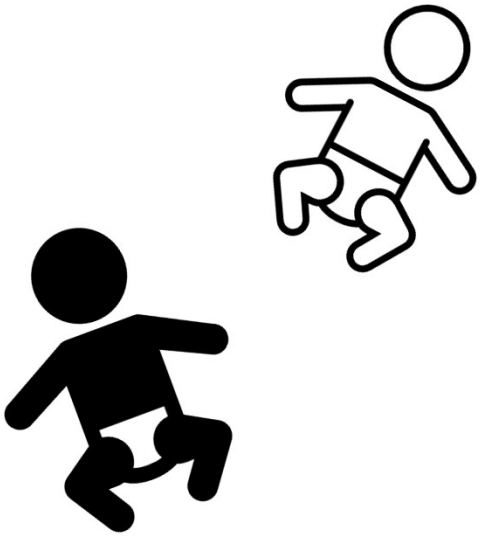
Same disease and different clinical presentations

Thaís Martins de Oliveira, MD

Hospital Materno Infantil Santa Catarina - HMISC - Criciúma – SC - Brazil

Same disease and different clinical presentations

Patient presentation

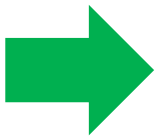


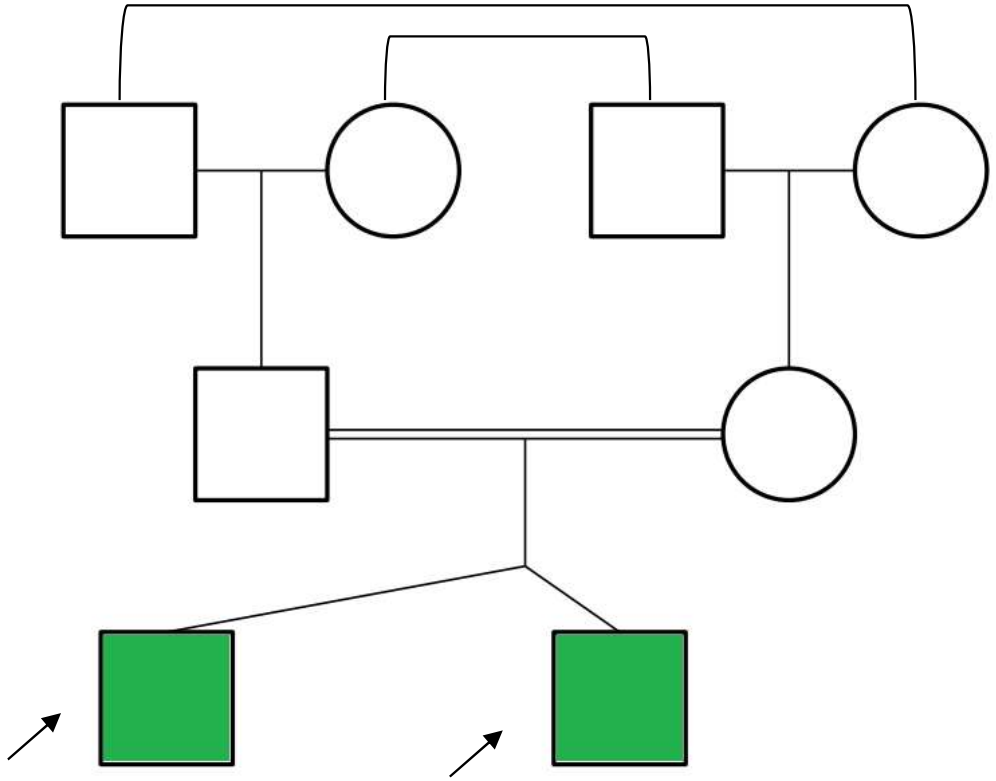
Dizygotic male twins
Gestational age 36+3: C-section due to symptoms of preeclampsia
First twin: birth weight of 2.8kg, length of 46,5 cm, head circumference of 33cm, Apgar 8/9.
Second twin: birth weight of 3.44kg, length of 46 cm, head circumference of 35cm, Apgar 6/9.

- Meconial amniotic fluid
- Hypotonic, cyanotic, HR under 100
- Good recovery after reanimation

Same disease and different clinical presentations

Family history





First Twin

Clinical presentation

After birth

- Hypoglycaemia
- Difficulty breastfeeding
- Discharged with infantile formula prescribed if necessary
- He had lost 9% of body weight

3 days of life

- Somnolence, food refusal, hypoactive, perioral cyanosis and dehydration
- Hypoglycaemia
- After an adequate offering of diet, the patient started to improve
- Blood culture – negative
- Discharged after 7 days of antibiotics

Second Twin

Clinical presentation

6 days of life

- He had lost approximately 20% of body weight
- Moans, grunting, retracting, hypothermia and dehydration

At the emergency room

- Non-invasive ventilation support
- Hypoglycaemia (glucose of 15)
- His respiratory status worsened and he was intubated
- Antibiotics
- Seizure

Second Twin

Clinical presentation

ABG: pH 6,89 pCO2 22,9 HCO3 4,4 BE -27,8

Intensive Care Unit

- Arterial blood gas = marked metabolic acidosis
- Cerebrospinal Fluid (CSF): increase of protein
- Bicarbonate replacement ➡ Dialysis?
- Dehydration ➡ Congenital adrenal Hyperplasia?
- Cranial sonography: brain swelling

Second Twin

Treatment

Consult called

- Inborn error of the metabolism group of small molecules
- Dialysis started on the same day
- The hospital didn't have the resources to do a metabolic test
- Diet with low protein intake

After 3 days of dialysis:
ABG: pH 7,41 pCO2 36 HCO3 22,5 BE -1,5

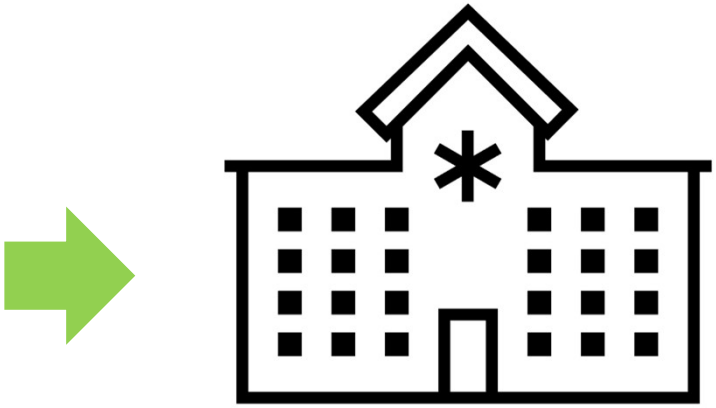
After 5 days, it was suspended and the patient extubated

Second Twin

Follow up

After 1 month and 20 days

- Discharged
- Low protein diet
- Referral to specialized centre
- Social problems



First Twin

Clinical presentation

30 days of life

Diarrhea with blood

6 months of life

Bronchiolitis without complications

7 months of life

Food introduction with good acceptancy

Two days of high fever (40ºC) due to vaccination

Tonic clonic seizure in the presence of fever at home

First Twin

Clinical presentation

Emergency room

- Dehydrated and lethargic
- Hypoglycaemia
- Respiratory arrest and HR < 100

- He was intubated, antibiotics were started in the first hour
- COVID19+
- Blood test: high lactate, very high transaminases, prolonged clotting time, and metabolic acidaemia

ABG: pH 7,08 pCO2 31,5
HCO3 9,2 BE -19,5
TGO 1031 (15-75 U/L)
TGP 716 (13-45 U/L)
Lactate 33,6 mg/dl (4,5-19,8 mg/dl)
RNI 2,8 (1-2)

First Twin

Treatment

Intensive Care Unit

- Dialysis
- Diet with low protein intake
- After 1 day of dialysis => Normal ABG
- After 5 days, dialysis suspended => extubated in the next day

First Twin

Treatment

D9 OF HOSPITALIZATION

Low protein diet
Hypercaloric:
0,5g/kg/day of protein
Maltodextrin
MTC

Hospital de Clínicas de Porto Alegre

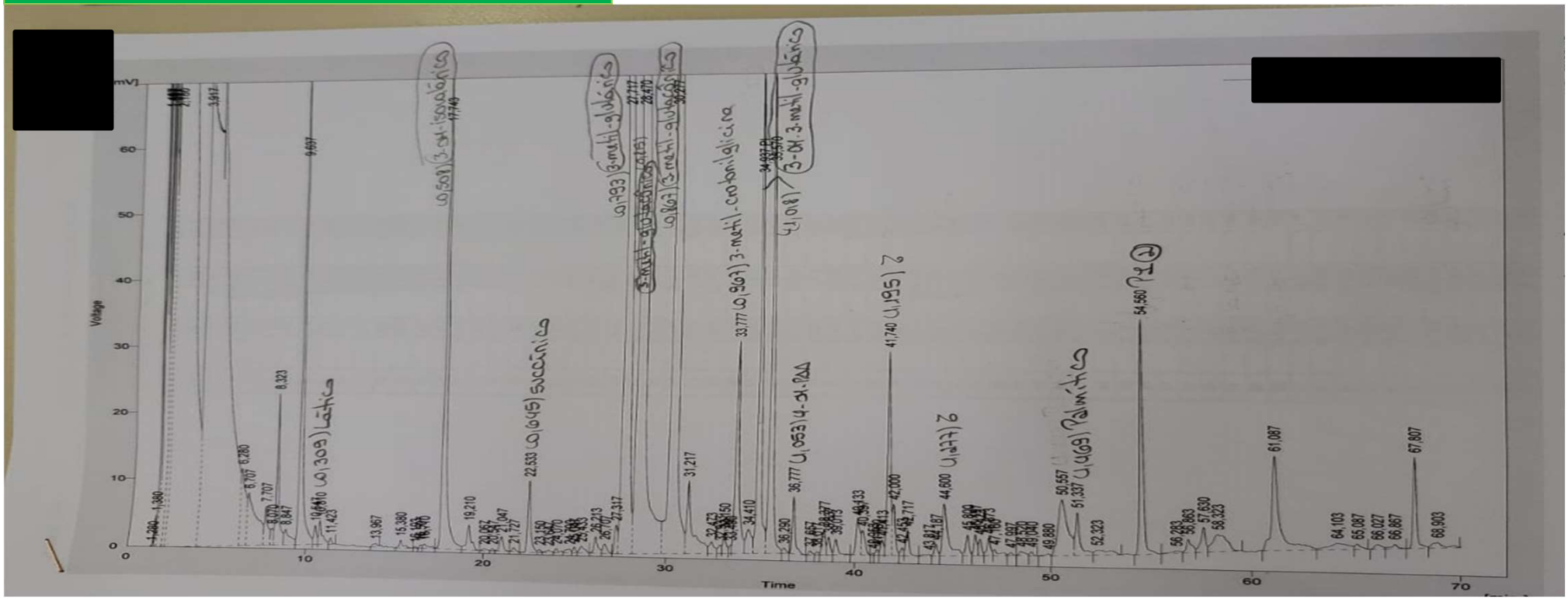
Amino acid Chromatography
Urinary organic acid profile

Donation:
- MSUD formula

First Twin

Metabolic Biochemistry

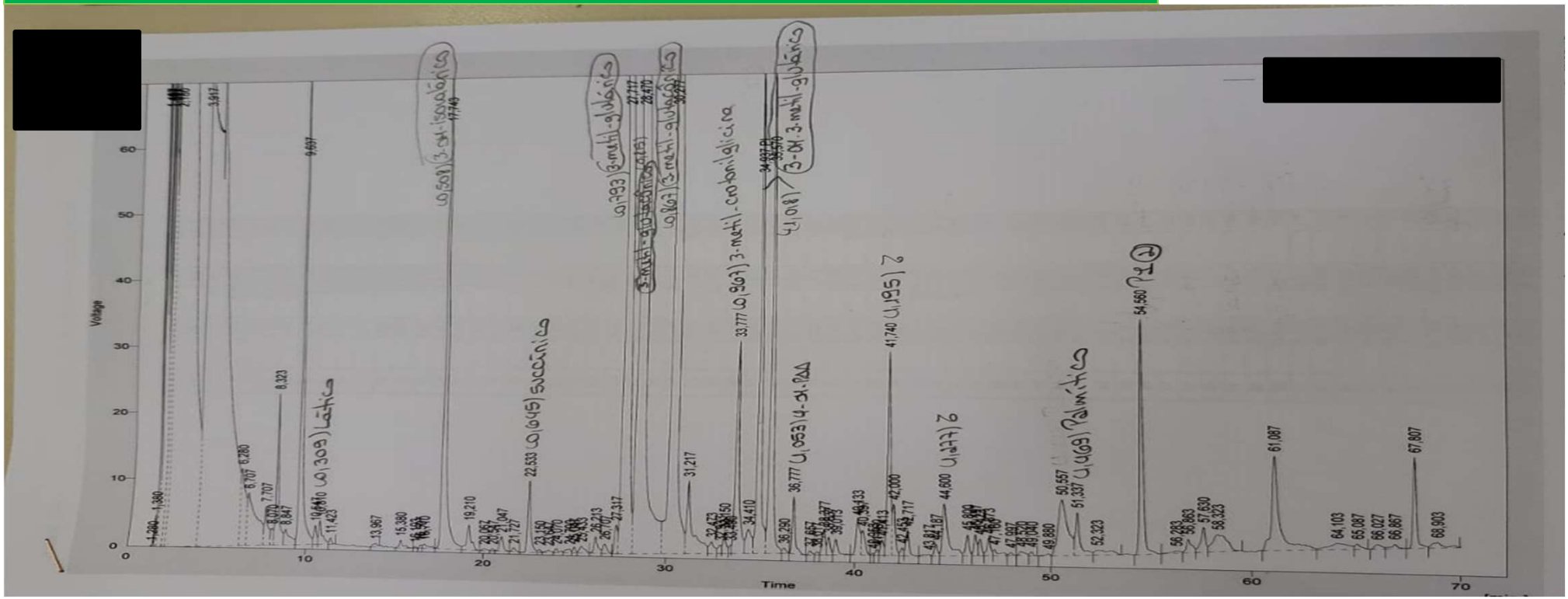
D11 OF HOSPITALIZATION



First Twin

Metabolic Biochemistry

3-HYDROXY-3-METHYLGLUTARIC ACIDEMIA



First Twin

Follow up

At discharge

- Protein 1,7g/kg/day
- L-carnitine 150 mg/kg/day

Diagnose explained
Instructions were given to the family
Emergency letter exhaustively explained

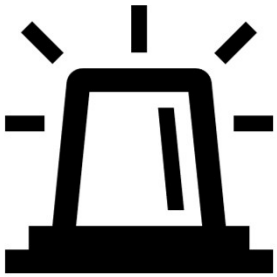
Appointment after 7 days

- Instructions reviewed
- Diet and L-carnitine adjusted for body weight
- Molecular test ordered

Molecular test result

HMGCL NM_000191.3: c.109G>T
p.Glu37* homozygous pathogenic

Conclusion



- It can be a life threatening disease
- Probably more common than we know
- Management Guidelines
- Call for colaboration

Acknowledgements

Thank you for your time!

My team:
Leon Iotti
Tamilis Borges
Luiz Cezar Tiberio

Genetic Service from Hospital de Clínicas de Porto Alegre

Questions?

Hypoglucemia in a 18-month-old female

Arantza Arza
Biochemistry Laboratory (Metabolic Diseases Unit)
Hospital Universitario Cruces. Barakaldo. Spain

The patient

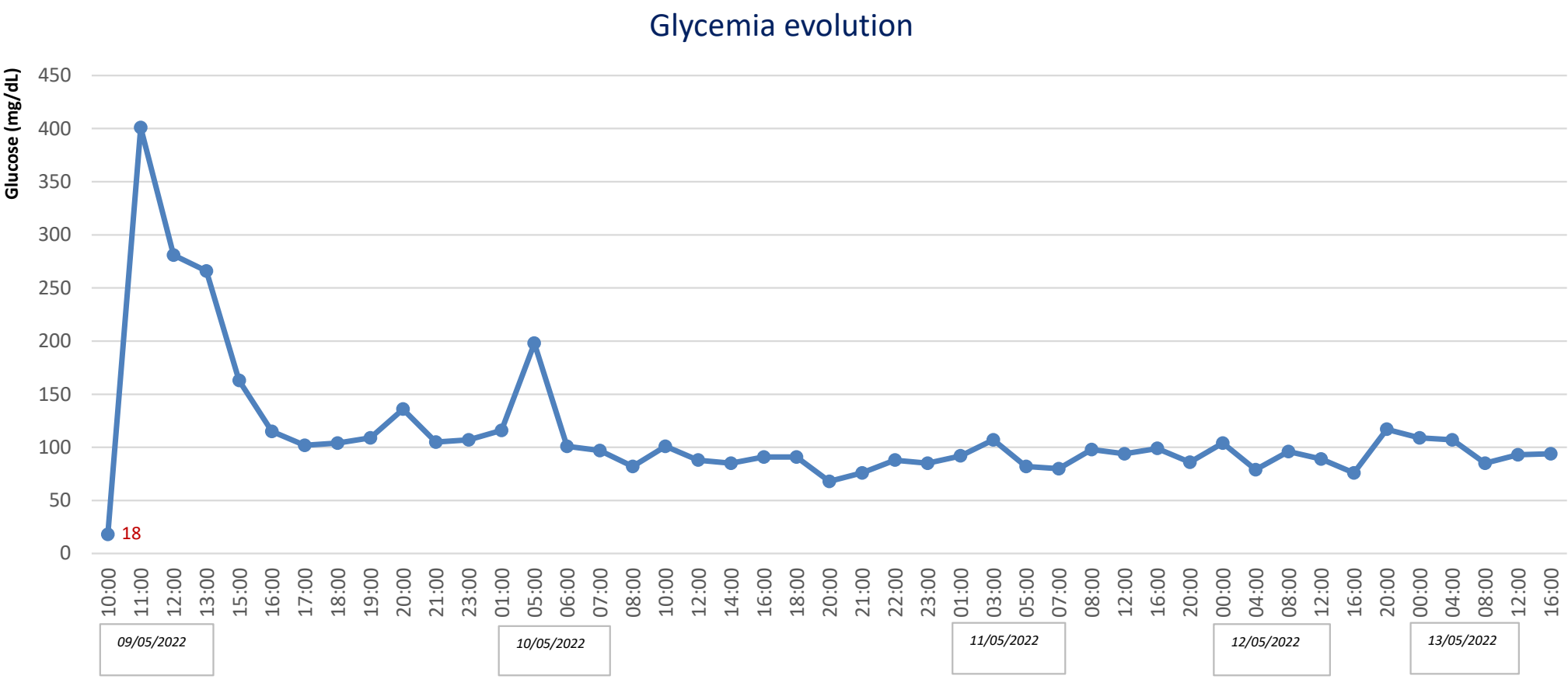
- *18-month-old female*
- *Patient referred to emergency department*
  - *Altered level of consciousness*
  - *Abnormal movements…*
- *Physical examination*
  - *Reactive miotic pupils*
  - *Hypotonic, hyporeactive with tonic movements*
  - *Low glucemia (18mg /dL), Ketone bodies neg*
  - *Intravenous Dextrose 10%, cefotaxime*
  - *Seizure episode: midazolam i.v 0,2mg/kg - PICU*

