# *Workshop organic acidemias*
# *Case 1*

# *Convulsions during rotavirus gastroenteritis?*

# *Clinical presentation*

- born at term after an uneventful pregnancy
- second child of healthy, non-related Swiss parents
- NBS was performed correctly with unremarkable results
- previous development was normal
- previous weight, height, head circumference were normal

# *Clinical presentation*

At the age of 9 months:

- presented to the emergency department with fever, vomiting, diarrhea, and mild dehydration
- body weight 62$^{nd}$ percentile, length 39$^{th}$ percentile, head circumference 63$^{rd}$ percentile
- admitted due to several short clonic seizures with eye deviation

What would you want to know?

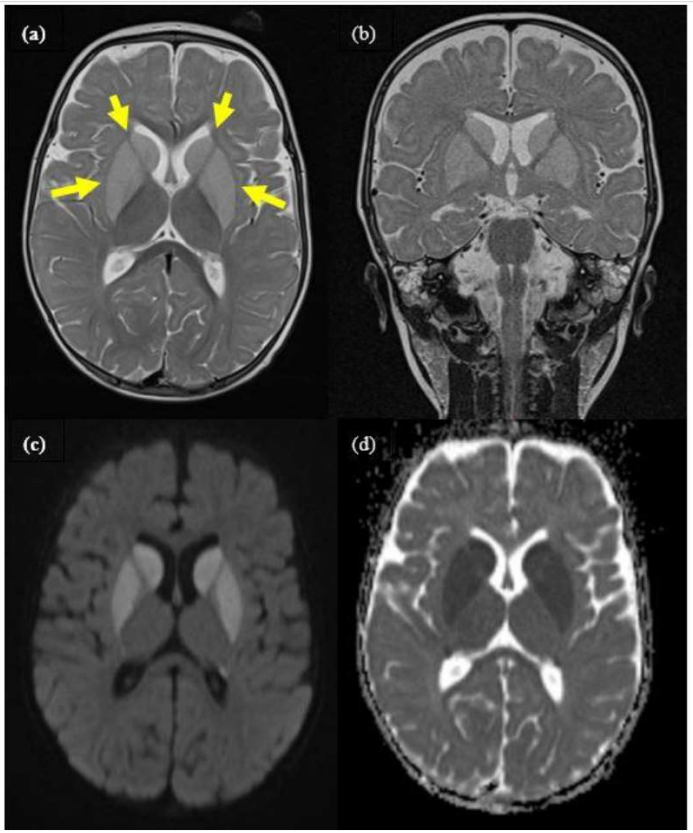
# *Investigations*

At the age of 9 months:

- Serum electrolytes and glucose were normal
- rotavirus antigen positive in feces

  → initial diagnosis: convulsions associated with rotavirus GE
- low-dose carbamazepine was started
- patient worsened next day with development of an acute encephalopathic crisis with muscular hypotonia, sleepiness, and repeated dystonic posturing with paroxysmal eye movements

# Brain MRI




bilateral signal alterations of basal ganglia affecting caudate, putamen, and pallidum

What is your diagnosis?

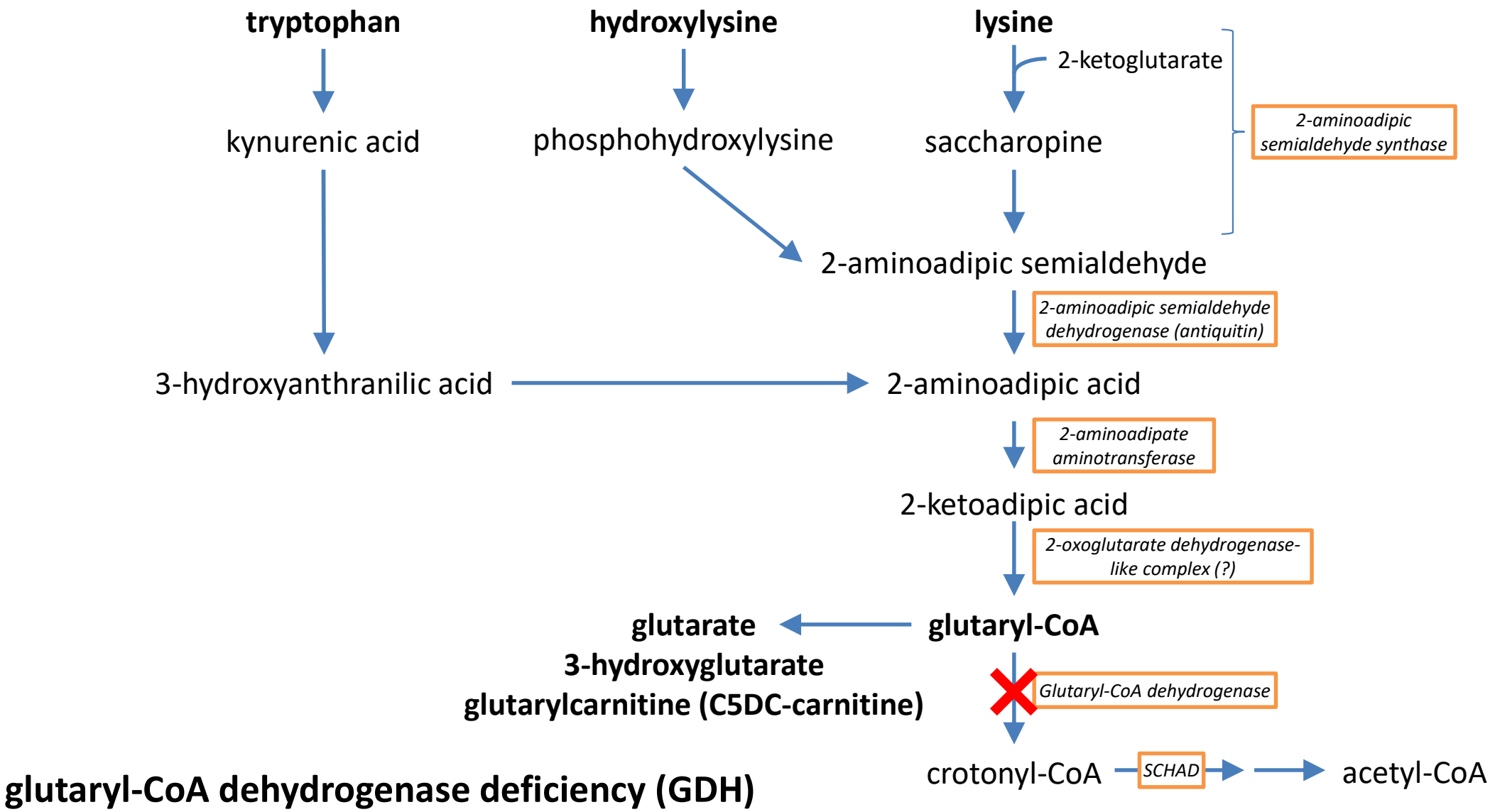
What investigation would you do?

# Metabolic screening

- Amino acids (plasma): normal
- Acylcarnitines (blood): normal (in total 14 tests over 8 years)
- Organic acids: elevation of urinary glutaric acid and 3-hydroxyglutaric acid

| Age | 4 d | Reference Range | 9 m | 10 m | 13 m | 15 m | 18 m | 24 m | Reference Range |
|---|---|---|---|---|---|---|---|---|---|
| **DBS** | | | | | | | | | |
| Free carnitine | 26 | 7–55 | 26 | 45 | 51 | 38 | 35 | 46 | 11–44 |
| Glutarylcarnitine | 0.5 | <0.53 | 0.03 | 0.04 | 0.04 | 0.06 | 0.01 | 0.04 | <0.06 |
| **Urine** | | | | | | | | | |
| Glutaric acid | n.d. | | 26 | <10 | <10 | n.d. | n.d. | <10 | 0–20 |
| 3-hydroxyglutaric acid | n.d. | | 22 | 19 | 23 | n.d. | n.d. | 17 | 0–10 |

Free carnitine & glutarylcarnitine in µmol/L - Glutaric acid & 3-hydroxyglutaric in mmol/mol creatinine


tryptophan
hydroxylysine
lysine
2-ketoglutarate
kynurenic acid
phosphohydroxylysine
saccharopine
2-aminoadipic semialdehyde synthase
2-aminoadipic semialdehyde
2-aminoadipic semialdehyde dehydrogenase (antiquitin)
3-hydroxyanthranilic acid
2-aminoadipic acid
2-aminoadipate aminotransferase
2-ketoadipic acid
2-oxoglutarate dehydrogenase-like complex (?)
glutarate
3-hydroxyglutarate
glutarylcarnitine (C5DC-carnitine)
glutaryl-CoA
Glutaryl-CoA dehydrogenase
crotonyl-CoA
SCHAD
acetyl-CoA
glutaryl-CoA dehydrogenase deficiency (GDH)