Intensive Unit Care

- *Brain CT scan*
  - No injuries identified
  - No evidence of ischemia or hemorrhage
- *Electroencephalography*
  - *Diffuse cerebral slowing reactive to stimuli*
  - *No crises*
- CSF study
- Infusion dextrose 5%, levetiracetam/12 hours
- Glycemic control

Hypoglucemia in a 18-month-old female

Intensive Unit Care



History

- *Term baby, unremarkable pregnancy*
- *Growth, development and health normal prior to presentation*
- *Family history*
  - *Second daughter born after two miscarriages*
  - *Non-consanguineous parents*
  - *No perinatal concerns*
  - *Normal newborn screening*

Hypoglucemia in a 18-month-old female

Initial Laboratory findings

Critical sample

Biochemistry		
Glucose (mg/dL)	18	70-110
ALT (U/L)	68	5-44
PCT (U/L)	17.04	
Ketones	Negative	
Gasometry		
pH	7.28	7,32 - 7.42
HCO3 (mmol/L)	17	24-28
EB (mmol/L)	-8,6	-3 - 3
Lactate (mmol/L)	1,4	0.5-2.2
Blood Count		
Platelets (x 103/uL)	593	135-450
Leukocytes (x 103/uL)	16.8	4-14
Neutrophils (x 103/uL)	13.41	1.5-9.5
%	79.9%	43-65
Hemostasis		
	Normal Profile	
Toxic in urine		
	Negative	

CFS STUDY		
Lactate (mmol/L)	1,1	(1,1-2,2)
Piruvate (mmol/L)	0,08	(0,05-0,15)
Amino acids	Normal profile	
PCR encephalitis and cultures	Negative	

Hypoglucemia in a 18-month-old female

Expanded metabolic screen

HORMONE PROFILE		
Cortisol (ug/dL)	53,9	(6,2-25)
GH (ng/mL)	21,5	(<5)
Insulin (uU/mL)	10,5	(<25)
C-Peptide (ng/mL)	1,1	(1,1-4,4)

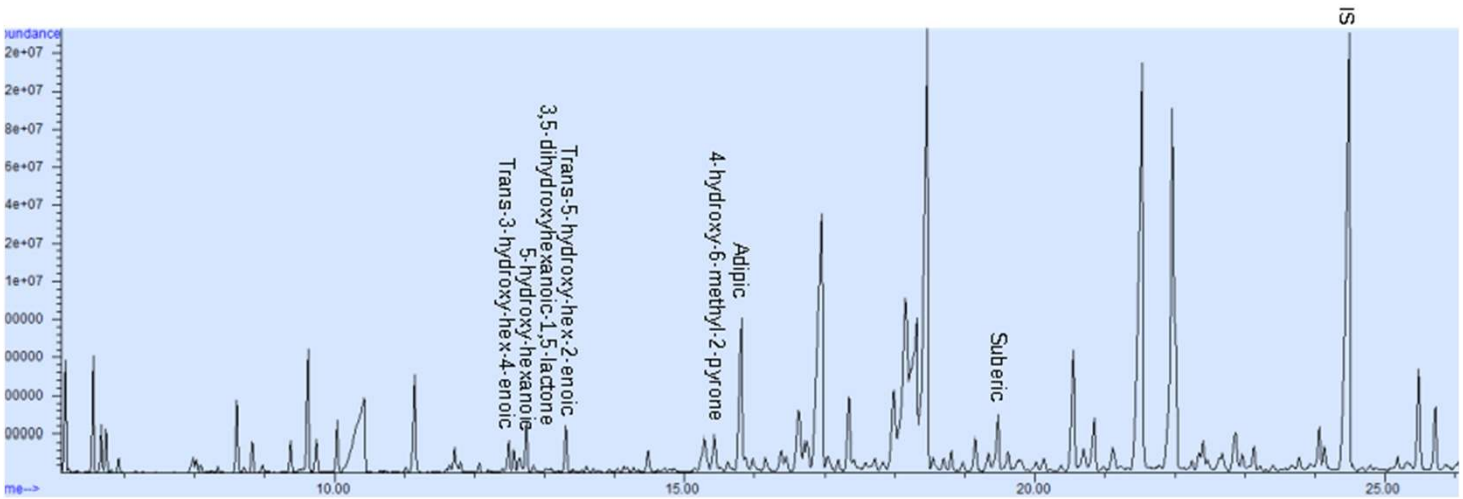
METABOLIC PROFILE				
Ammonia (umol/L)	26	(19-87)		
Lactate (mmol/L)	1,16	(0,7-1,8)		
Piruvate (mmol/L)	0,1	(0,034-1,02)		
B-Hydroxybut (mmol/L)	0,35	(0,02-0,67)		
Acetoacetate (mmol/L)	0,24	(0,01-0,2)		
B-HBT/ACA	1,5	<2,5		
Plasma Aminoacids	Hypoaminoacidemia, increased branched chain amino acids (Leu, Isoleu, Val)			
Plasma Acylcarnitines	Increase in acetylcarnitine (**C2: 22,1 umol/L**) and decrease in free carnitine(**C0: 7 umol/L**). Slight increase in long-chain acylcarnitines (C14:1, C18, C18:1, C18:2)			
	Normalization of the profile after providing carnitine and resolution of the process			
Urine organic acids	Dicarboxilic aciduria (**adipic acid**) and several unusual **trans-hydroxyhexenoic acids**			

Hypoglucemia in a 18-month-old female

Organic acid profile

- *Dicarboxilic aciduria:*
  - ***adipic**, suberic,*
  - *5-hydroxyhexanoic acid*

- *Several unusual trans-hydroxyhexenoic acids:*
  - *3,5-dihydroxyhexanoic-1,5-lactone*
  - *trans-5-hydroxyhex-2-enoate*
  - ***4-hydroxy-6-methyl-2-pyrone***
  - *5-hydroxy-3-ketohexanoic*
  - *3,5-dihydroxyhexanoic*
  - *trans -3-hydroxy-hex- 4-enoic*
  - *cis-5-hydroxy-2-enoic*



Hypoglucemia in a 18-month-old female

Genetic study

GENETICA MOLECULAR

Estudio genético enfermedad metabolica	Estudio genético Hipoglucemia
Alteración ADN	c.(821G>A) ; c.(1270C>T)
Alteración proteína	p.(Arg274His) ; p.(Arg424Ter)
Estatus	Doble Heterocigoto

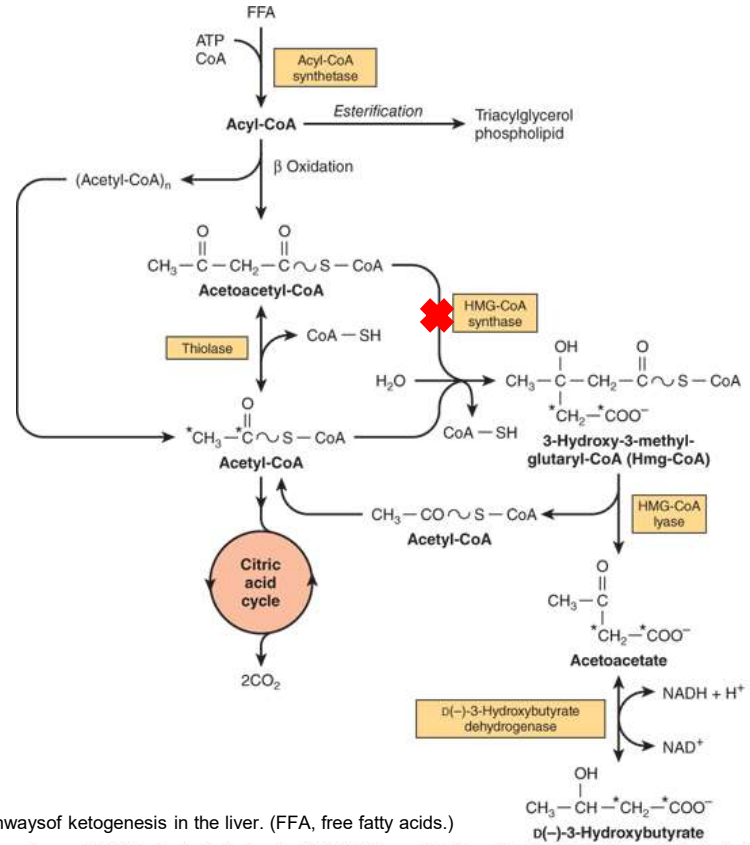
Interpretación

INTERPRETACION:

Se ha identificado las variantes c.821G>A (p.Arg274His) y c.1270C>T (p.Arg424Ter) en heterocigosis en el gen HMGCS2. El cambio c.821G>A da lugar a la sustitución de arginina por histidina en la posición 274 de la proteína (p.Arg274His), no está descrito en HGMD pero sí en ClinVar (VCV001327464.1). Los predictores de patogenicidad (www.varsome.com) utilizando los criterios del American College of Medical Genetics (ACMG), la clasifican como variante probablemente patogénica. El cambio c.1270C>T da lugar a la sustitución de arginina por un codón stop prematuro en la posición 424 de la proteína (p.Arg424Ter), está descrito en HGMD y en ClinVar (VCV000009258.4). Los predictores de patogenicidad (www.varsome.com) utilizando los criterios del American College of Medical Genetics (ACMG), la clasifican como variante patogénica. El gen HMGCS2 (MIM* 600234) codifica para la 3-hidroxi-3-metilglutaril CoA sintasa. Mutaciones en este gen causan la deficiencia de 3-hidroxi-3-metilglutaril CoA sintasa (MIM# 605911) que se hereda de forma recesiva.

Hypoglucemia in a 18-month-old female

3-hydroxy-3-methyl-CoA synthase def



Pathwaysof ketogenesis in the liver. (FFA, free fatty acids.)

- Rare AR disorder of ketone body metabolism
- Presents in the first years of life
- Characterized by episodes of decompensation presenting with vomiting, lethargy, hepatomegaly, nonketotic hypoglycemia, and rarely coma
- Most of those affected asymptomatic between acute episodes
- It requires early diagnosis to avoid hypoglycemic crises that can cause irreversible brain damage or death.

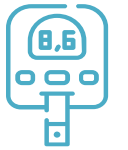
Treatment

- *Avoid fasting periods of more than 6 to 8 hours*
- *Corn starch (1 g/kg) before going to sleep*
- *During intercurrences, or days when eat worse*
  - ✓ *Dextrinomaltose*
    1-2 years: 15%
    2-10 years: 20%
    >10 years: 25%
- *Capillary glycaemia control at home*
- *In case of poor general condition, go to the hospital (rule out metabolic acidosis and hypoglycemia)*
- *Carnitine (50 mg/kg/d)*

Clinical evolution

- *Subsequent hypoglycaemic episode*
  - ➢ *At 23 months, a new episode of hypoglycemia (69 mg/dL) in the context of a viral infection*

- *Good glycemic management at home. Sensor free + capillary blood glucose*
- *Child has developed normally, no neurologic impairment*
- *Considering a low fat diet.*



Conclusions

- ✓ *mHMG-CoA synthase is required for the generation of KB that provide energy to the brain and other organs at times of fasting*
- ✓ *The diagnosis is made by evaluating various markers of glucose homeostasis during hypoglycaemia (free fatty acids and KB). It is recently found to have presenting laboratory abnormalities (elevation of acetyl carnitine and specific urine organic acid profile)*
- ✓ *Probably it will be more frequently diagnosed due to the availability of exome sequencing*

Acknowledgements

- *Emergency Unit and Neonatal Intensive Unit Care. H.U Basurto*
- *Dr Nuñez FJ (Endocrinology Unit Care. H.U Basurto)*
- *Dr de las Heras J (Pediatrics Metabolic Unit. H.U Cruces)*
- *Dra Unceta M (Biochemistry Laboratory. Metabolic Diseases Unit. H.U Cruces)*
- *Dra Gort, Dra.Garcia (H.Clinic. Barcelona)*

Questions?

The risk of arrhythmia persists despite good metabolic control

Paula Sánchez Pintos, PhD

Diagnosis and Treatment Unit of Congenital Metabolic Diseases.
Clinical University Hospital of Santiago de Compostela, Spain.
C.S.U.R. MetabERN.

Case

Male.
Born after an uneventful full-term pregnancy (39 weeks of GA) by eutocic delivery. Apgar score 9/10.
Birth biometries according to gestational age: weight 3090g, height 52cm, HC 35cm.
He received breastfeeding with correct grip tolerance.

Familial history: first child of healthy consanguineous parents from Morocco (cousins). No other relevant data.

Case

Parents warned due to partial rejection of the last feed and **hiporeactivity**.

Physical examination: stood out low response to stimuli, continuous moaning, skin pallor and bradycardia (HR 65 bpm) with **severe hypoketotic hypoglycemia** (blood glucose in capillary sample: 10mg/dL- 0.56 mmol/L).

IV bolus of dextrose 10% (2 cc/kg) was administered without recovery, and the patient was urgently transfered to NICU.

Case

- Continuous dextrose IV infussion was started with progressively glucemic recuperation. Maximum dextrose needs: 10mcg/kg/min.
- A decrease in ammonia levels was observed until its normalization at 8 hours of admission without the use of ammonia scavenger drugs.
- He needed respiratory support with nasal High-Flow and hemodinamic support with continuous iv infussion of dopamine and noradrenaline, due to the maintenance of bradicardia and hypotension, during the first 3 days.

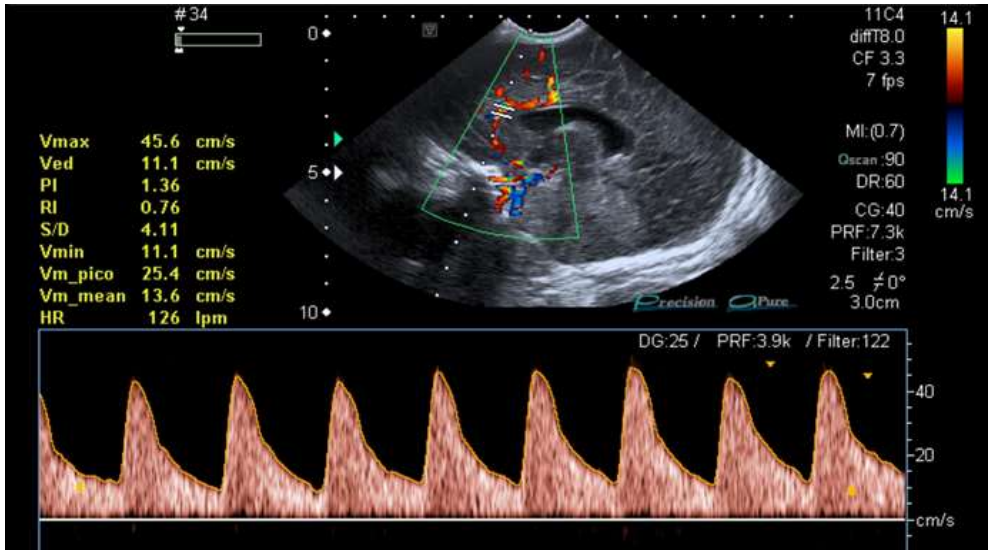
Case

Ecocardiography: structurally normal heart with normal contractility. FOP.

He mantained lethargic with slow gradually improvement.

- **CFM**: continuous normal voltage pattern. No clinical or electric seizures.

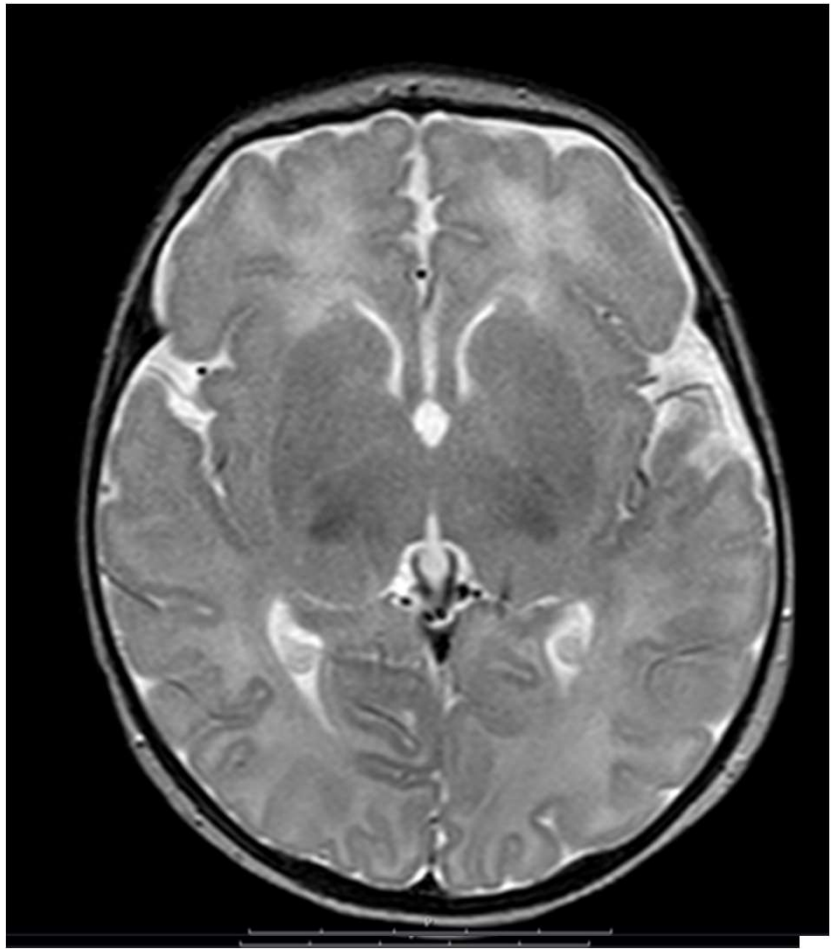
Doppler MCA: high and diastolic flow attenuation pulsatility, suggestive of cerebral edema.