# Further course

- Carnitine supplementation and a protein-restricted diet were started according to current guidelines
- family received an emergency plan

# Genetic confirmation

- compound heterozygous *GCDH* genotype:
- pathogenic variants c.722G>T, p.Gly241Val (not previously described) and c.1169G>C, p.Gly390Ala

Alternative for confirmation: enzymatic studies (fibroblasts or leukocytes)

# Outcome

- developed dystonic movement disorder
- global developmental delay
- age of 23 months: not able to sit, but was not dependent on tube feeding and had preserved good social contact
- age of 5 years: dyskinetic movement disorder but good social contact, still not dependent on tube feeding

# GA1 – adults

**Late-onset (adult) presentations (<10-20% of cases)**

No dystonia or striatal injury

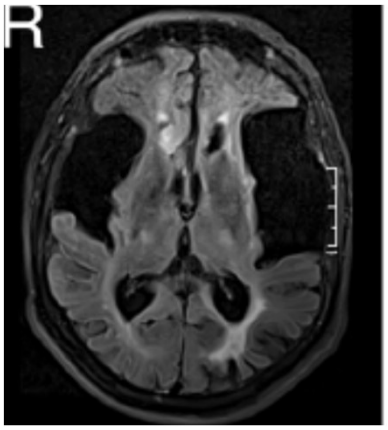
**Clinical:**

Asymptomatic (mother of newborns - picked up on newborn screening)

Mild (headache) → more severe neurology (dementia, tremor, epilepsy)

**MRI:**

Frontotemporal hypoplasia, white matter changes, subependymal lesions




Boy *et al*, OJRD 2017

# *Take home message*

- GA-1 can be missed in NBS (see: Int. J. Neonatal Screen. 2021, 7, 32. https://doi.org/10.3390/ijns7020032 reporting 4 cases)
- a normal C5DC after a borderline elevated screening result cannot exclude GA-1
- low excretors can have normal C5DC even on carnitine
- urinary glutarylcarnitine may be useful for low excretors
- If clinical suspicion of GA-1, complete workup, including urinary glutarylcarnitine and genetic/enzymatic testing, is necessary

# *A newborn girl with poor feeding*

# History & examination

- Born at term, normal pregnancy & delivery, 3.20 kg
- 1st child of healthy non-consanguineous parents
- Discharged after 24 hours
- Readmitted on day 3 with poor feeding
- Sleepy, hypotonia, 2.84 kg

# Investigations

- Blood glucose low: 1-2 mmol/L (20 -35 mg/dl)
- Blood gases: pH 7.1 (7.32-7.42), BE – 12 mmol/L (-2 to +2)
- Lactate: 7 mmol/L (<2.4)
- Calcium (ionized):  0.9 mmol/L (1.16-1.31)
- Hb 160 g/L (134-199), leukocytes 2.5 x $10^6$/L (9-30), platelets 100 x $10^6$/L (150-450)
- Ammonia 405 µmol/l (< 50)

What are the differential diagnoses of neonatal hyperammonemia?

# DD for neonatal hyperammonemia

- Branched-chain organic acidemias
- Urea cycle disorders
- Fatty acid oxidation disorders
- Pyruvate carboxylase deficiency etc.
- Transient hyperammonemia of newborn
- Non-metabolic causes, e.g. disseminated herpes simplex

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REVIEW ARTICLE

JIMD SSIEM WILEY

**Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision**

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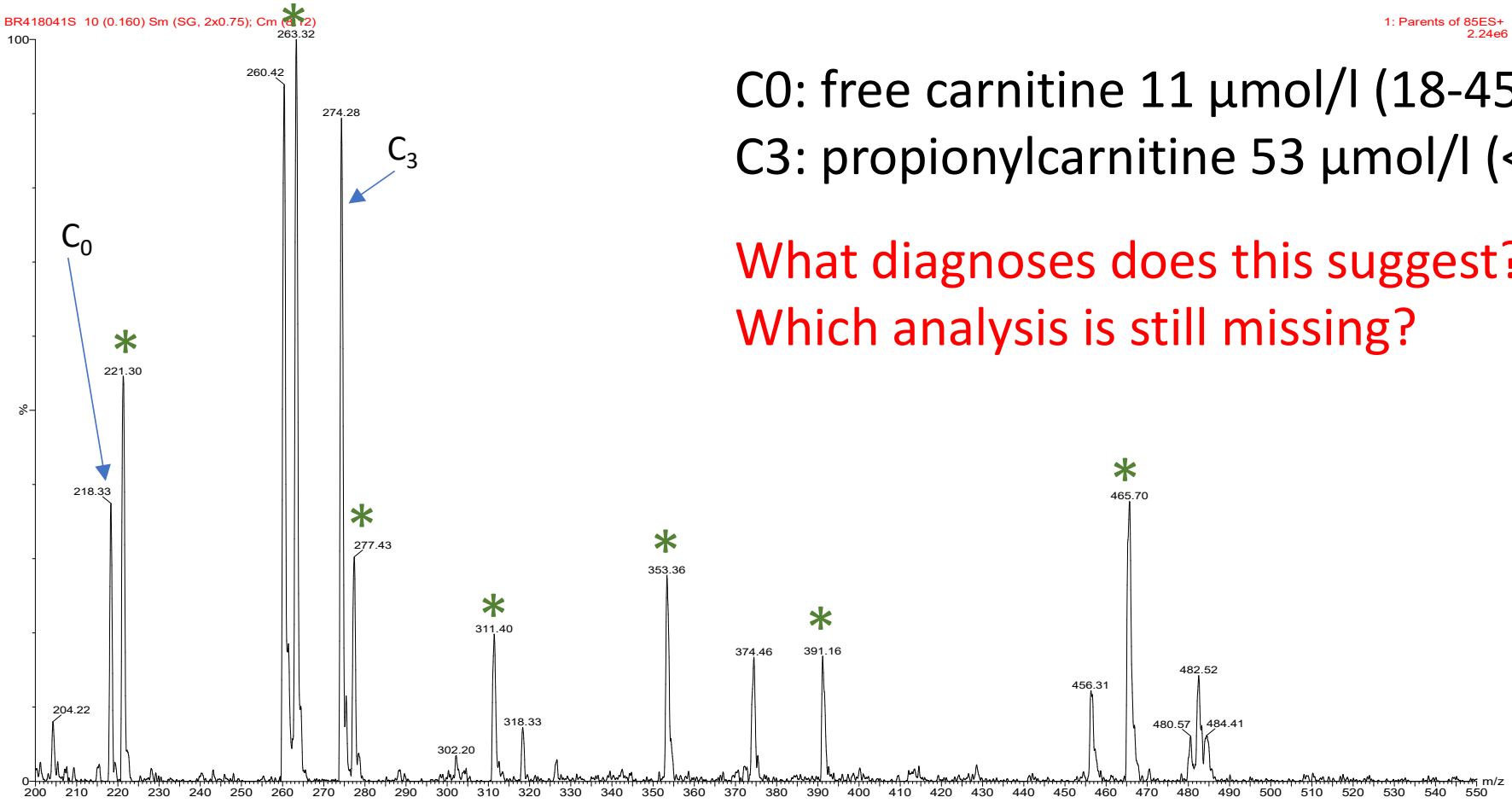
Which is most likely & why?

Which further analyses would you do?

# Further analyses

- Plasma amino acids: nonspecific changes, glutamine normal
- Total homocysteine: normal

# Acylcarnitine profile



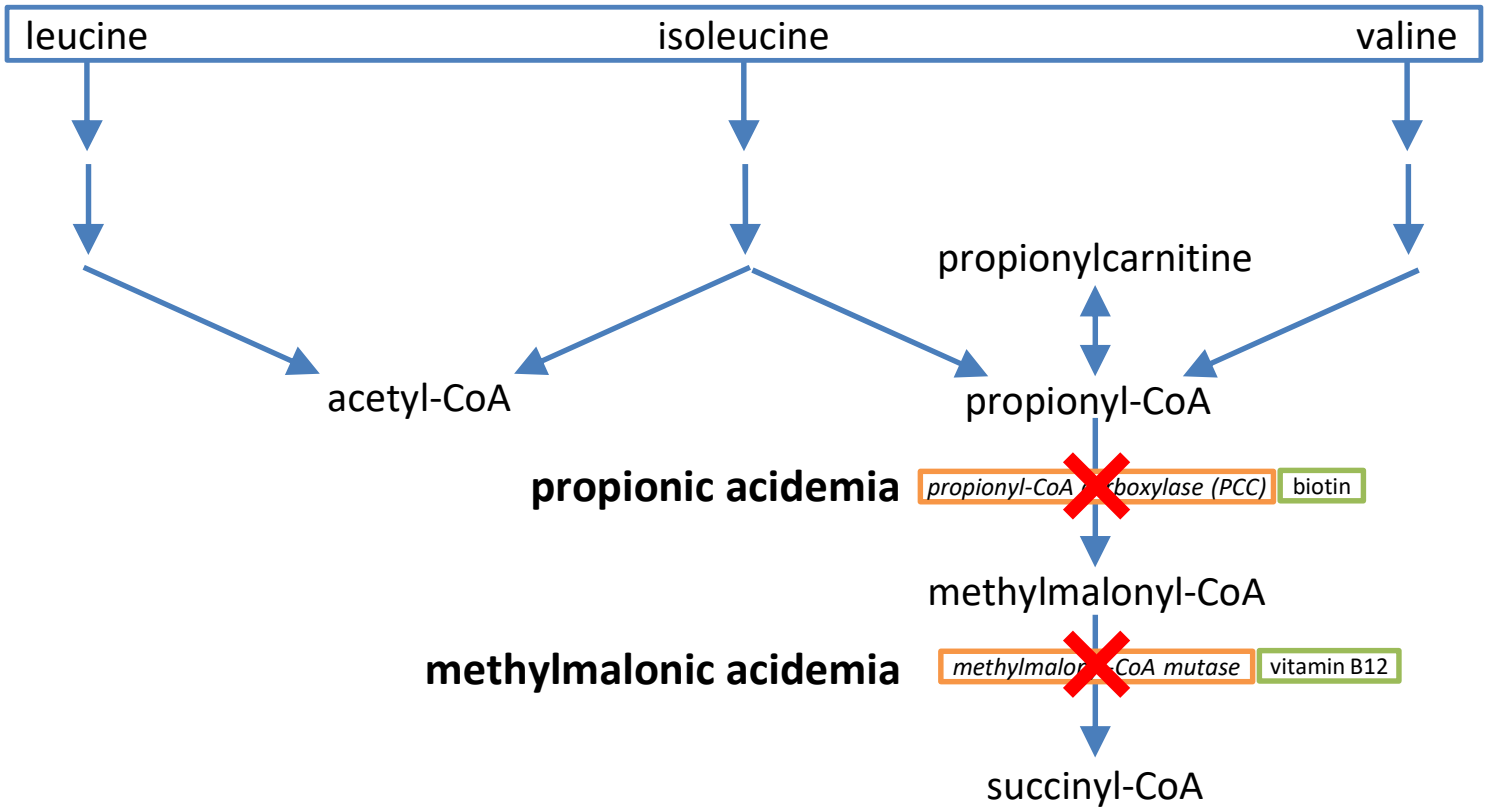

C0: free carnitine 11 µmol/l (18-45)

C3: propionylcarnitine 53 µmol/l (< 3)

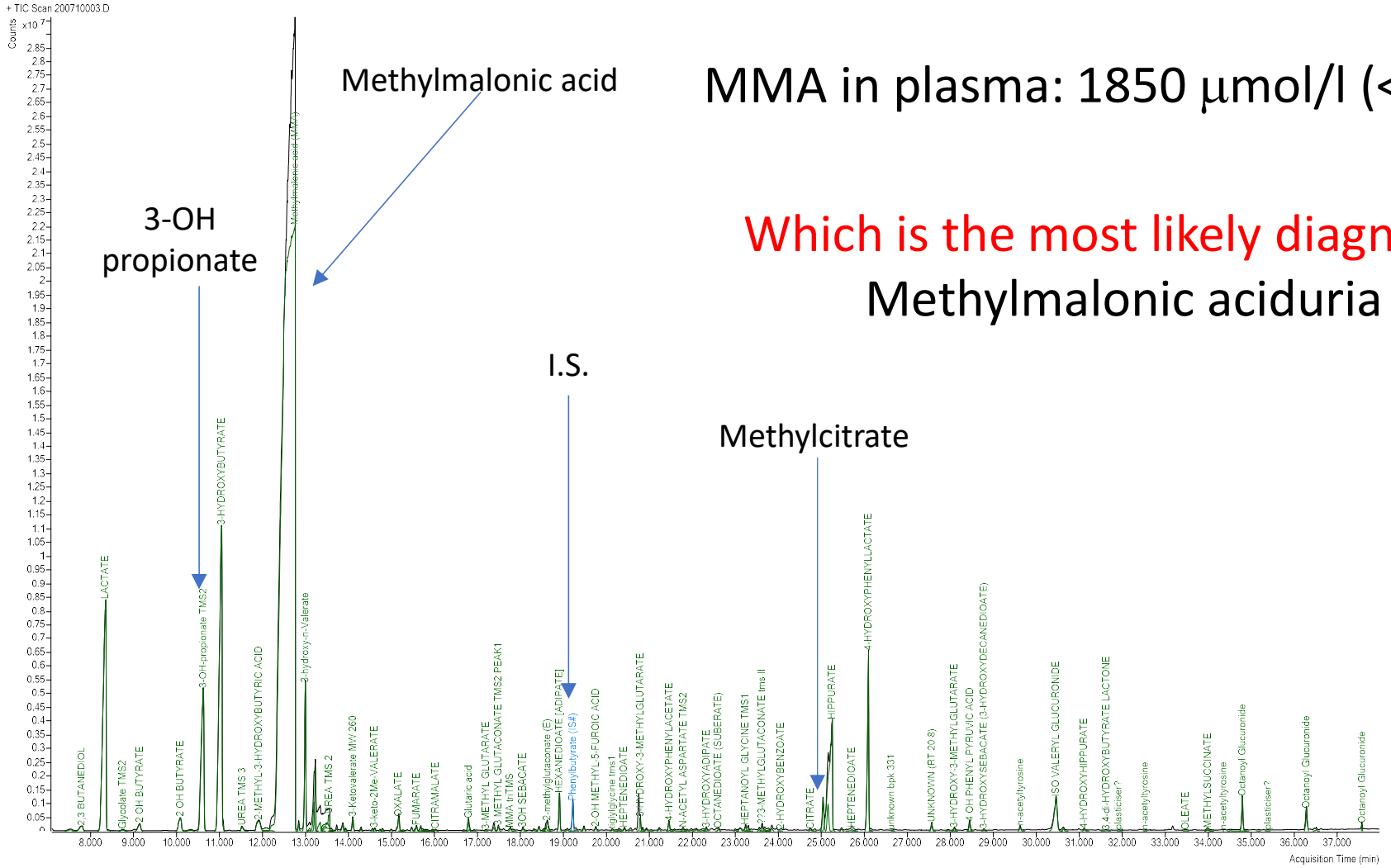
What diagnoses does this suggest?

Which analysis is still missing?