Case

Brain US: periventricular hyperechogenicity

Brain MRI: alteration of the signal and punctate lesions in the white matter of the border territories of both cerebral hemispheres in possible relation to **sequelae of damage hypoxic-ischemic.**



Case

Metabolic studies:

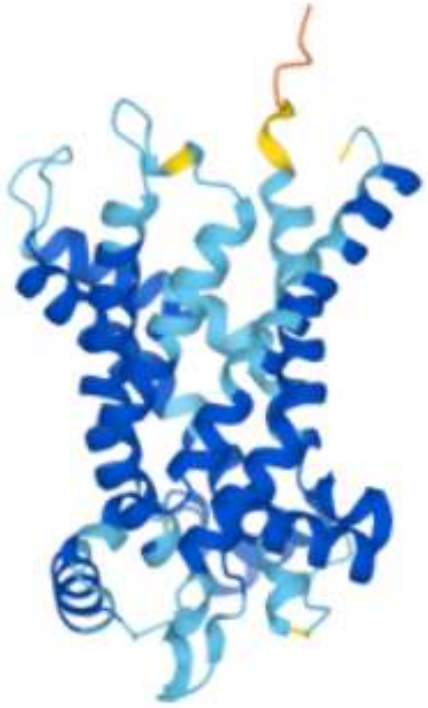
- Normal plasma and urine amino acids profile.
- Normal organic acids in urine.
- Acylcarnitines profile: **increase levels of LC - acylcarnitines**:
  - **C10**: 0.71umol/L (< 0.35)
  - **C14**: 1.27umol/L (< 0.56)
  - **C16**: 16.39umol/L (0.41-7.1)
  - **C16:1**: 1.45umol/L (< 0.49)
  - **C18**: 2.83umol/L (0.24-2)
  - **C18:1**: 4umol/L (<2.8)

Increased ratios: C0/C16+C18:1: 1.12 (4.1-33)
C16+C18/C2: 1.06 (0.09-0.25).

C0: 7.02umol/L.

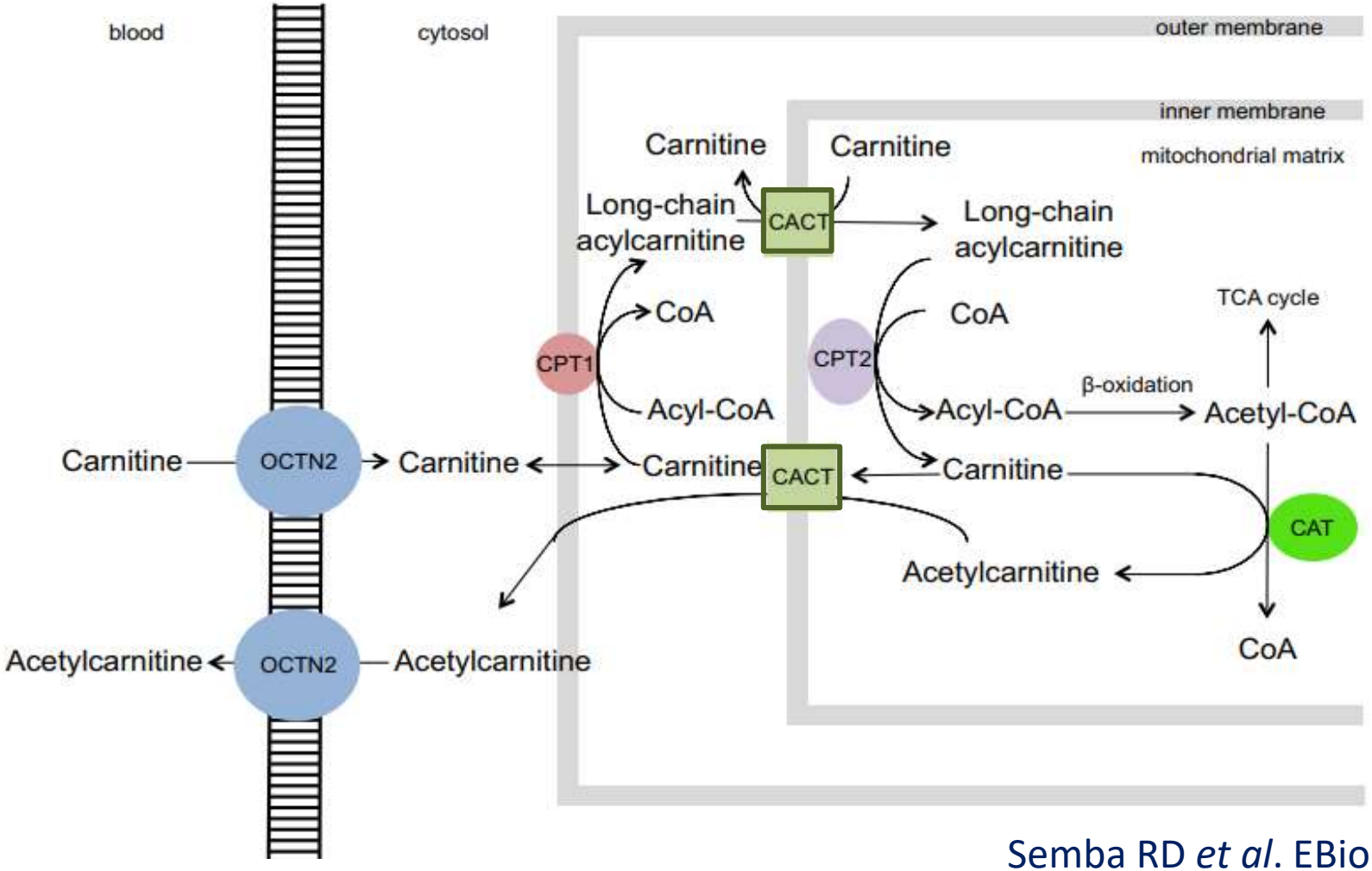
Genetical study showed the previously described mutation c.532C>T (p.R178*) in homozigosity in ***SLC25A20*** **gene** related with **CACTD**.

Turkish girl who ter birth seizures and apnoea, who requ lation. She developed hy nol/L), lactic acidaemia (7.9 mmol/L), hyp mol/L), mildly elevated transaminases an .



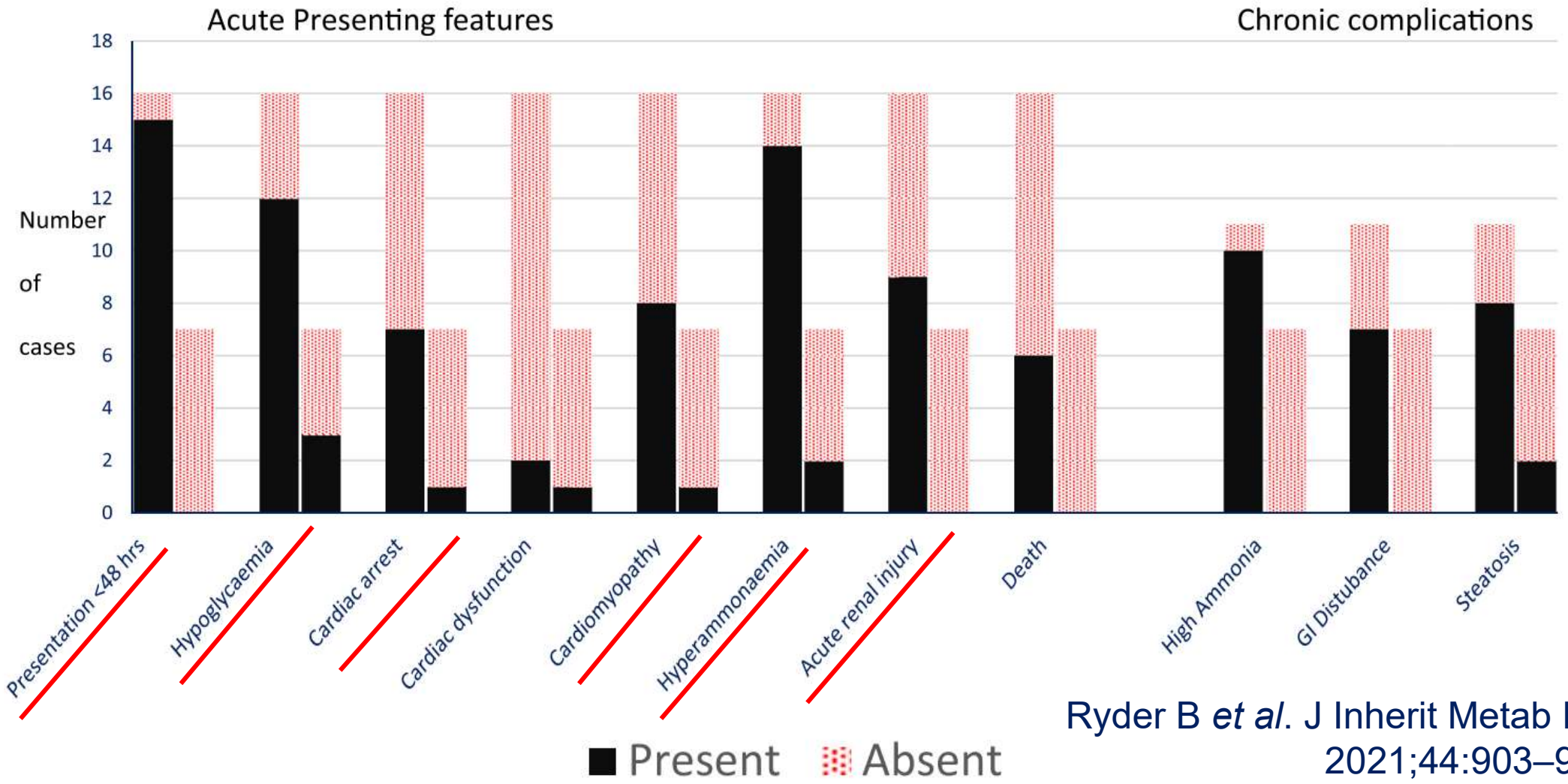
Rubio-Go ca 2003;92:501-504.

The carnitine shuttle



Semba RD *et al*. EBioMedicine 17; 2017: 57–66

CACTD



Ryder B *et al*. J Inherit Metab Dis. 2021;44:903–915.

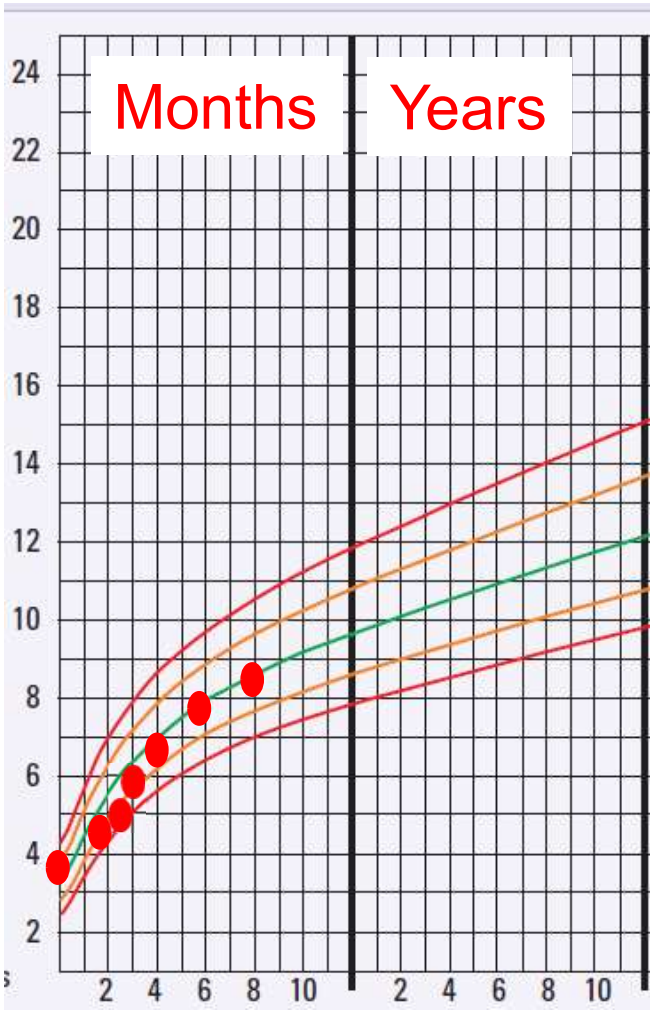
Case

Dietary management with low fat-high carbohydrates diet (LCT at 10% of total caloric intake) and supplementation with medium chain triglycerides allow a progressively clinical improvement. He received nocturnal continuous enteral feeding during the first six months of age.

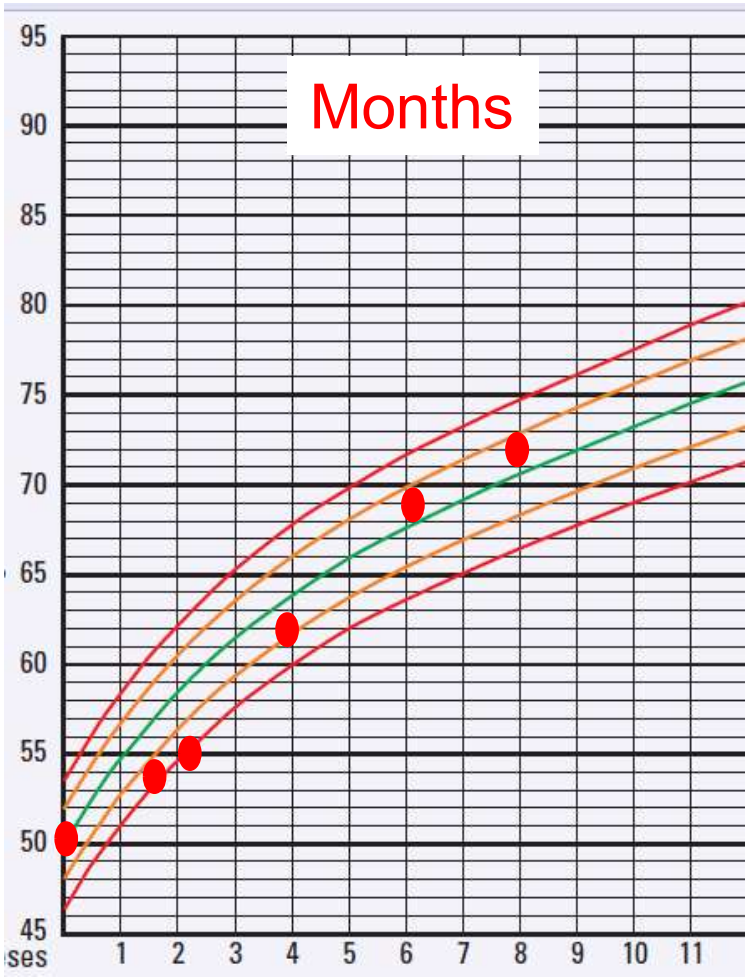
Despite feeding difficulties with poor suction, frequent vomiting and intermitent diarrhoea, as described in classical CACTD cases, that conduce to gastrostomy tube placement, a normal growth and a normalization of hepatic dysfunction and acycarnitines profile was achieved.

Case

Weight

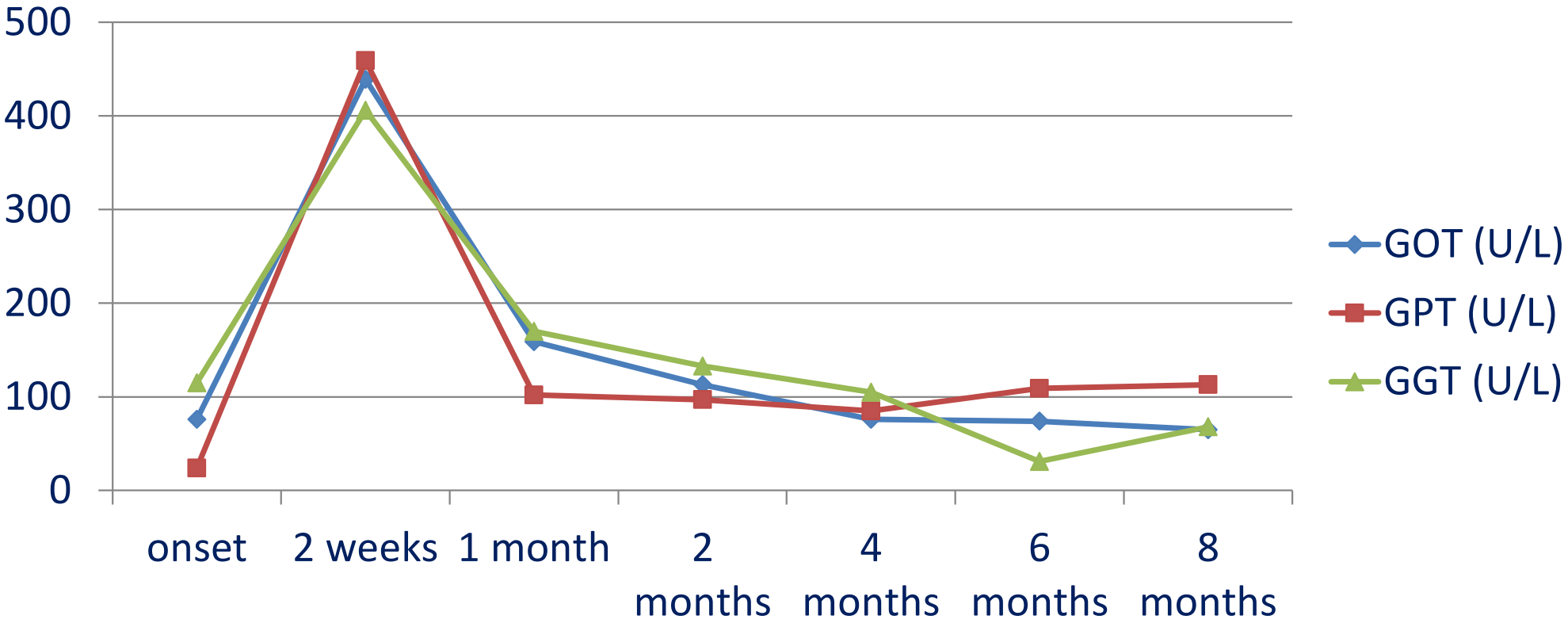


Height

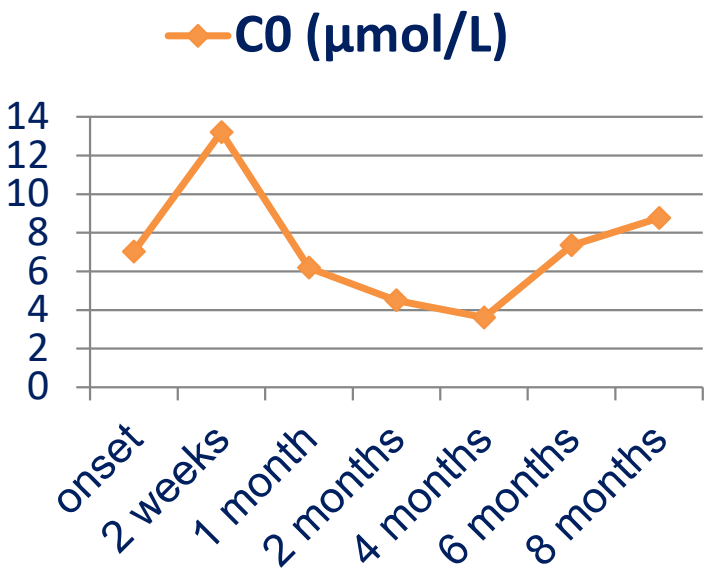


Case

Evolution of liver function



Evolution of acylcarnitines profile



	C0	C16	C16:1	C16:1-OH	C18	C18:1:0H
onset	7.03	**9.86**	**0.75**	0.13	**2.5**	0.06
2 weeks	13.20	**9.41**	**0.99**	0.13	1.94	0.03
1 month	6.19	4.78	**0.50**	0.08	1.51	0.03
2 months	4.5	2.22	0.25	0.06	0.56	0.02
4 months	3.61	2.41	0.34	0.04	0.71	0.02
6 months	7.35	3.01	0.55	0.05	1.18	0.04
8 months	8.77	2.15	0.41	0.04	0.94	0.05

Case

One hour after the arrival he presented a **fatal episode of cardiac arrest related to arrhythmia.** He developed **successive episodes of ventricular fibrillation and pulseless ventricular tachycardia** with no response to advanced cardiopulmonary resuscitation, epinephrine and amiodaron therapy, and repeated cardioversion.