# Propionic aciduria or methylmalonic aciduria



# Organic acids in urine

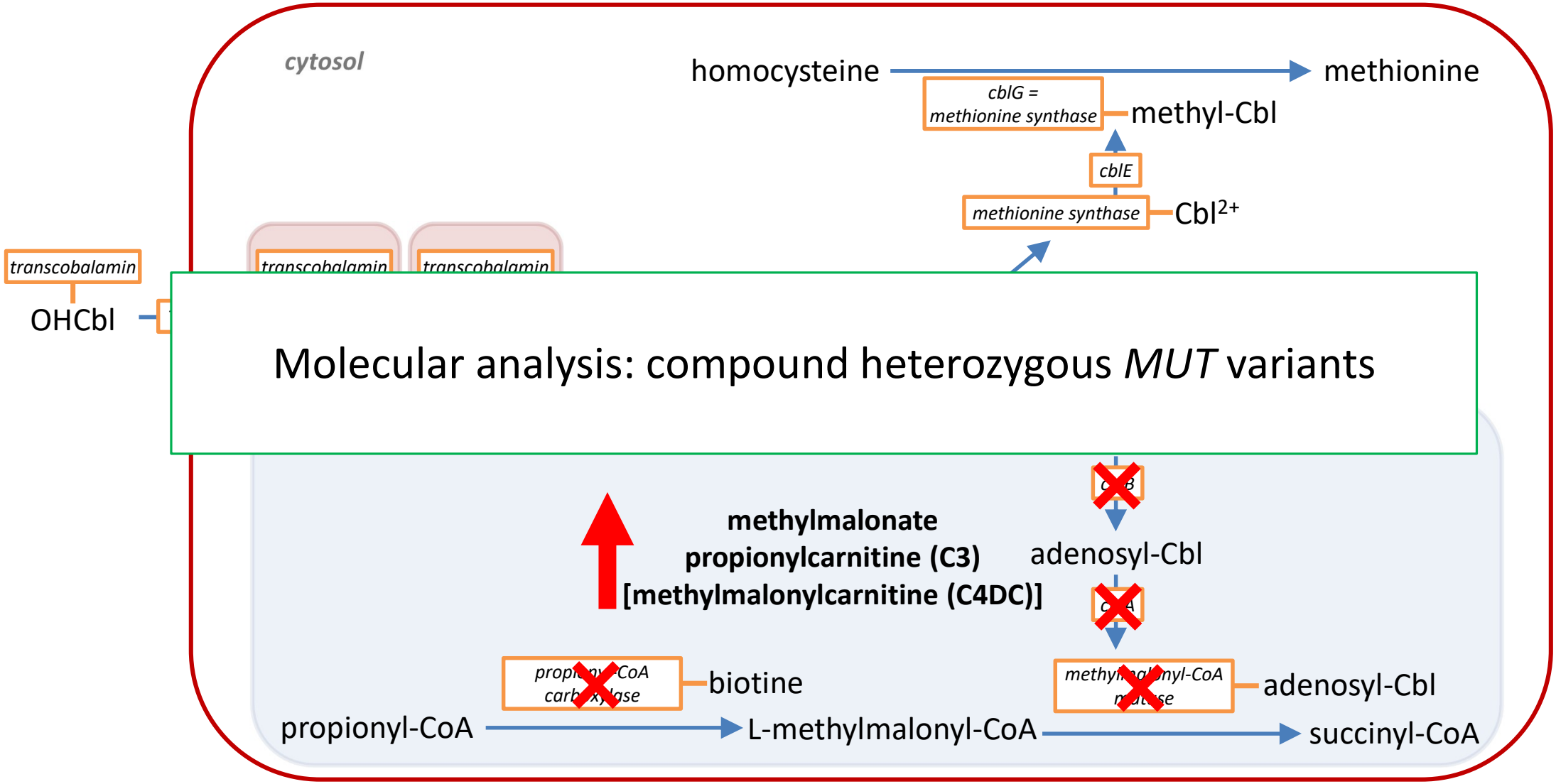



MMA in plasma: 1850 µmol/l (<0.28)

Which is the most likely diagnosis?