- Myoglobine 1554ng/mL
- Troponine 1533ng/L (p99:71)

The necropsy is still in progress but there was **no evidence of viral myocarditis.**

CACTD and cardiac affectation

CACTD has the highest rate of cardiac arrhythmia and mortality among FAO disorders

Classical (n=16)

Attenuated (n=7)

- No cardiomyopathy at onset with normal follow up 5/7
- Asystolic arrest requiring CPR at 2 months of age with longer development of hypertrophic cardiomyopathy 1/7.

Ryder B *et al*. J Inherit Metab Dis. 2021;44:903–915.

Doubts that we raised to discuss

- Has carnitine supplementation played a role in arrhythmogenesis? or

- Would higher levels of free carnitine have allowed him to tolerate the infection better at the cardiac level?

- This patient presented mild previous respiratory infection by sars-CoV-2 at 6 months of age without clinical decompensation or cardiac affectation. Is the risk of arrhythmia somehow dependent on type of causal virus?

Acknowledgements

Diagnosis and Treatment Unit of Congenital Metabolic Diseases.
Clinical University Hospital of Santiago de Compostela, Spain.

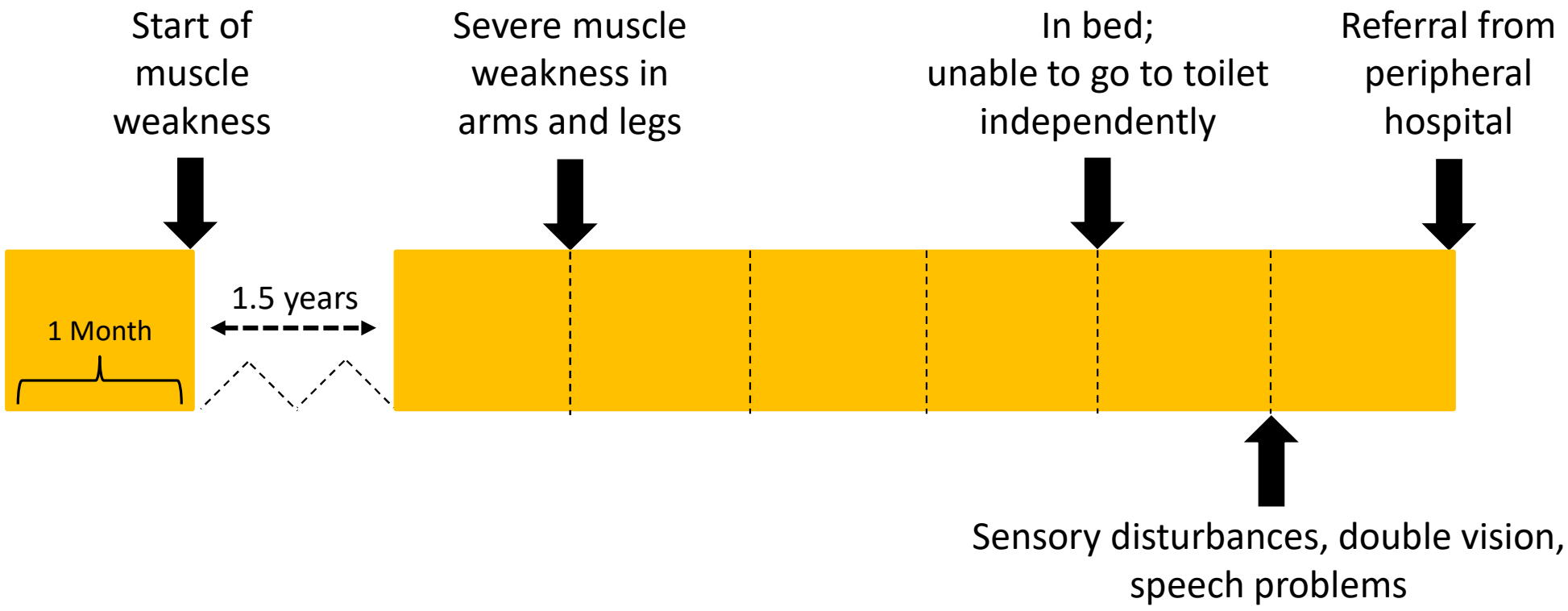
Questions?

Muscle weakness in a person on a very restricted diet

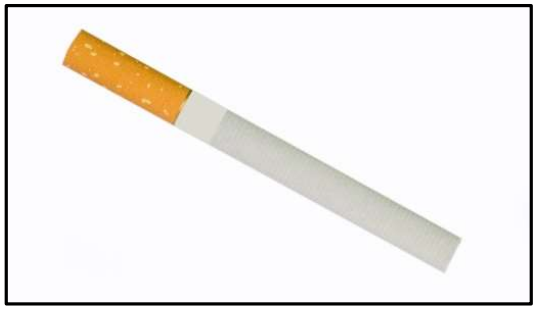
Marie van Dijk

Laboratory Genetic Metabolic Diseases, Amsterdam UMC

Case: middle-aged person



Case diet



X 40 per day

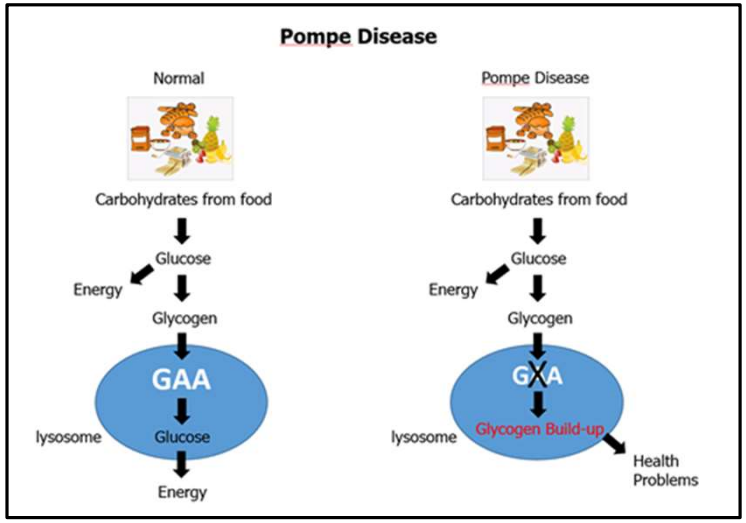
Differential diagnosis

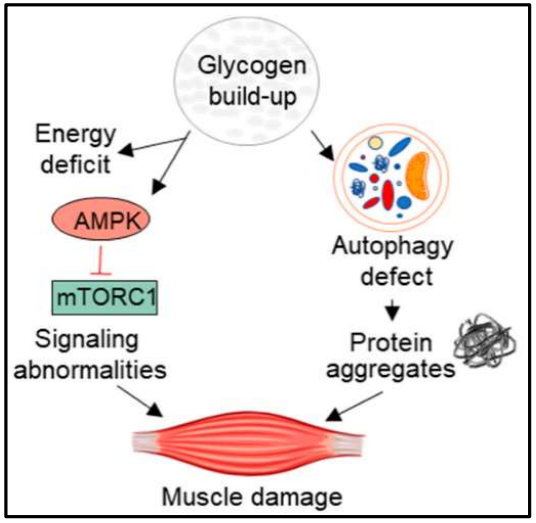
- *Neuromuscular transition disease*
  - *Myasthenia gravis (autoimmune)*
  - *Lambert-Eaton myasthenic syndrome (autoimmune; 50% also lung cancer)*
- *Limb-girdle muscular dystrophy (genetic)*
- *Chronic inflammatory demyelinating polyneuropathy (autoimmune)*

Diagnostics

- *Trial use of Mestinon to treat Myasthenia gravis → No effect, only side effects*
- *MRI → Muscle edema*
- *PET-CT → Potential myositis*
- *Muscle biopsy → Initial review: glycogen accumulation? Pompe disease?*

Pompe diagnostics





Materiaal	Testnaam/Ziektebeeld	Uitslag	Ref. Waarden	Eenheid
LEU	b-D-galactosidase GM-1 gangliosidose	139	80 - 240	nmol/mg.uur
LEU	a-D-glucosidase Glycogenose type II (M. Pompe)	130	60 - 250	nmol/mg.uur
LEU	a-D-glucosidase (met acarbose) Glycogenose type II (M. Pompe)	90	30 - 160	nmol/mg.uur

Biopsy further review
Fat deposits (not glycogen)

- *Late-onset Pompe disease rejected*

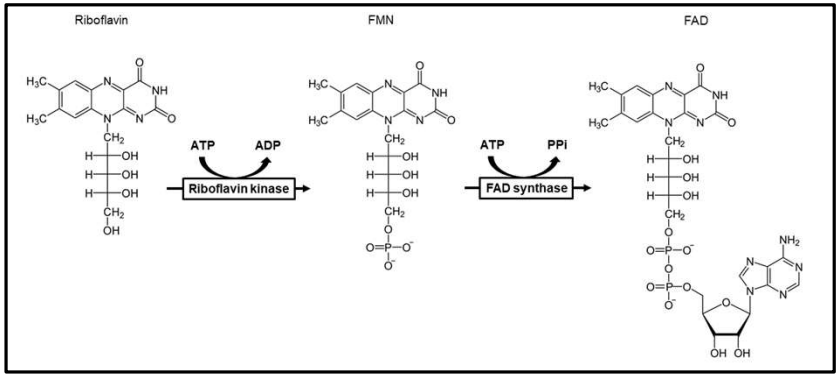
Fat deposits: Acylcarnitines

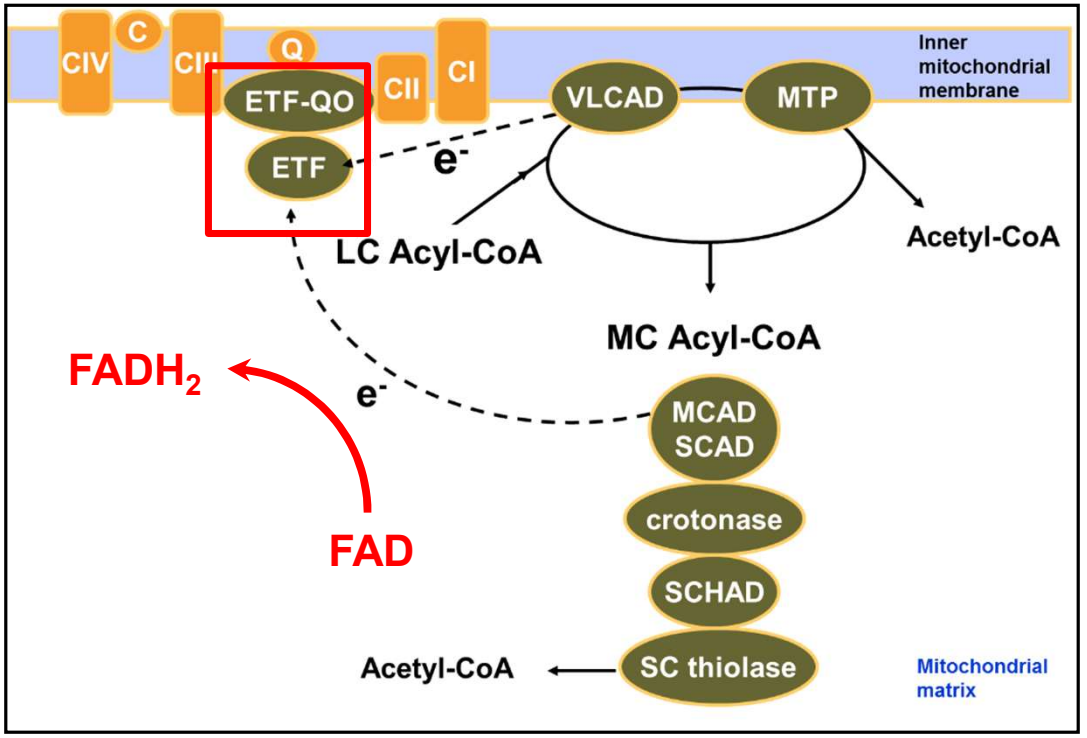
	µmol/l		Referentiewaarden		
Vrij carnitine	24.95		22.30	-	54.80
C2-carnitine	4.64		3.40	-	13.00
C3-carnitine	0.18		0.14	-	0.94
C4-carnitine	0.50		0.07	-	0.58
C5:1-carnitine	0.00		0.00	-	0.04
C5-carnitine	0.19		0.04	-	0.22
C4-3-OH-carnitine	0.03		0.00	-	0.15
C6-carnitine	0.25	+	0.02	-	0.12
C5-OH-carnitine	0.01	-	0.02	-	0.06
C8-carnitine	0.44	+	0.04	-	0.22
C3-DC-carnitine	0.01	-	0.02	-	0.08
C10:1-carnitine	0.08		0.04	-	0.22
C10:0-carnitine	0.60	+	0.04	-	0.30
C4-DC-carnitine	0.04		0.02	-	0.06
C5-DC-carnitine	0.03		0.02	-	0.06
C12:1-carnitine	0.09		0.02	-	0.14
C12:0-carnitine	0.43	+	0.04	-	0.14
C6-DC-carnitine	0.02		0.00	-	0.06
C12:1-OH-carnitine	0.01	-	0.02	-	0.08
C12-OH-carnitine	0.01		0.00	-	0.06
C5-3M-3OH-carnitine	0.00		0.00	-	0.02
C14:2-carnitine	0.17	+	0.02	-	0.08
C14:1-carnitine	1.15	+	0.02	-	0.18
C14:0-carnitine	0.87	+	0.00	-	0.08
C8-DC-carnitine	0.04		0.00	-	0.04
C14:1-OH-carnitine	0.03		0.00	-	0.04
C14-OH-carnitine	0.00		0.00	-	0.04
C16:1-carnitine	1.40	+	0.02	-	0.08
C16:0-carnitine	1.48	+	0.06	-	0.24
C10-DC-carnitine	0.07	+	0.00	-	0.04
C16:1-OH-carnitine	0.04	+	0.00	-	0.02
C16-OH-carnitine	0.00		0.00	-	0.02
C18:2-carnitine	0.38	+	0.02	-	0.18
C18:1-carnitine	1.83	+	0.06	-	0.28
C18:0-carnitine	0.53	+	0.02	-	0.10
C18:2-OH-carnitine	0.00		0.00	-	0.02
C18:1-OH-carnitine	0.01		0.00	-	0.02
C18-OH-carnitine	0.00		0.00	-	0.04

- *Elevated saturated mid-chain acylcarnitines*
- *Elevated saturated and unsaturated long-chain acylcarnitines*
- *Normal hydroxyacylcarnitines*

Differential diagnosis 2

- *Acquired or inherited functional riboflavin (vitamin B2) deficiency*
- *Deficiency in riboflavin dependent enzymes*





Flavin measurements

	nmol/l	Referentiewaarden
Flavine adenine dinucleotide (FAD)	93.0	46.0 - 114.0
Flavine mononucleotide (FMN)	4.2	2.8 - 21.4
Riboflavine	2.2 -	3.9 - 49.0

2 weeks later:

	nmol/l	Referentiewaarden
Flavine adenine dinucleotide (FAD)	104.7	46.0 - 114.0
Flavine mononucleotide (FMN)	2.3 -	2.8 - 21.4
Riboflavine	1.7 -	3.9 - 49.0

- *Riboflavin supplementation was started*
- *Mutation analysis of ETFA, ETFB and ETFDH was ordered*

2 weeks after supplementation

	nmol/l	Referentiewaarden		
Flavine adenine dinucleotide (FAD)	98.5	46.0	-	114.0
Flavine mononucleotide (FMN)	14.3	2.8	-	21.4
Riboflavine	14.5	3.9	-	49.0

	µmol/l			Referentiewaarden		
Vrij carnitine	7.77	-	←	22.30	-	54.80
C2-carnitine	0.98	-		3.40	-	13.00
C3-carnitine	0.04	-		0.14	-	0.94
C4-carnitine	0.07			0.07	-	0.58
C5:1-carnitine	0.00			0.00	-	0.04
C5-carnitine	0.01	-		0.04	-	0.22
C4-3-OH-carnitine	0.00			0.00	-	0.15
C6-carnitine	0.06			0.02	-	0.12
C5-OH-carnitine	0.00	-		0.02	-	0.06
C8-carnitine	0.37	+	←	0.04	-	0.22
C3-DC-carnitine	0.01	-		0.02	-	0.08
C10:1-carnitine	0.09			0.04	-	0.22
C10:0-carnitine	0.46	+	←	0.04	-	0.30
C4-DC-carnitine	0.04			0.02	-	0.06
C5-DC-carnitine	0.01	-		0.02	-	0.06
C12:1-carnitine	0.04			0.02	-	0.14
C12:0-carnitine	0.08			0.04	-	0.14
C6-DC-carnitine	0.00			0.00	-	0.06
C12:1-OH-carnitine	0.00	-		0.02	-	0.08
C12-OH-carnitine	0.00			0.00	-	0.06
C5-3M-3OH-carnitine	0.00			0.00	-	0.02
C14:2-carnitine	0.04			0.02	-	0.08
C14:1-carnitine	0.18			0.02	-	0.18
C14:0-carnitine	0.08			0.00	-	0.08
C8-DC-carnitine	0.00			0.00	-	0.04
C14:1-OH-carnitine	0.01			0.00	-	0.04
C14-OH-carnitine	0.00			0.00	-	0.04
C16:1-carnitine	0.11	+	←	0.02	-	0.08
C16:0-carnitine	0.17			0.06	-	0.24
C10-DC-carnitine	0.01			0.00	-	0.04
C16:1-OH-carnitine	0.01			0.00	-	0.02
C16-OH-carnitine	0.00			0.00	-	0.02
C18:2-carnitine	0.04			0.02	-	0.18
C18:1-carnitine	0.21			0.06	-	0.28
C18:0-carnitine	0.06			0.02	-	0.10
C18:2-OH-carnitine	0.00			0.00	-	0.02
C18:1-OH-carnitine	0.00			0.00	-	0.02
C18-OH-carnitine	0.00			0.00	-	0.04

- *Flavins normalized*
- *Acylcarnitines normalized?*
  → Very low free carnitine!