Methylmalonic aciduria

## DD MMAuria without hyperhomocysteinemia

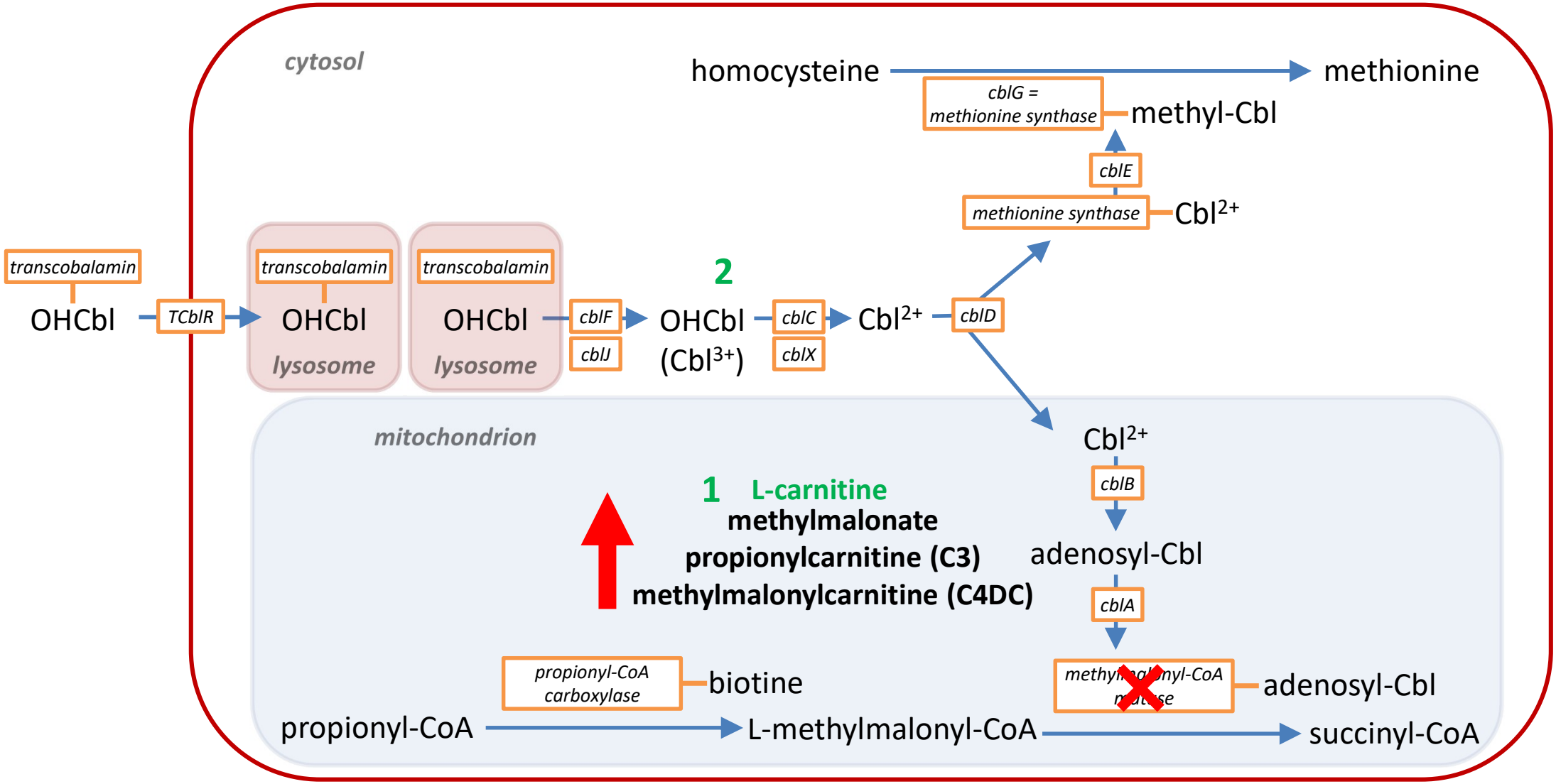


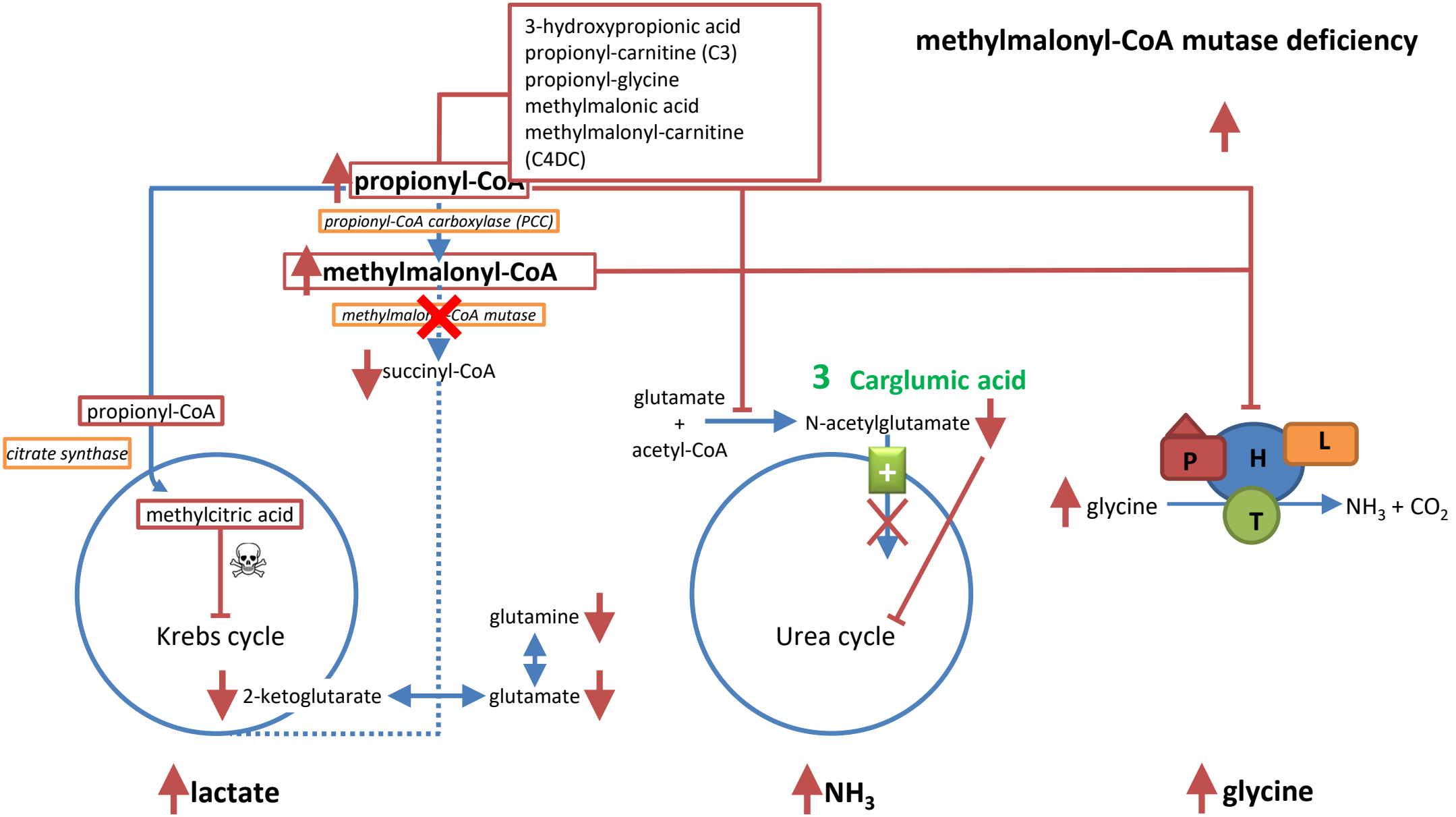
# Acute treatment

- Follow guidelines for neonatal hyperammonemia
- Consider preparation for hemofiltration (start at which NH3 value?)

- N-Carbamylglutamate (Carglumic acid): structural analog of N-acetylglutamate (NAG), anaplerotic effect?
- Sodium phenylbutyrate not suitable

# DD MMAuria without hyperhomocysteinemia



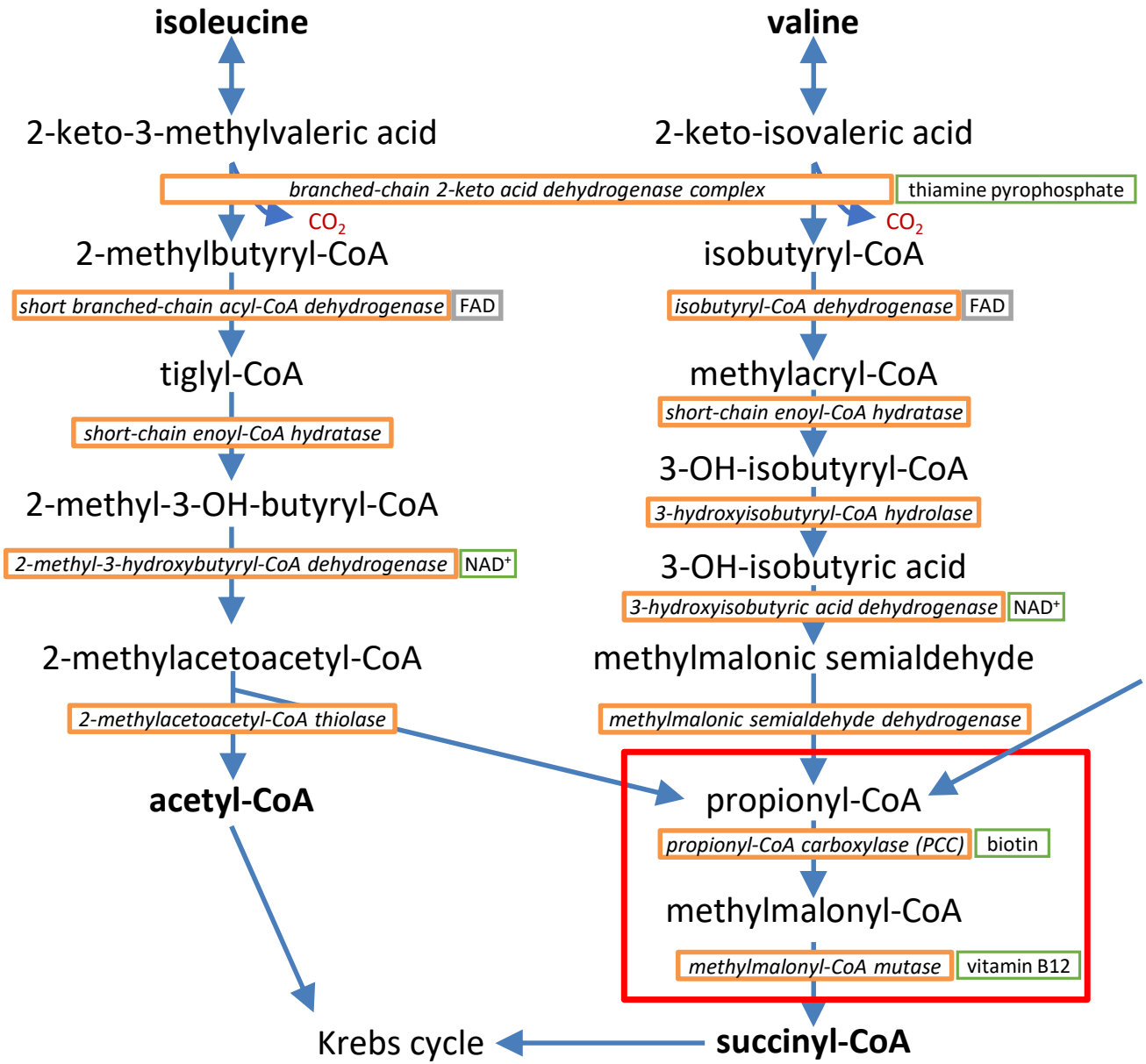

methylmalonyl-CoA mutase deficiency
3-hydroxypropionic acid
propionyl-carnitine (C3)
propionyl-glycine
methylmalonic acid
methylmalonyl-carnitine
(C4DC)
propionyl-CoA
propionyl-CoA carboxylase (PCC)
methylmalonyl-CoA
methylmalonyl-CoA mutase
succinyl-CoA
propionyl-CoA
citrate synthase
methylcitric acid
Krebs cycle
2-ketoglutarate
glutamine
glutamate
glutamate
+
acetyl-CoA
N-acetylglutamate
3 Carglumic acid
Urea cycle
glycine
P
H
L
T
NH3 + CO2
lactate
NH3
glycine

# Long-term treatment

- Natural protein-restricted diet
- Methionine/threonine/valine/isoleucine-free amino acid supplements (controversial)
- L-carnitine 100 mg/kg/day
- Avoid catabolism & dehydration: emergency regimen with adequate fluid for illness

**Consider**

- Vitamin B12 – most neonatal-onset cases unresponsive
- Metronidazole courses (10 days/month, CAVE: peripheral polyneuropathy)
- Carglumic acid (or sodium benzoate) if NH3 >250 µmol/l
- Sodium bicarbonate if renal tubular acidosis
- Renal (+liver?) transplantation (most adequate age?)