➢ *Preliminary conclusion: acquired riboflavin deficiency due to poor diet*

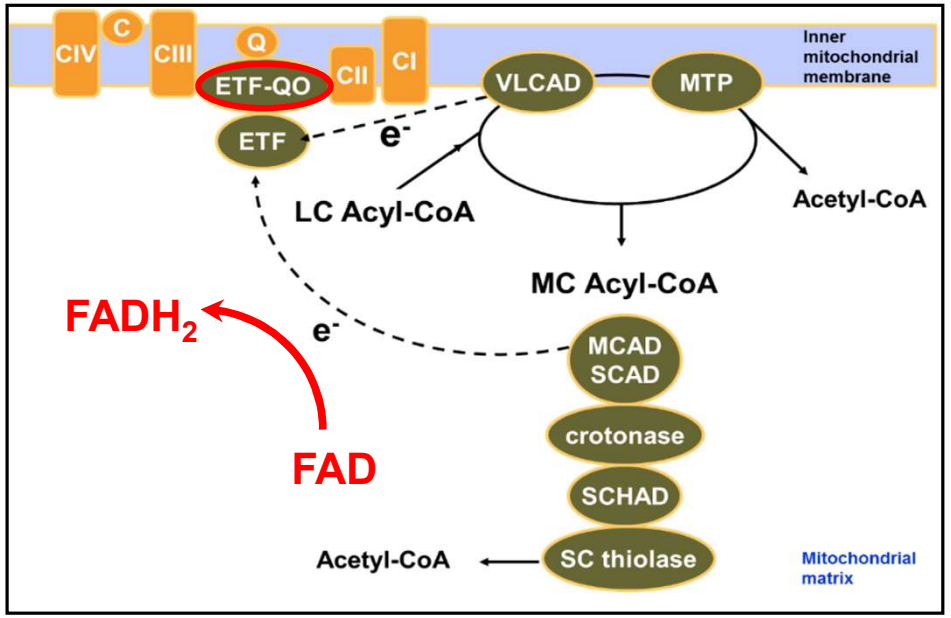
Results mutation analysis

- *ETFA & ETFB no variants*
- *ETFDH c.1255_1258del (p.(Thr419Valfs*9); class 5 variant)*
- *ETFDH c.1514T>C (p.(Ile505Thr); class 5 variant)*

- *Father is carrier of ETFDH c.1514T>C (p.(Ile505Thr)*
  → variants are located on different alleles (in trans)

Diagnosis

- *Multiple acyl-CoA dehydrogenase deficiency (MADD)*



Mutations phenotype

- *ETFDH mutations generally associated with late-onset disease*
- *ETFDH c.1255_1258del – Late-onset riboflavin-responsive phenotype*
  (Wang et al., J Mol Med (Berl), 2011)
- *ETFDH c.1514T>C – Late-onset riboflavin-responsive phenotype*
  (Wang et al., Mol Med Rep, 2020)

Upon diet improvement

		10 Months later		2 Months later	
Vrij carnitine	→	38.75	*18.7 - 49.2*	18.97	*- 22.3 - 54.8*
C2-carnitine		4.87	*3.4 - 13*	2.79	*- 3.4 - 13*
C3-carnitine		0.35	*0.14 - 0.94*	0.16	*0.14 - 0.94*
C4-carnitine		0.35	*0.07 - 0.58*	0.18	*0.07 - 0.58*
C5:1-carnitine		0.01	*0 - 0.04*	0.00	*0 - 0.04*
C5-carnitine		0.10	*0.04 - 0.22*	0.08	*0.04 - 0.22*
C4-3-OH-carnitine		0.04	*0 - 0.15*	0.02	*0 - 0.15*
C6-carnitine	→	0.41	*+ 0.02 - 0.12*	0.20	*+ 0.02 - 0.12*
C5-OH-carnitine		0.02	*0.02 - 0.06*	0.00	*- 0.02 - 0.06*
C8-carnitine	→	1.31	*+ 0.04 - 0.22*	0.83	*+ 0.04 - 0.22*
C3-DC-carnitine		0.02	*0.02 - 0.08*	0.01	*- 0.02 - 0.08*
C10:1-carnitine		0.20	*0.04 - 0.22*	0.12	*0.04 - 0.22*
C10:0-carnitine	→	1.47	*+ 0.04 - 0.3*	1.07	*+ 0.04 - 0.3*
C4-DC-carnitine		0.06	*0.02 - 0.06*	0.05	*0.02 - 0.06*
C5-DC-carnitine		0.03	*0.02 - 0.06*	0.02	*0.02 - 0.06*
C12:1-carnitine		0.09	*0.02 - 0.14*	0.08	*0.02 - 0.14*
C12:0-carnitine	→	0.26	*+ 0.04 - 0.14*	0.40	*+ 0.04 - 0.14*
C6-DC-carnitine		0.02	*0 - 0.06*	0.01	*0 - 0.06*
C12:1-OH-carnitine		0.02	*0.02 - 0.08*	0.01	*- 0.02 - 0.08*
C12-OH-carnitine		0.01	*0 - 0.06*	0.00	*0 - 0.06*

		10 Months later		2 Months later	
C14:2-carnitine	→	0.08	*0.02 - 0.08*	0.12	*+ 0.02 - 0.08*
C14:1-carnitine	→	0.38	*+ 0.02 - 0.18*	0.58	*+ 0.02 - 0.18*
C14:0-carnitine	→	0.15	*+ 0 - 0.08*	0.32	*+ 0 - 0.08*
C8-DC-carnitine		0.01	*0 - 0.04*	0.01	*0 - 0.04*
C14:1-OH-carnitine		0.01	*0 - 0.04*	0.01	*0 - 0.04*
C14-OH-carnitine		0.01	*0 - 0.04*	0.00	*0 - 0.04*
C16:1-carnitine	→	0.11	*+ 0.02 - 0.08*	0.29	*+ 0.02 - 0.08*
C16:0-carnitine	→	0.18	*0.06 - 0.24*	0.26	*+ 0.06 - 0.24*
C10-DC-carnitine		0.02	*0 - 0.04*	0.02	*0 - 0.04*
C16:1-OH-carnitine		0.01	*0 - 0.02*	0.01	*0 - 0.02*
C16-OH-carnitine		0.01	*0 - 0.02*	0.00	*0 - 0.02*
C18:2-carnitine		0.04	*0.02 - 0.18*	0.07	*0.02 - 0.18*
C18:1-carnitine	→	0.16	*0.06 - 0.28*	0.30	*+ 0.06 - 0.28*
C18:0-carnitine		0.05	*0.02 - 0.1*	0.09	*0.02 - 0.1*
C18:2-OH-carnitine		0.00	*0 - 0.02*	0.00	*0 - 0.02*
C18:1-OH-carnitine		0.00	*0 - 0.02*	0.00	*0 - 0.02*
C18-OH-carnitine		0.00	*0 - 0.04*	0.00	*0 - 0.04*

- *MADD acylcarnitine profile*

Upon diet improvement

	10 Months later			2 Months later	
Flavine adenine dinucleotide (FAD)	213.4	+	*46 - 114*	70.2	*46 - 114*
Flavine mononucleotide (FMN)	9.3		*2.8 - 21.4*	4.1	*2.8 - 21.4*
Riboflavine	166.8	+	*3.9 - 49*	6.6	*3.9 - 49*

- *Patient has no more muscle pains*
- *Patient is able to walk again and go outside*

→ Now has emergency protocol

Take home messages

- *Late-onset MADD can be easily misdiagnosed*
  (Myasthenia gravis, progressive muscular dystrophy, myositis, Pompe disease)
- *ETFDH mutations are associated with late-onset riboflavin-responsive MADD*
- *Very low free carnitine can mask abnormal acylcarnitines*
- *Proper diet (with sufficient riboflavin intake) can largely reverse symptoms*

Acknowledgements

- *Barbara Sjouke – Internist-endocrinologist IEM, Amsterdam UMC*
- *Mirjam Langeveld – Internist-endocrinologist IEM, Amsterdam UMC*

- *Susan Goorden – Clinical biochemist IEM, Erasmus MC Rotterdam*
- *Merel Ebberink – Clinical laboratory geneticist IEM, Amsterdam UMC*
- *Fred Vaz – Clinical biochemist IEM, Amsterdam UMC*

Organic acids

	µmol/l	mmol/mol kreatinine		Referentiewaarden		
Glycolzuur	451	51		20	-	140
3-Hydroxypropionzuur	154	17		1	-	18
Methylmalonzuur	5	1		0	-	5
3-Hydroxyisovaleriaanzuur	54	6		0	-	53
Ethylmalonzuur	107	12	+ ←	0	-	8
Glutaarzuur	24	3		0	-	8
Adipinezuur	208	23	+ ←	2	-	9
2-Hydroxyglutaarzuur	304	34	+ ←	6	-	17
3-Hydroxy-3-methylglutaarzuur	44	5		4	-	9
Suberinezuur	44	5		1	-	10
Melkzuur	558	63	+	7	-	36
Acetoacetaat	v					
3-Hydroxyisoboterzuur	1377	155				
3-Hydroxyboterzuur	1661	187				
2-Hydroxyisovaleriaanzuur	v					
Benzoezuur	81	9				
2-Ketoglutaarzuur	149	17				
Hexanoylglycine	v		←			
para-Hydroxyfenylazijnzuur	74	8				
Octeendizuur	v					
4-Acetylaminofenol	v					
Deceendizuur	v					
2-Hydroxyisoboterzuur	v					
3-Hydroxyadipinezuurlacton	v					

- ✓ *Ethylmalonic acid*
- ✓ *2-hydroxyglutaric acid*
- ✓ *C4-C8 Acylglycines*
- ✓ *C6-C10 dicarboxylic acids*
- ✗ *Glutaric acid*

Questions?

The benefit of triheptanoin supplementation in a patient with long-chain fatty acid oxidation disorder

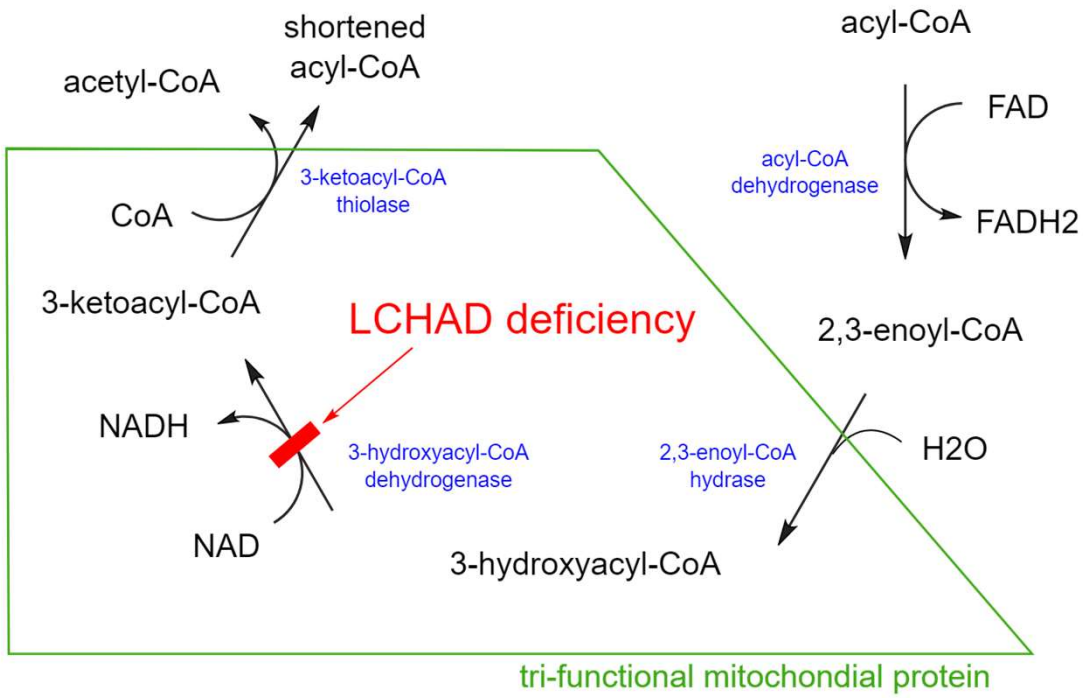
Dr. Francesco Tagliaferri, MD

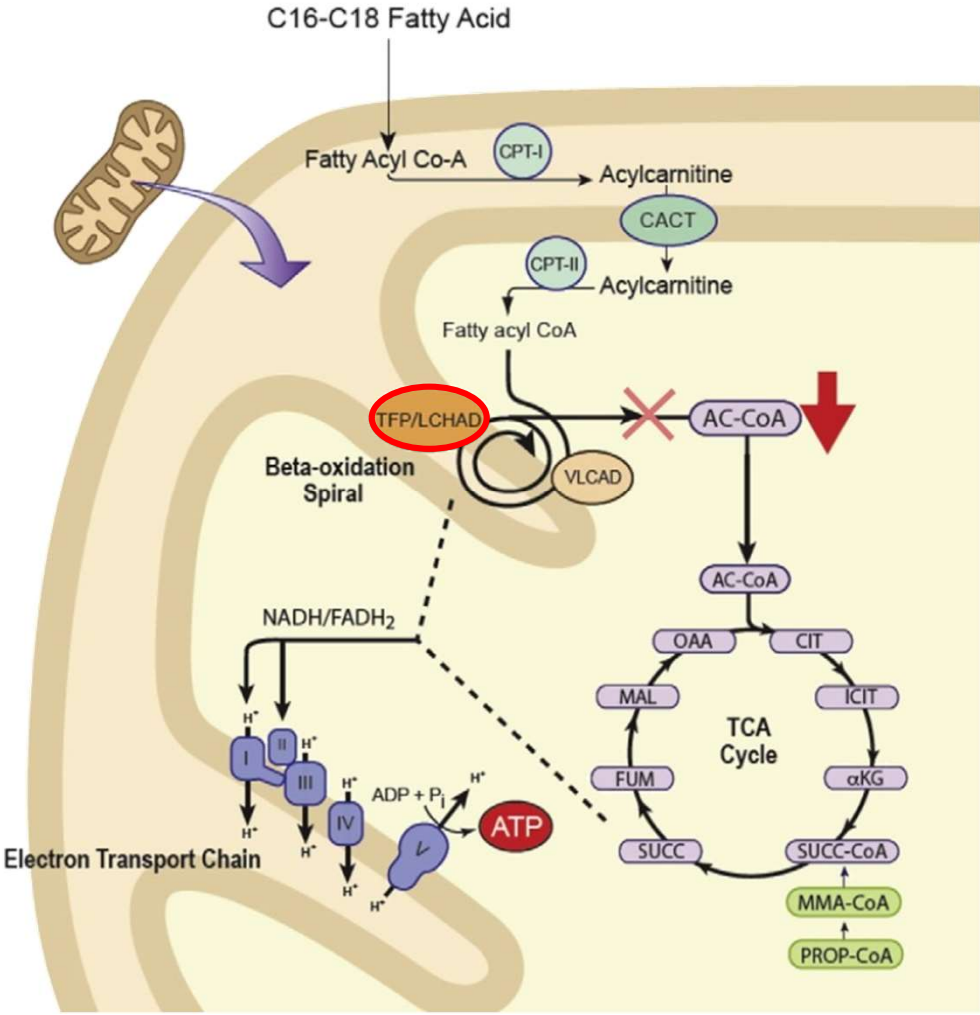
Division of Metabolism, Ospedale Pediatrico
Bambino Gesù, IRCCS, Rome, Italy

Case presentation

- *Female, born April 2015*
- *NBS positive for LCHAD deficiency*
- *2 months: vomiting and hyporexia*
  - *Blood gas analysis: pH 7.13, BE -12, Lac 9.1*
  - *Apnea → ET intubation*
  - *↑ ↑ CK, ↑ LDH, ↑ ALT-AST*
  - *Hypocalcemia, hyperphosphatemia*

LCHAD deficiency

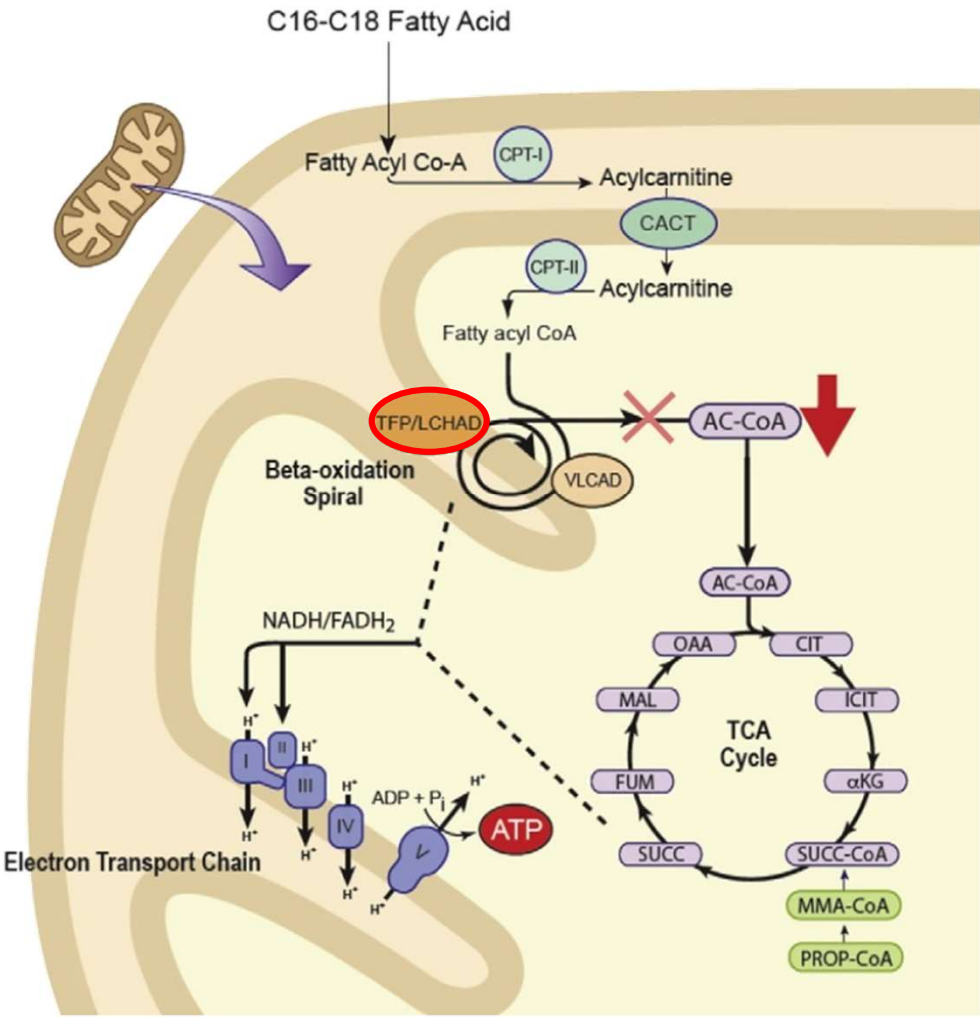




Modified from Vockley et al., 2015

LCHAD deficiency

- Hypoglycemia
- Liver dysfunction
- Lactic acidosis
- Rhabdomyolysis
- Cardiomyopathy
- Hypoparathyroidism
- Acute respiratory distress syndrome



Modified from Vockley et al., 2015

LCHAD deficiency

Diagnosis

- Plasma ACs:

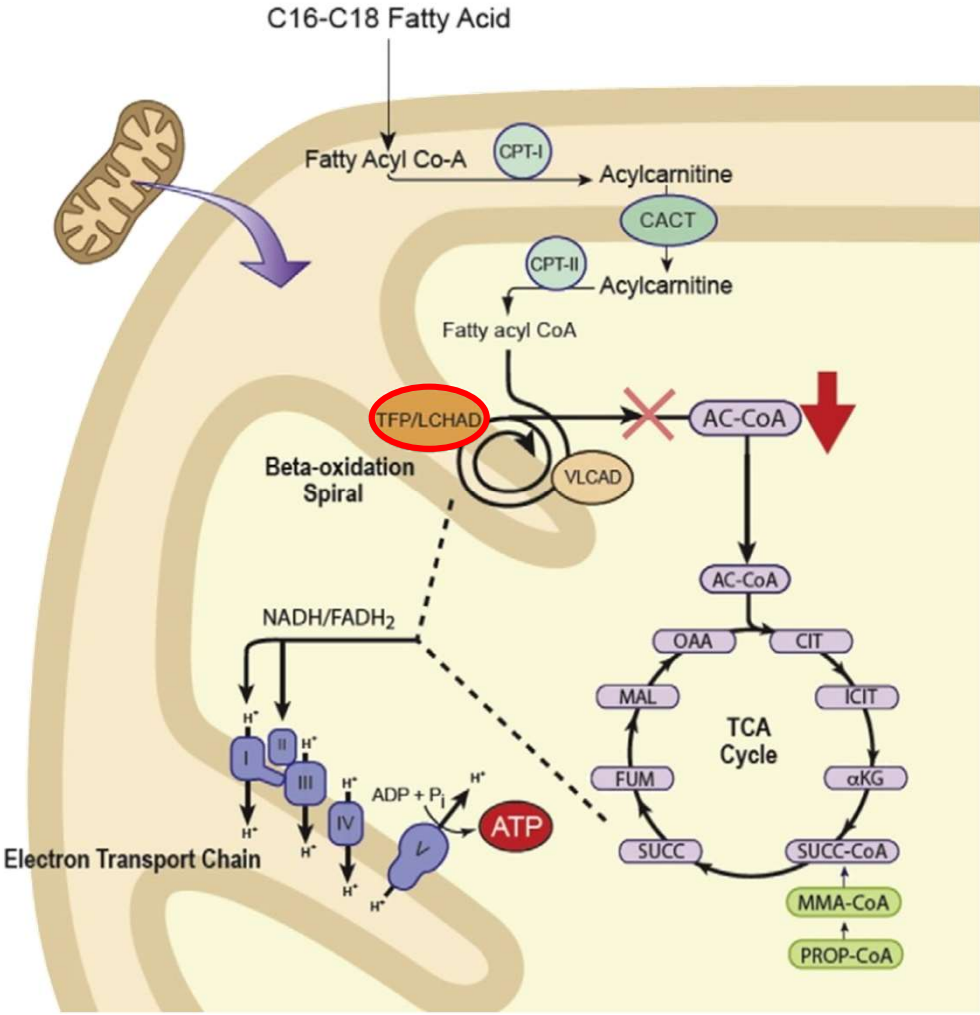
↑C16-OH, ↑C18-OH, ↑C18:1-OH,
↑C16-OH/C16 and C18-OH/C18 ratios

- Urine OAs:

↑3-hydroxy-dicarboxylic acids and lactic acid

Treatment

- low fat isocaloric nutrition,
- with regular food intake and
- MCT supplementation



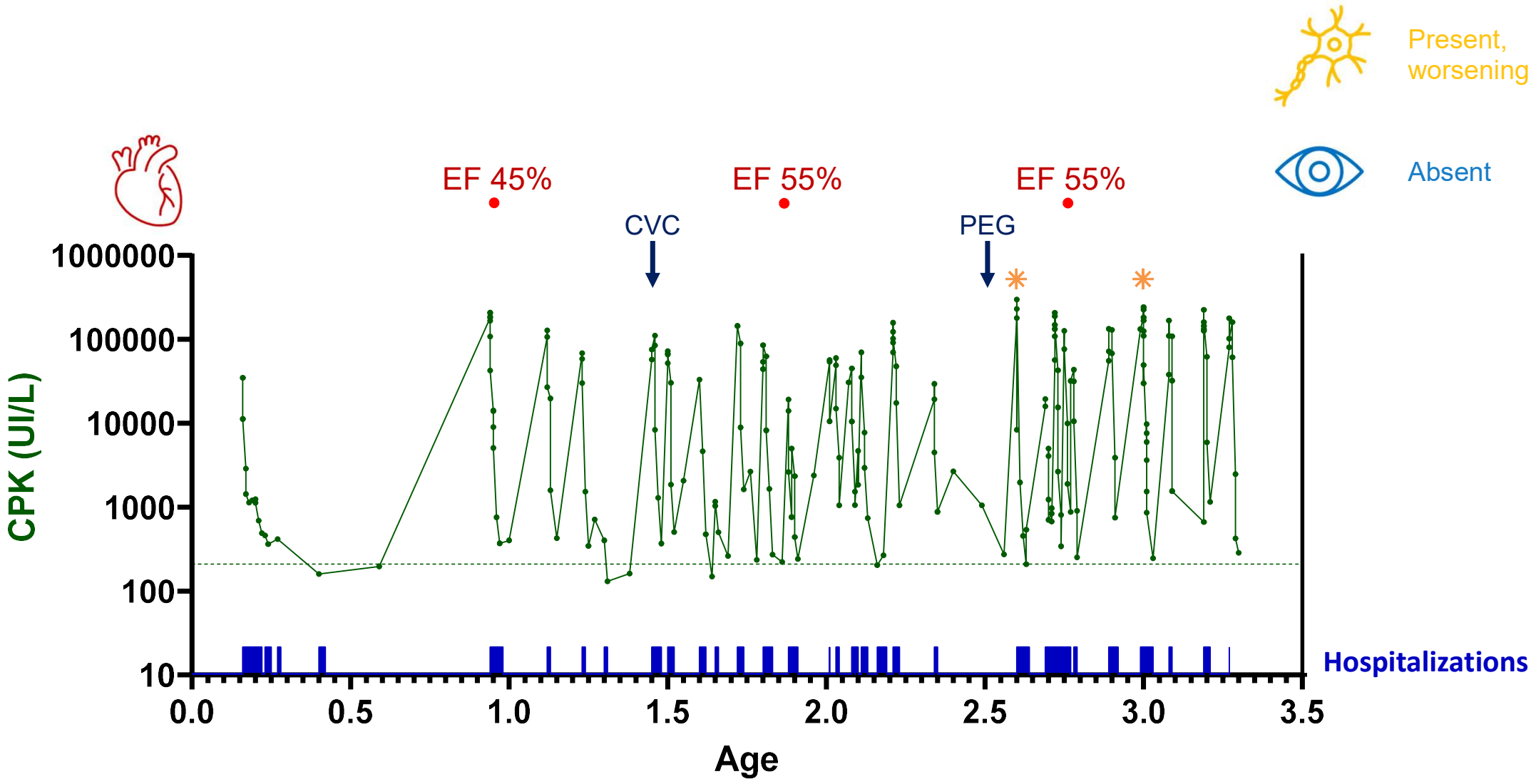
Modified from Vockley et al., 2015

Management

Diet therapy was started with special low-lipid content milk, MCT integration for 30% of kcal/die, bedtime cornstarch integration

Monitor long-term complication




Present, worsening
Absent
EF 45%
EF 55%
EF 55%
CVC
PEG
1000000
100000
10000
1000
100
10
CPK (UI/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Age
Hospitalizations

Triheptanoin (C7)

Case Reports › J Clin Invest. 2002 Jul;110(2):259-69. doi: 10.1172/JCI15311.

Treatment of cardiomyopathy and rhabdomyolysis in long-chain fat oxidation disorders using an anaplerotic odd-chain triglyceride

Charles R Roe [1], Lawrence Sweetman, Diane S Roe, France David, Henri Brunengraber

Molecular Genetics and Metabolism 116 (2015) 53–60

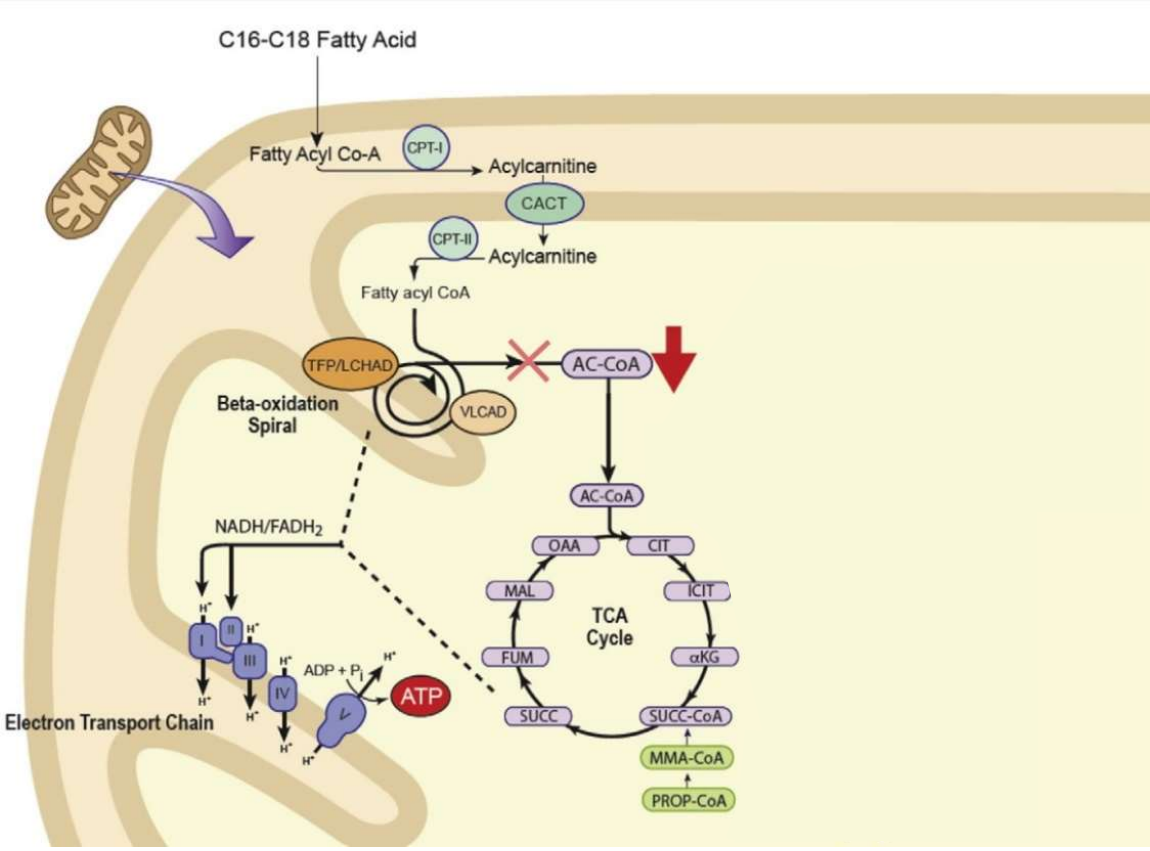
Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

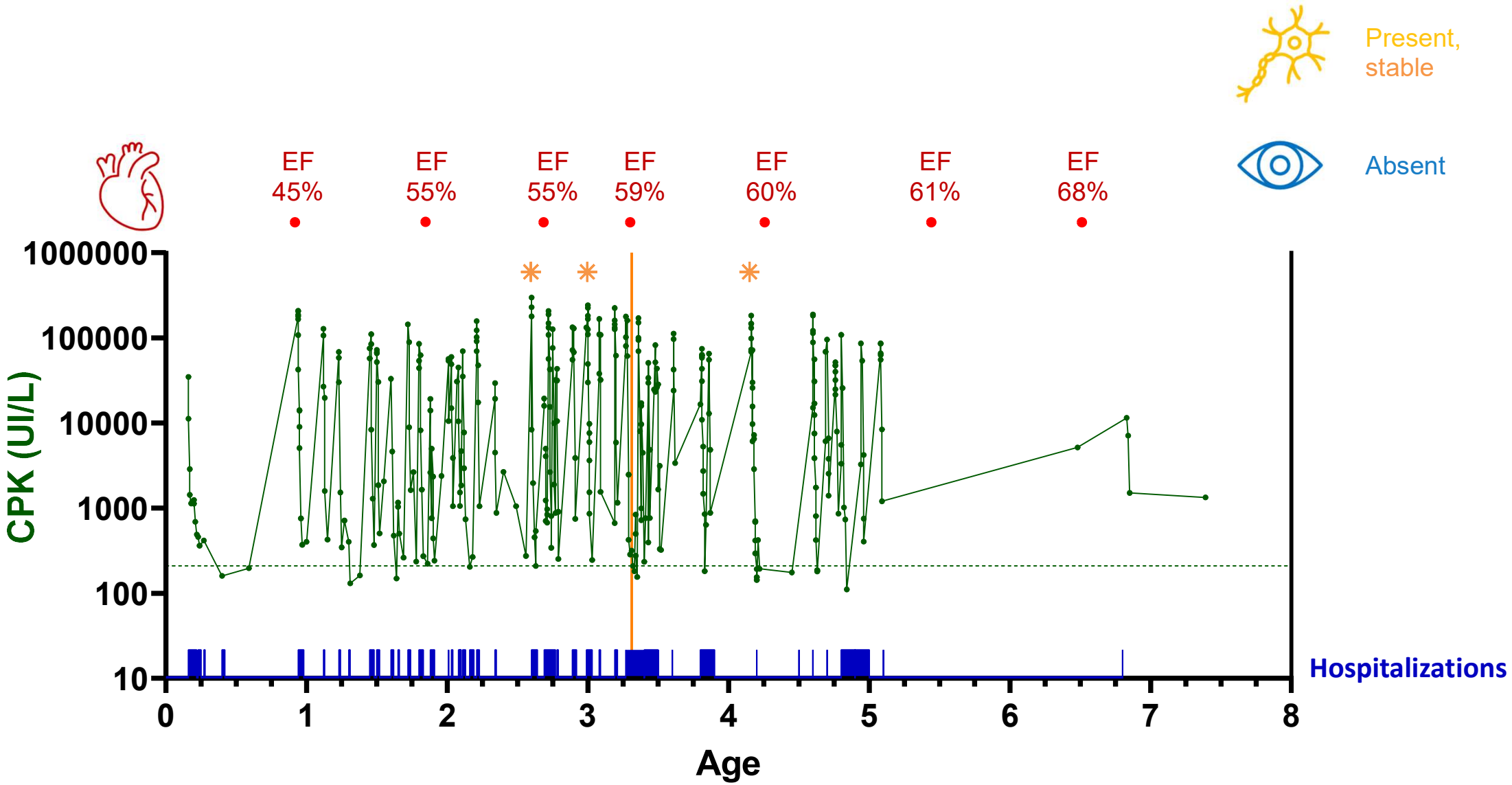
journal homepage: www.elsevier.com/locate/ymgme

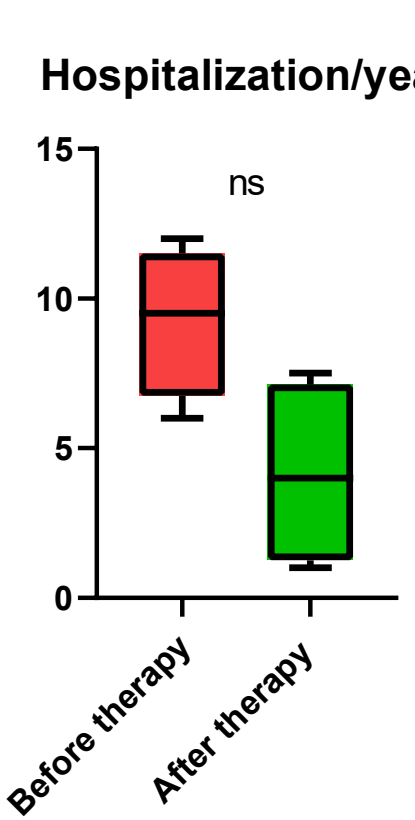
Long-term major clinical outcomes in patients with long chain fatty acid oxidation disorders before and after transition to triheptanoin treatment—A retrospective chart review