**(other) sources of propionyl- and methylmalonyl-CoA**

- isoleucine
- valine
- threonine
- methionine
- branched-chain fatty acids
- odd-chain fatty acids
- bacterial metabolism in the gut

# Monitoring

- Growth
- Urate, lactate
- MMA in plasma and/or urine
- Renal function
- Calcium and vitamin D metabolism, hemoglobin
- Psychomotor evaluation
- Ophthalmology (optic atrophy)

# Take home messages

- Methylmalonic aciduria often presents in the neonatal phase with acute decompensation, likely before the NBS result is available
- Hyperammonemia in methylmalonic aciduria responds well to carglumic acid, phenylbutyrate is not suitable
- Renal function often deteriorates with increasing age. Liver transplantation should be considered before (otherwise combined liver-kidney transplantation is necessary)
- MMA measurements in plasma and/or urine allow to distinguish between methylmalonic and propionic aciduria

# *A girl with vomiting and severe metabolic acidosis*

# Clinical presentation

- 3 years old girl
  - 7 day history of upper respiratory infection
  - 48 hours vomiting with fever, developed bilious vomiting
- Admission investigations
  - Glucose 2.9 mmol/L
  - Ketones 4.4 mmol/L (point of care)
  - Ammonia 160 µmol/L
  - pH 7.16, PC02 5.4 kPa, base excess –19 mmol/L, bicarbonate 8.9 mmol/L, lactate 3.8 mmol/L
  - Liver function tests normal
  - Full blood count normal
- Treated with rehydration IV fluids, slowly recovered.
- Repeat ammonia 47 µmol/L pre-discharge

**What else would you like to know?**

# Clinical presentation

- Birth history
  - 39/40, 3.63 kg
  - Noted to have dilated bowel on USS at 36/40
  - Surgical correction of “duodenal atresia” on day 1
  - Had a period of time on TPN (total parenteral nutrition)
  - Initial formula fed but suspected cow’s milk protein intolerance → Neocate

- Normal cognitive developmental history
- Poor growth with weight on 0.4$^{th}$ – 2$^{nd}$ centile
- Over last 18 months often seemed tired/ lethargic

**What metabolic investigations would you suggest?**

## *What metabolic investigations would you suggest?*

- **Urine organic acids:**
  - Urine MMA ↑↑ 7440 µmol/mmol creatinine (ref 0 – 30)
  - Grossly raised **methylmalonate** and **methylcitrate** with strongly raised **3-hydroxypropionate**.
  - Strongly raised glutarate, 3-methylglutaconate with mildly raised tiglylglycine.
  - Very strongly raised lactate, pyruvate and 2-oxo-isocaproate.
  - Grossly raised 3-hydroxybutyrate and acetoacetate with moderately raised adipate, suberate, sebacate, 3-hydroxy-C10 dicarboxylate, C8:1 dicarboxylate. Strongly raised 3-hydroxyisovalerate.
  - Moderately raised 4-hydroxyphenyllactate and 4-hydroxyphenylpyruvate.

- **Bloodspot Acylcarnitine profile:**
  - Free Carnitine ↓ 13 (ref 17 – 55) µmol/L
  - C3 (Propionyl) carnitine ↑ 9.4 (0.27 - 1.84) µmol/L
  - C4-OH (Hydroxybutyryl) carnitine 0.27 µmol/L (0-0.15)

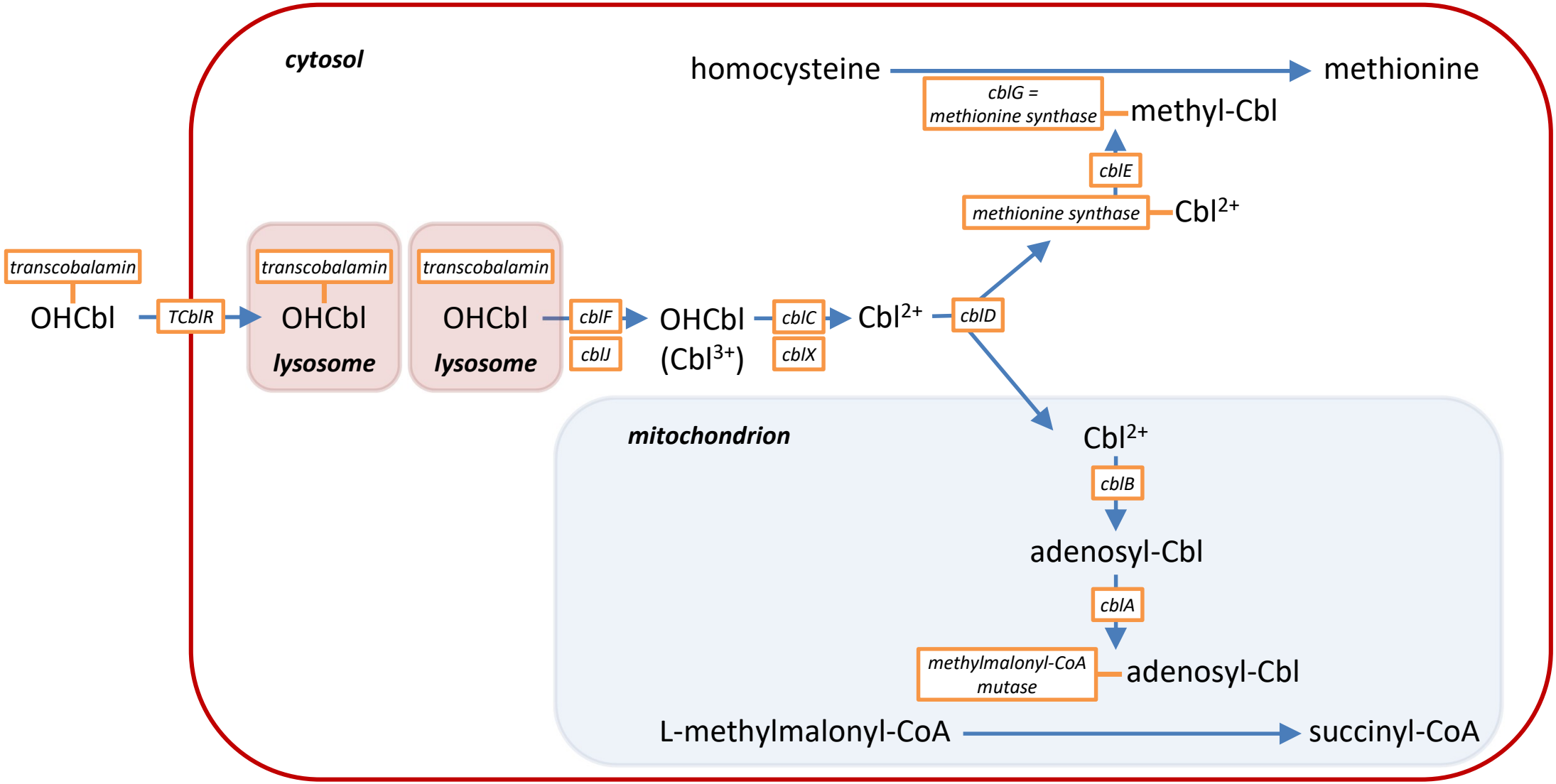
**What's the diagnosis? What further investigations would you do?**

# *What metabolic investigations would you suggest?*

| **Plasma Amino Acids** | | |
|---|---|---|
| Glycine | **240** | *100 - 330 µmol/l* |
| Serine | **90** | *90 - 290 µmol/l* |
| Threonine | **96** | *70 - 220 µmol/L* |
| Proline | **122** | *85 - 290 µmol/L* |
| Leucine | **167** | *65 - 220 µmol/l* |
| Isoleucine | **103** | *26 - 100 µmol/l* |
| Valine | **262** | *90 - 300 µmol/l* |
| Allo-isoleucine | **4** | *0 - 5 µmol/l* |
| Alanine | **196** | *150 - 450 µmol/l* |
| Glutamine | **421** | *480 - 800 µmol/l* |
| Arginine | **54** | *40 - 120 µmol/l* |
| Ornithine | **38** | *25 - 120 µmol/l* |
| Citrulline | **7** | *25 - 40 µmol/l* |
| Lysine | **174** | *100 - 300 µmol/l* |
| Methionine | **27** | *10 - 60 µmol/l* |
| Taurine | **114** | *40 - 140 µmol/l* |
| Phenylalanine | **73** | *35 - 100 µmol/l* |
| Tyrosine | **76** | *30 - 120 µmol/l* |
| Tryptophan | **20** | *30 - 80 µmol/l* |
| Histidine | **69** | *30 - 150 µmol/l* |
| Glutamic acid | **33** | *25 - 130 µmol/l* |
| **Total Homocysteine** | **64 ↑** | ***5 - 15 µmol/l*** |

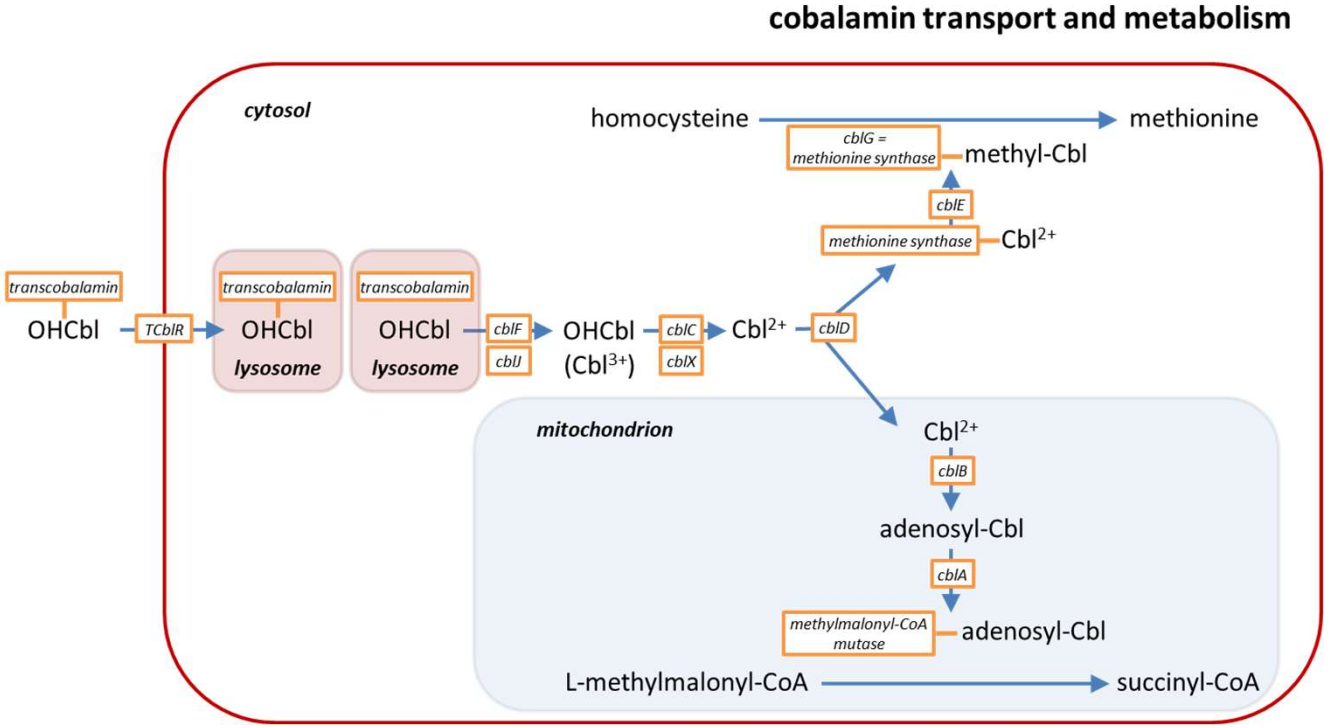
**What's the differential diagnosis?**
**What further investigations would you do?**

# cobalamin transport and metabolism



# *Combined methylmalonic aciduria and homocystinuria*

- B12 dietary deficiency
- B12 absorption defect
  - Intrinsic factor deficiency
  - Imerslund Gräsbeck
  - Ileal pathology
- Transport defect
  - Transcobalamin/ receptor defect
- Cobalamin metabolism
  - cblC
  - cblF
  - cblD (some forms)
  - cblJ
  - cblX




**What further investigations would you do?**

# What further investigations would you do?

- Vitamin B12: **<150 pg/ml** (ref 416-1,210)
- Why is the B12 level so low?

- Dietary history (not vegan diet)
- Auto-antibodies
  - Gastric parietal cell antibodies negative
- (Schilling test – not done (obsolete))
- Urine albumin/creatinine: normal

- Molecular genetic studies?
  - AMN/CBL – Imerslund Gräsbeck
  - Cbl genetics

***Further history….***

- *Careful review of past medical history:*
- *"Duodenal atresia" actually ileal atresia with resection of terminal ileum*

***Diagnosis….***

- *Severe B12 deficiency due to absence of terminal ileum*
- *Secondary combined methylmalonic aciduria/homocystinuria*

# What treatment would you instigate?

- **B12 replacement**
  - Parenteral (IM) hydroxocobalamin
  - Initially 3 times per week but now every 3 months – long term treatment.
- Carnitine until replete
- "Metabolic management" (glucose polymer) not needed.

| Treatment Response | Acute | Baseline | +4 weeks |
|---|---|---|---|
| Urine MMA (μmol/mmol creatinine) | 7440 | 126 | |
| Urine methylcitrate (μmol/mmol creatinine) | 268 | 21 | |
| Plasma MMA μmol/L (0-0.28) | | 8.54 | 0.15 |
| Bloodspot C3 carnitine μmol/L (0.27-1.84) | 9.4 | 8.41 | 0.84 |
| Bloodspot free carnitine μmol/L (17-55) | 13 | 12 | 36 |
| Plasma total homocysteine μmol/L (5-15) | 64 | 168 | 13 |
| Vitamin B12 pg/ml (416-1210) | | <150 | >1000 |

## *Take home message*

- Take a careful history!
- Pronounced metabolic biochemical abnormalities not always due to primary inherited disorder
- Differential for combined methylmalonic / homocystinuria

# Workshop on organic acidemias

## Case 4

## Adolescent with breathlessness and swollen ankles

# History & examination

18 year old man presented with

- Worsening breathlessness for 48 hours
- Ankle swelling for 1 month
- Language delay since 3 years of age

Brother died at 18 years with renal failure & hypertrophic cardiomyopathy

- Afebrile
- Hypertension 210/100 mm Hg
- Tachypnoea 26/minute
- Basal crackles over lung bases bilaterally
- Ankle oedema

# Investigations

- Haemoglobin 78 g/L (controls >130)
- Platelets 141 x $10^9$/L (controls >150)

- LDH 787 IU/L (controls <250)
- Haptoglobin <0.1 g/L (markedly decreased)

- Plasma creatinine 700 µmol/L (controls <120)
- Haematuria 100/$mm^3$
- Proteinuria 5.9 g/d (nephrotic range)

→ Haemolytic uraemic syndrome (HUS)/ renal thrombotic microangiopathy (TMA)

# Further investigations

- CT scan: pulmonary oedema, normal renal appearances
- Echocardiography: left ventricular hypertrophy, normal systolic function, raised pulmonary artery pressure
- Renal histology: arterial stenosis due to fibroproliferative lesions, thickened glomerular basement membranes with IgM deposits; consistent with HUS/TMA

Causes of HUS/TMA include Shiga toxin-producing *Escherichia coli* (STEC) infection, thrombotic thrombocytopenic purpura (TTP) & complement mutations

- STEC PCR: negative
- ADAMTS13 activity: 60% normal (<10% in TTP, so excludes hereditary TTP)
- Autoantibody & complement screening: no significant abnormalities

# Management

- IV methylprednisolone
- Plasma exchange
- Haemodialysis

What other investigations would you do?