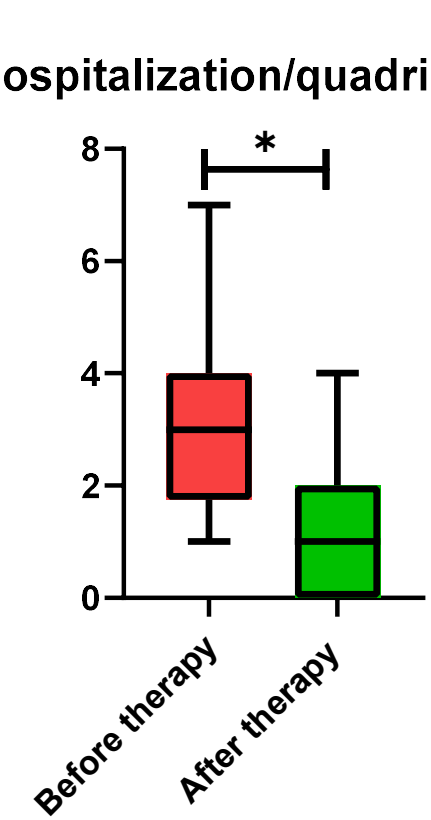
Jerry Vockley [a,b,*], Deborah Marsden [c], Elizabeth McCracken [a], Stephanie DeWard [a], Amanda Barone [a], Kristen Hsu [c], Emil Kakkis [c]

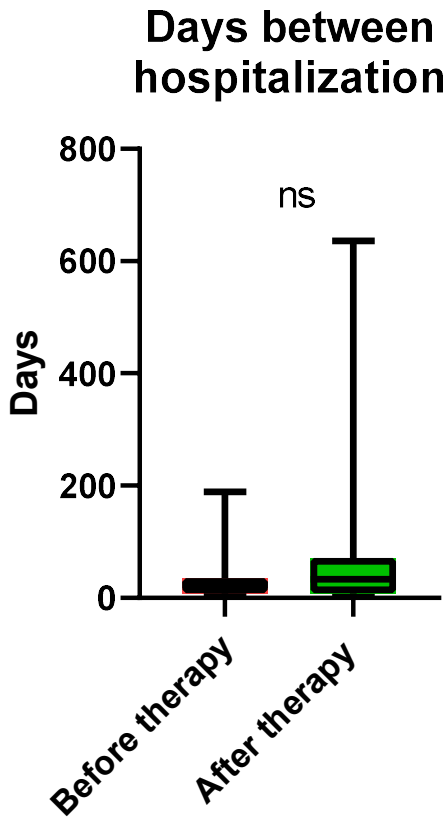


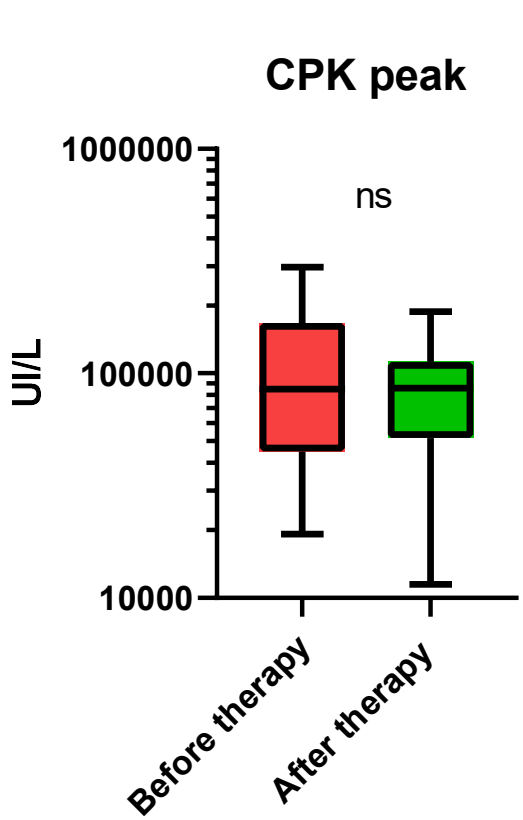
Modified from Vockley et al., 2015


Present, stable
Absent
EF 45%
EF 55%
EF 55%
EF 59%
EF 60%
EF 61%
EF 68%
1000000
100000
10000
1000
100
10
CPK (UI/L)
Hospitalizations
0
1
2
3
4
5
6
7
8
Age


Hospitalization/year
15
10
5
0
ns
Before therapy
After therapy


Hospitalization/quadrimester
8
6
4
2
0
*
Before therapy
After therapy


Days between hospitalization
800
600
400
200
0
Days
ns
Before therapy
After therapy


CPK peak
1000000
100000
10000
UI/L
ns
Before therapy
After therapy

Take home messages

- *Triheptanoin is effective in reducing number of decompensations (hospitalizations), primarily rhabdomyolysis*
- *C7 seems to improve cardiomyopathy (effects on other long term complications?)*
- *Cost-effectiveness and availability*

Acknowledgements

Dott. Carlo Dionisi-Vici

Medical team

Roberta Taurisano
Giorgia Olivieri
Arianna Maiorana
Federica Deodato
Diego Martinelli
Barbara Siri
Elsa Bevivino
Giovanna Cotugno

Laboratory team

Sara Boenzi
Cristiano Rizzo
Bianca Goffredo
Giulio Catesini
Giulia Tozzi
Elisa Sacchetti
Sara Cairoli
Anna Sidorina

Questions?

Radboudumc

Inconclusive laboratory results:

fatty acid oxidation disorder or prematurity-related complications?

Dr. Marloes Michels
Translational Metabolic Laboratory
Radboud university medical centre
Nijmegen, the Netherlands

Case presentation

- *Positive newborn screening in a 6-day old boy*
- *NBS:*

Marker	Concentration	Reference
Free carnitine	7.4 µmol/L	
C14:1	0.52 µmol/L	<0.60 µmol/L
C16-OH	**0.56** µmol/L	<0.08 µmol/L
C14:1 / C2	**0.090**	<0.023

- *→ suspicion of fatty acid oxidation (FAO) disorder*

Clinical case presentation

- *Prematurely born 24+2 wks (spontaneous delivery)*
- *Severe respiratory failure & perinatal asphyxia*
- *Anaemia*
- *Multiple hematomas*
- *Large intraventricular haemorrhages in the cerebrum*
- *Sepsis (& possibly meningitis)*

- *Hypoglycaemia (1st measurement) → hyperglycaemia*
- *Persistent lactate acidosis*

What should we do (first)?

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- *Omit fatty acids from nutrition & ensure intake*
- *Start further metabolic work-up*
- *Check further clinical parameters*

Mitochondrial fatty acid oxidation

C12- C22 → *C4-C12*

①	VLCAD	MCAD	SCAD
②	LCEH	EH (crotonase)	
③	LCHAD	HAD (SCHAD)	
④	LCKAT	KAT (M/SCKAT)	

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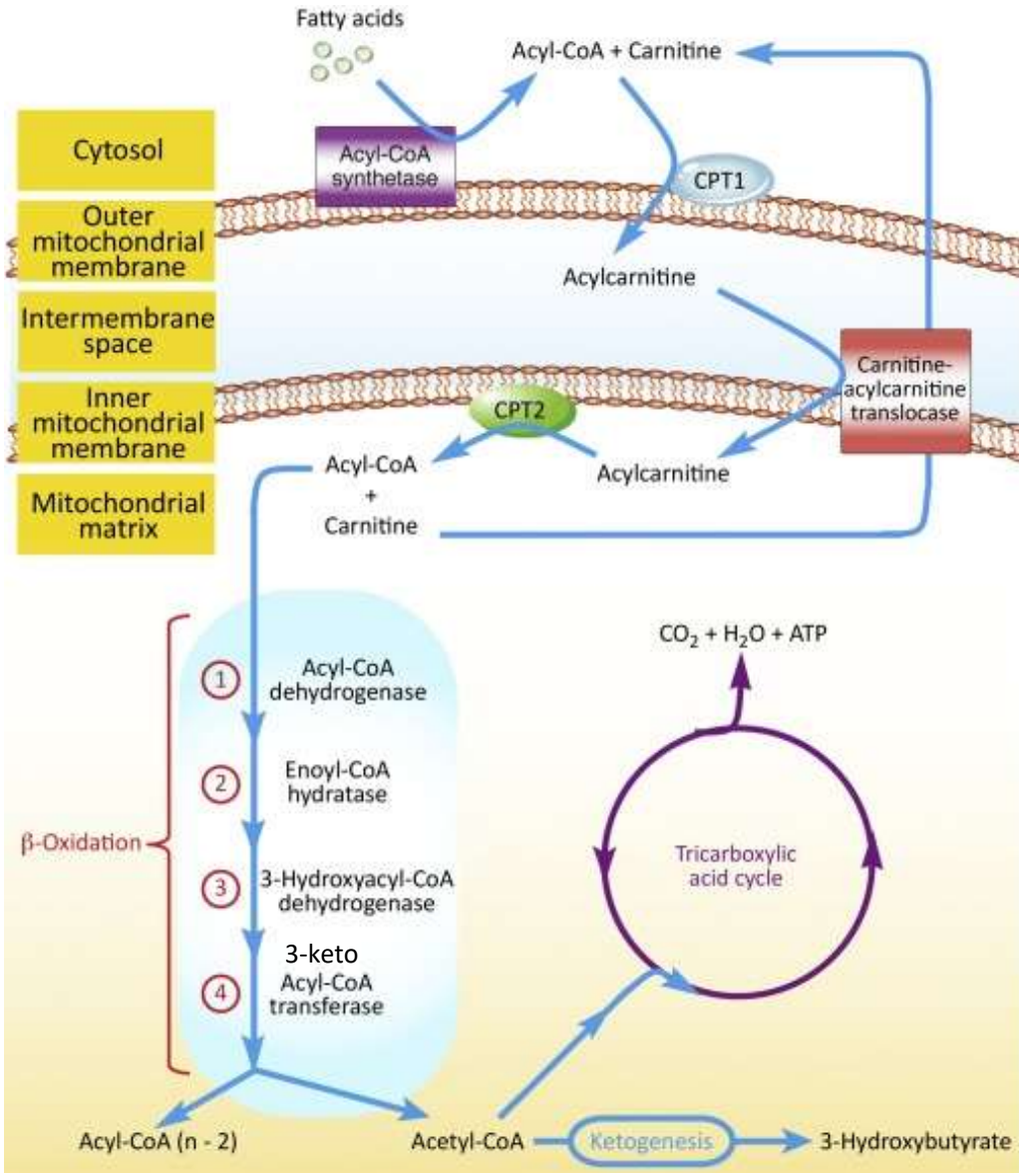
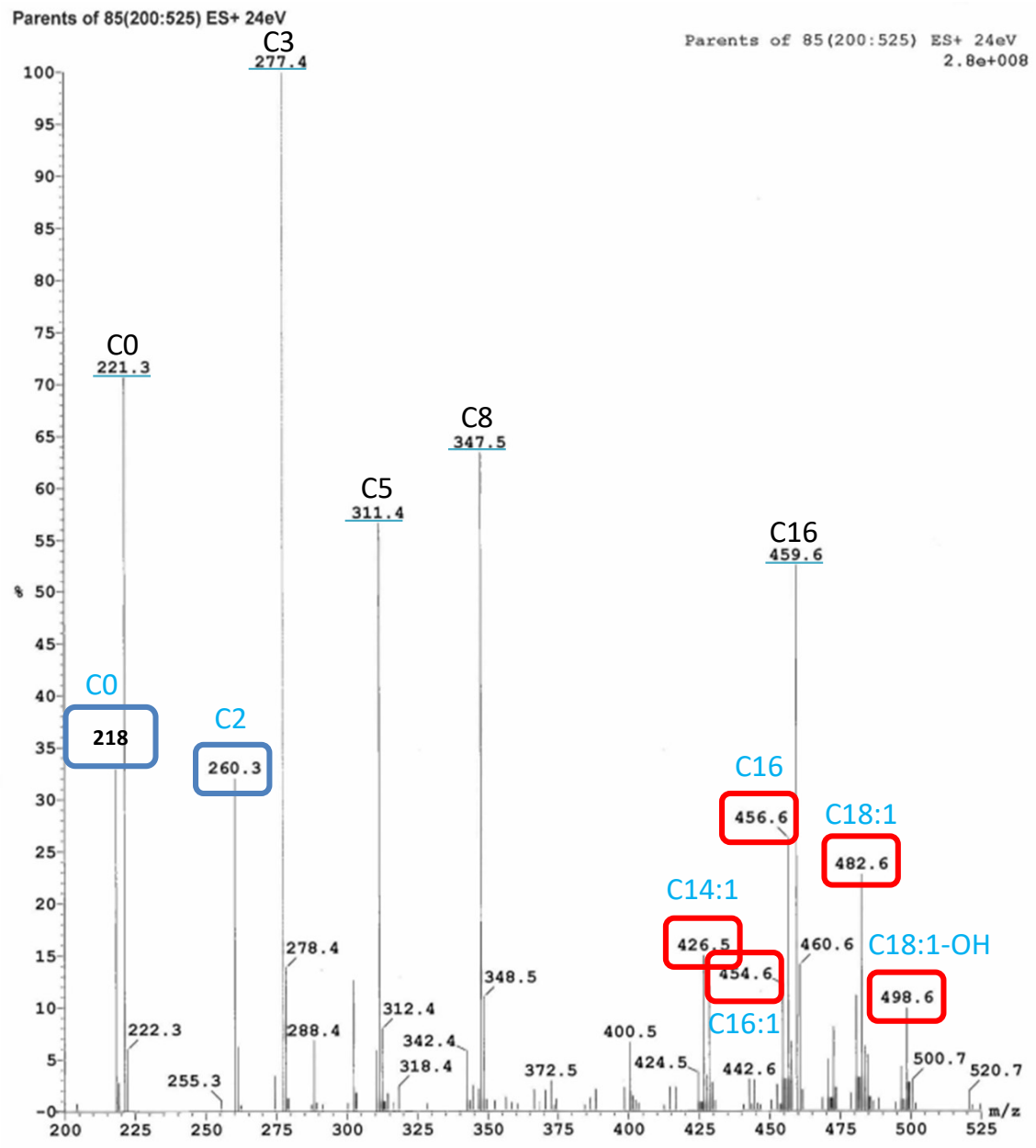


Extended laboratory investigations

- *Acylcarnitine profiling:*
  → *measure fatty acid derived acylcarnitines in blood*

<u>Underlined:</u>
internal standards (d_3)



Acylcarnitine profile

Marker	Concentration	Reference
Total carnitine	**21.63** µmol/L	25 – 65 µmol/L
Free carnitine	**11.64** µmol/L	20 – 55 µmol/L
C14	**0.44** µmol/L	<0.13 µmol/L
C14:1	**0.54** µmol/L	<0.17 µmol/L
C16	**1.05** µmol/L	<0.23 µmol/L
C16:1	**0.49** µmol/L	<0.08 µmol/L
C16:1-OH	**0.20** µmol/L	<0.02 µmol/L
C16-OH	**0.33** µmol/L	<0.02 µmol/L
C18	**0.24** µmol/L	<0.09 µmol/L
C18:1	**1.01** µmol/L	<0.28 µmol/L
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Extended laboratory investigations

- *Acylcarnitine profiling:*
  → *Elevated long chain acylcarnitines in blood*

- *Enzyme studies*
  → *measure mFAO enzymes in lymphocytes*

Lymphocytes

mFAO enzyme studies

Enzyme	Activity (nmol/ min.mg protein)	Reference
VLCAD	***3.90***	2.15 – 3.79
LCHAD	**19**	22 – 74
LCKAT	**4**	23 – 43
SCHAD	122	77 – 185

MTP

Extended laboratory investigations

- *Acylcarnitine profiling:*
  → *Elevated long-chain fatty acids*

- *Enzyme studies (lymphocytes):*
  → *high-normal VLCAD activity*
- → *decreased LCHAD and especially LCKAT activity*

- *Whole exome sequencing*

Whole exome sequencing

- *1 variant of unknown significance (class III) in* ACADVL
  → *gene encoding VLCAD*

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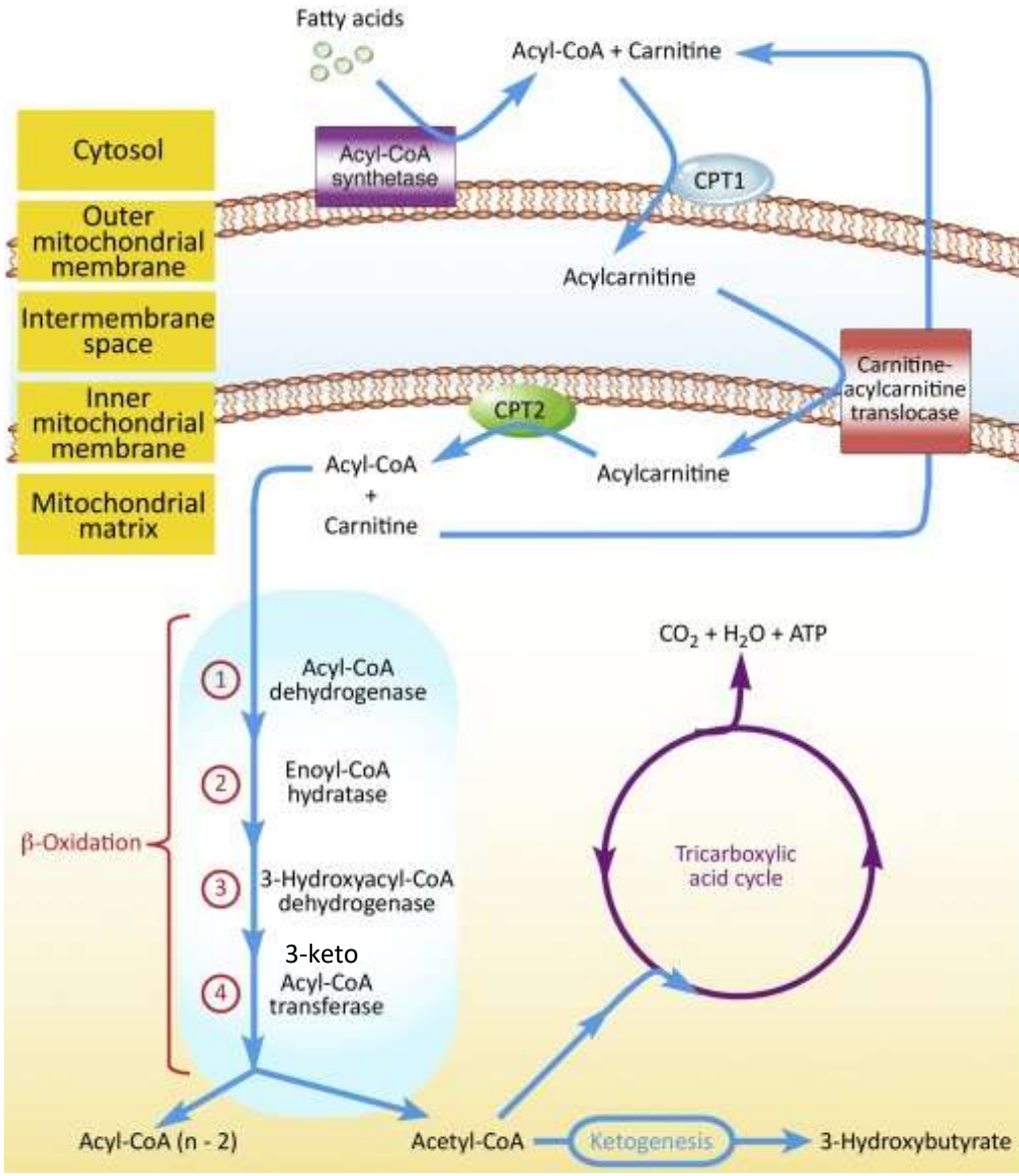

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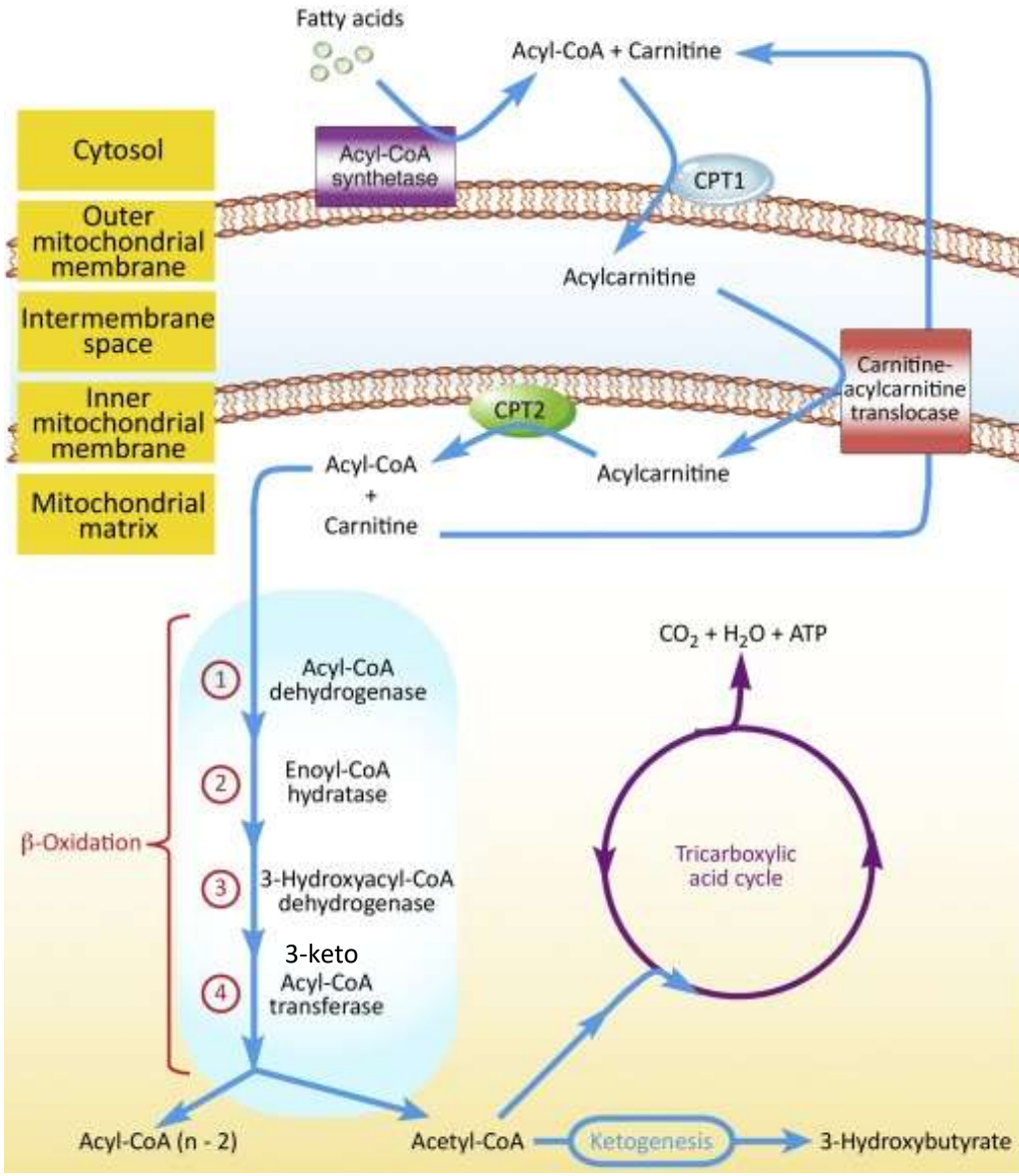

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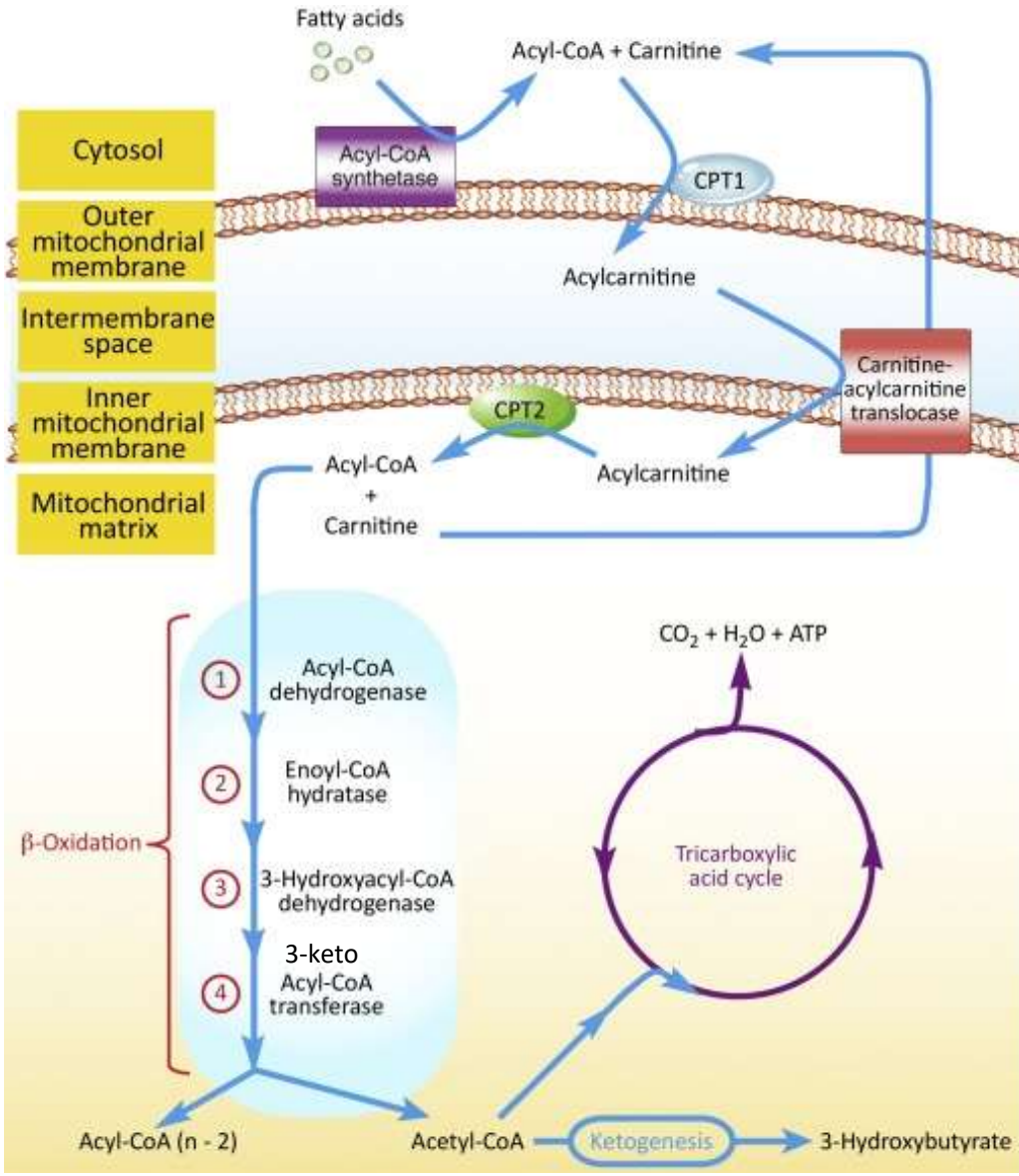

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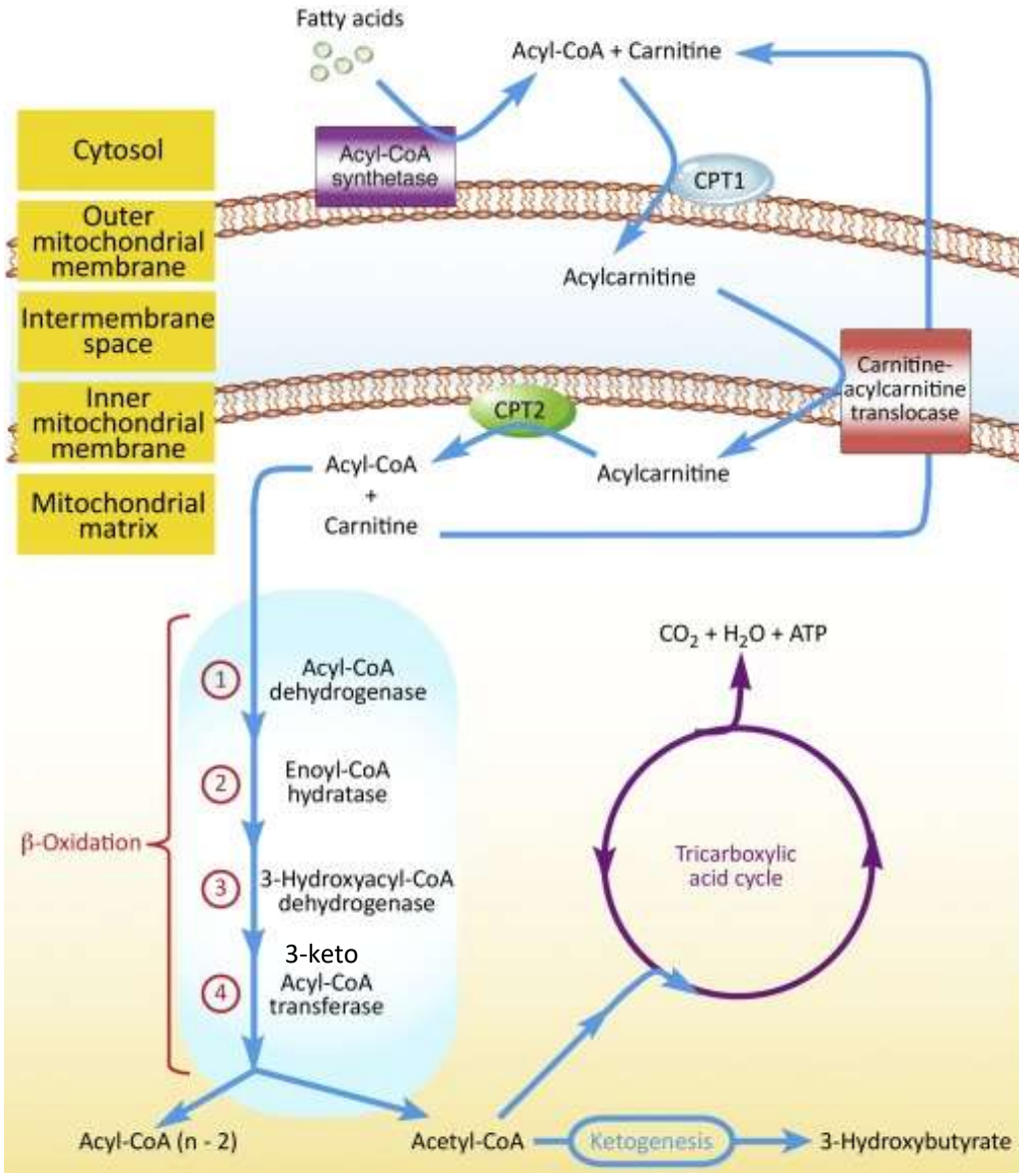


Clinical follow-up

- *Infection (largely) overcome*
- *Respiratory problems slightly improved*
- *Normoglycaemic (→ hyperglycaemic)*
- *Cerebral hemorrhages*
  → multidisciplinary evaluation of neurological damage and expected (neurological) outcome

- *Day 12: discontinuation of treatment due to disproportionality in relation to expected outcome*

mFAO disorder or prematurity-related complications?

- *Biochemical clues:*
- *Genetic clues:*
- *Clinical clues:*
  - *→ complex due to extreme prematurity and other complications*
  - *no cardiomyopathy, liver failure, …*

Fibroblasts

mFAO enzyme studies

	Enzyme	Activity (nmol/ min.mg protein)	Reference
	VLCAD	3.35	1.38 – 5.72
MTP	LCHAD	**19**	34 – 114
MTP	LCKAT	**6**	58 – 110

Long Template PCR

- *HADHA and HADHB*
  → encoding MTP (mitochondrial trifunctional protein)

- *Coding areas (exons) and non-coding areas (introns)*

Mitochondrial fatty acid oxidation

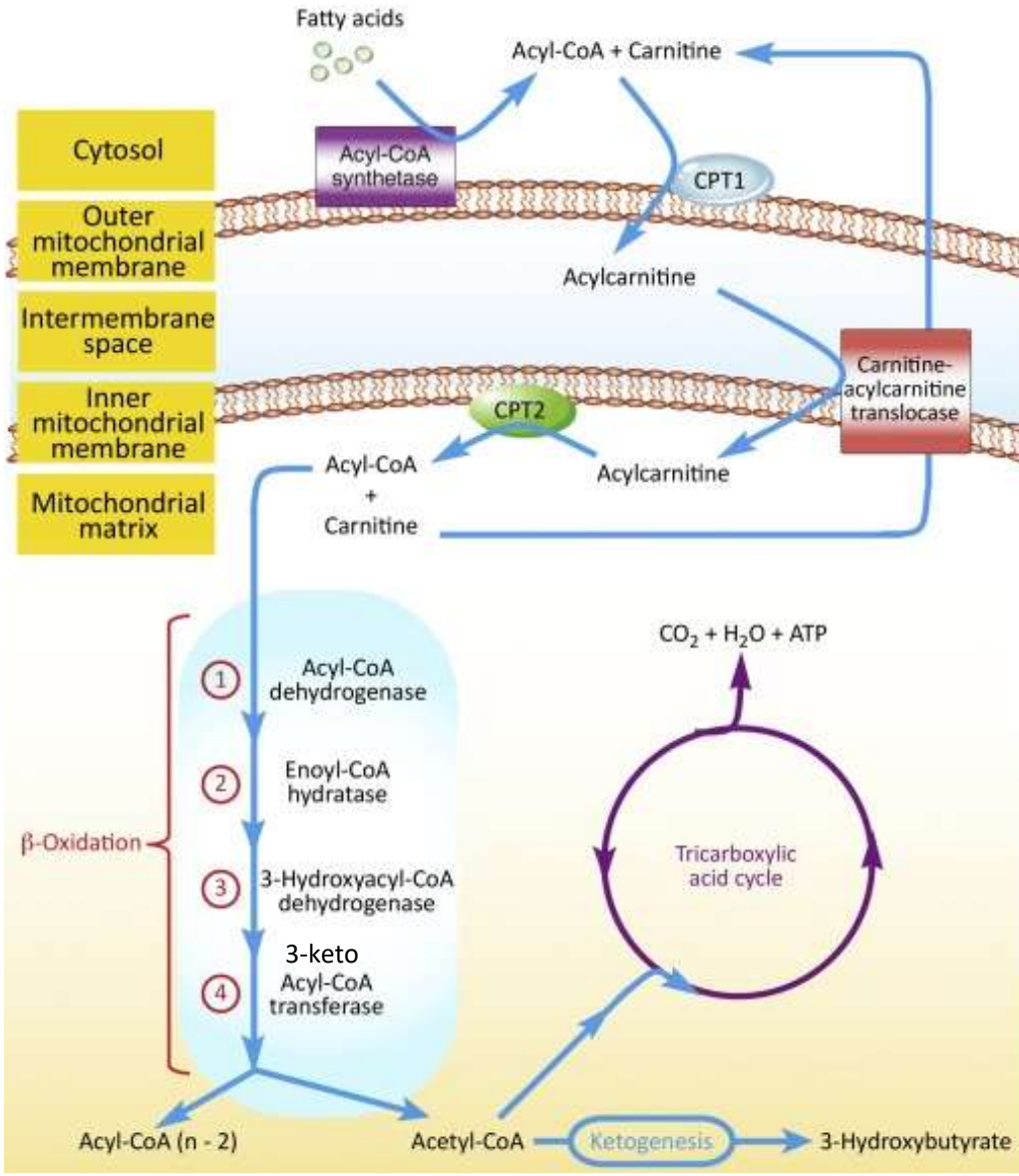
C12- C22 → *C4-C12*

1. VLCAD — MCAD — SCAD
2. LCEH — EH (crotonase)
3. LCHAD — HAD (SCHAD)
4. LCKAT — KAT (M)

MTP

α-unit — *HADHA*

β-unit — *HADHB*



Long-read sequencing

- *2 intronic variants in HADHB (class III):*
  - *c.1390-515_1390-499del (p.?)*

Conclusion

- *Biochemical clues:*
- *Genetic clues:*
- *Clinical clues:*

- ***LCKAT/MTP deficiency***

Acknowledgements

- *Dr. Marleen Huigen*
- *Dr. Leo Kluijtmans*
- *Dr. Maaike de Vries*
- *Prof. dr. Bert van den Heuvel*
- *Dr. Richard Rodenburg*
- *Dr. Sacha Ferdinandusse*

Questions?