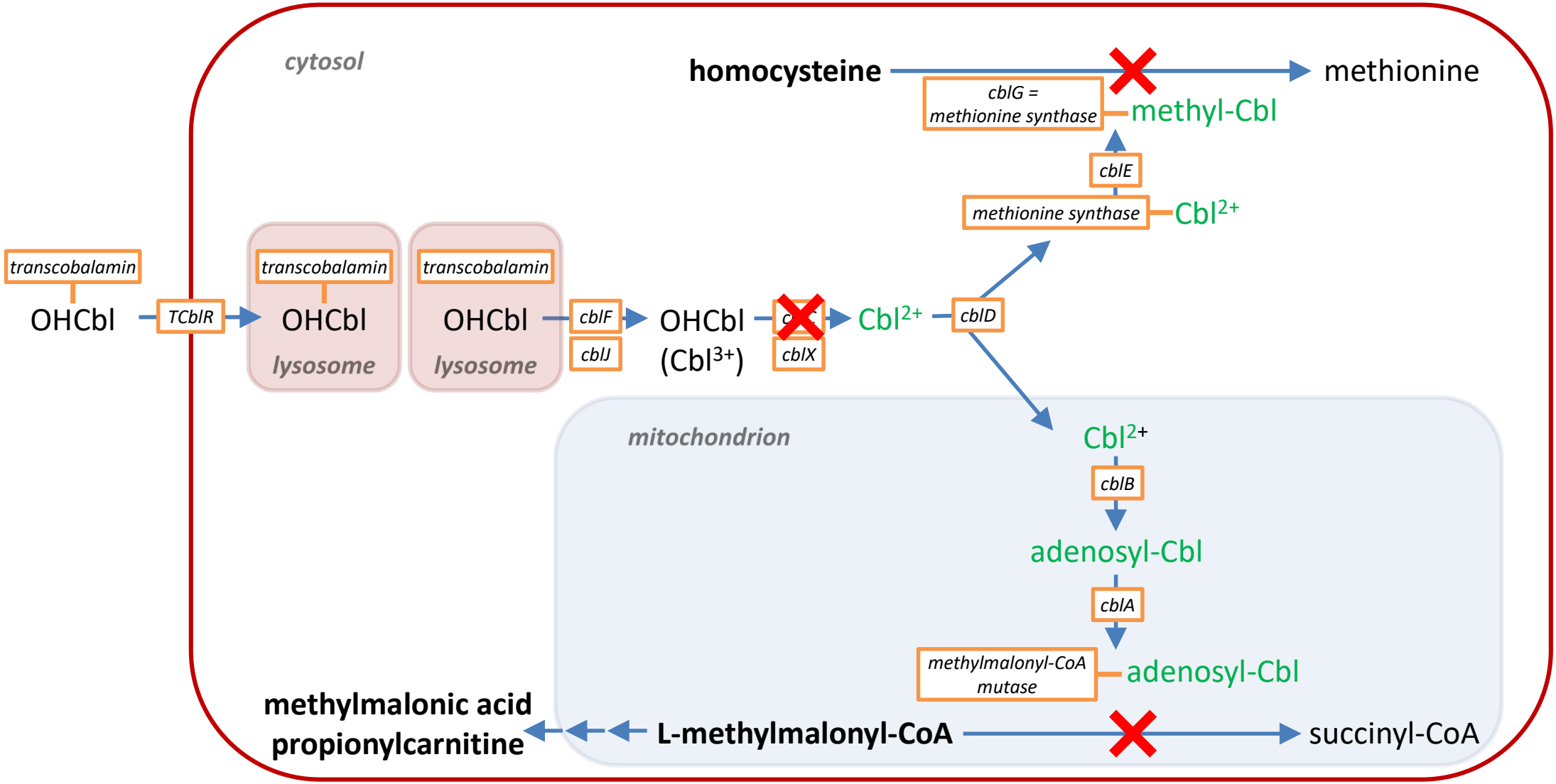
# Metabolic investigations

- Plasma total homocysteine 73 µmol/L (controls <17)
- Plasma amino acids
  - Homocystine: 2 µmol/L (controls <1)
  - Homocysteine-cysteine mixed disulfide: 20 µmol/L (controls: undetectable)
  - Methionine: 19 µmol/L (controls 17-36)
- Bloodspot acylcarnitines: propionylcarnitine 5 µmol/L (controls <0.75)
- Plasma methylmalonic acid (MMA): 20.6 µmol/L (controls <0.5)
- Organic acids in urine: MMA clearly elevated, methylcitrate & 3-OH propionate slightly elevated

What is the commonest cause for these metabolic results?

- Plasma B12 412 pmol/L (controls 141-489)

# cobalamin transport and metabolism – CblC deficiency



# How would you treat this patient?

- Hydroxocobalamin 5mg IM, initially daily
- Betaine 3 g twice daily
- Calcium folinate 15 mg daily

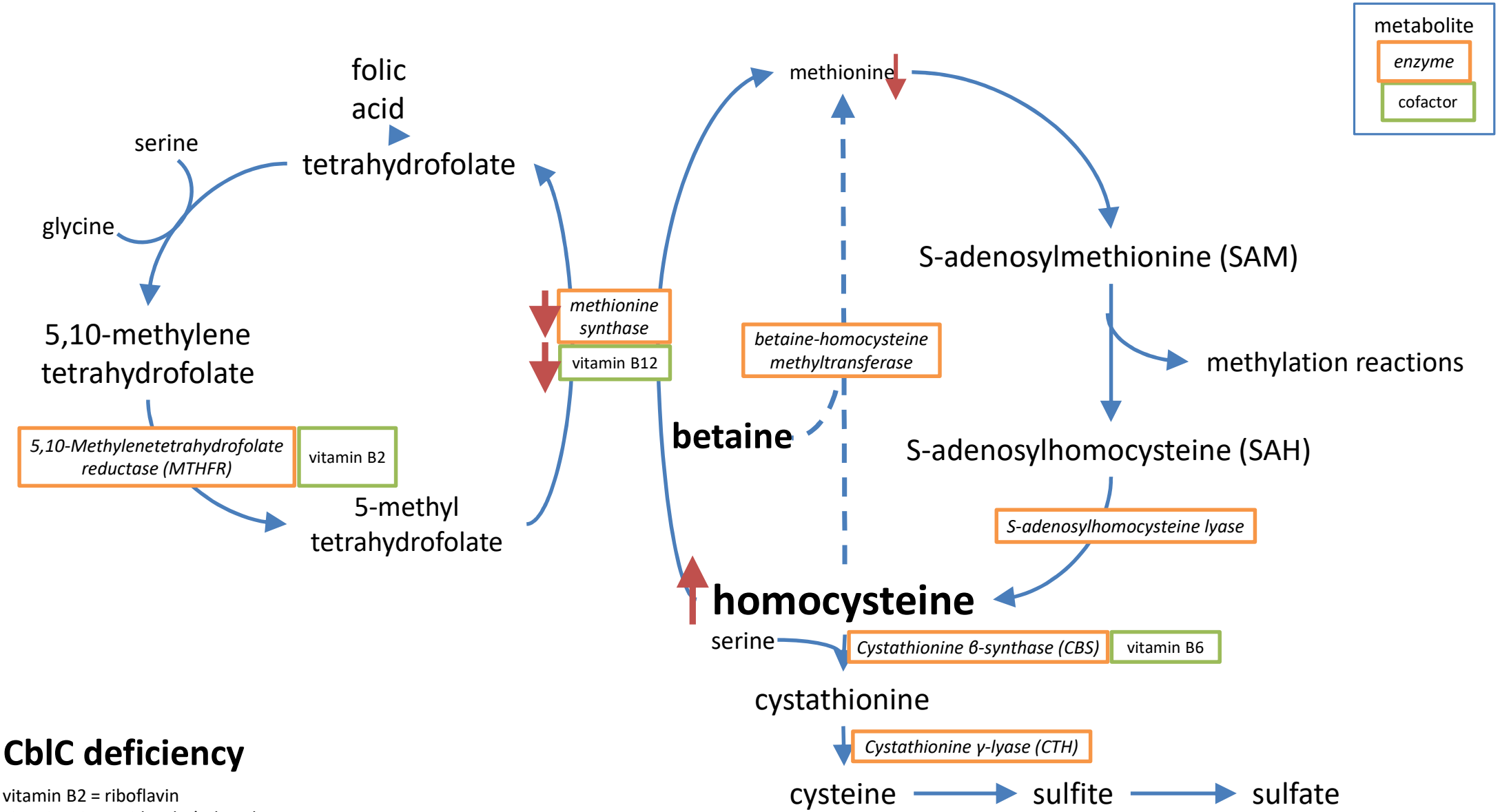
Why is Hydroxocobalamin given IM?

What Hydroxocobalamin preparations do you have available?

Can it be given intranasally?

Can cyanocobalamin be given in this condition? (No!)

Why were betaine & calcium folinate given?




## CblC deficiency

vitamin B2 = riboflavin
vitamin B6 = pyridoxal-5'-phosphate
vitamin B12 in this cycle = methylcobalamin

# Outcome

- Haemolysis improved within a week
- Plasma total homocysteine fell to 33 µmol/L
- Pulmonary CT: no veno-occlusive disease
- Brain MRI: normal
- Dialysis stopped after 5 months, plasma creatinine 147 µmol/L (<120)
- *MMACHC* sequencing: compound heterozygous variants
  - c.271dupA (commonest, frameshift variant usually associated with early onset)
  - c.82-12_82-9delTTTC (3 other reports, intronic deletion presumed to cause abnormal splicing)

Grangé et al, Lancet (2015) 386: 1011-2

# CblC – adults

Prominent **neurological and neuropsychiatric** features in adult (late) onset disease include:

- neuropsychiatric symptoms (behavioural change, hallucinations, psychosis),
- progressive cognitive decline,
- thromboembolic complications,
- and/or subacute combined degeneration of the spinal cord

Brain MR may show leukodystrophy – isolated periventricular or more diffuse white matter disease

Visual loss and ocular complications are less frequent in late onset forms
(but surveillance is still important)

Remind your adolescent and young adults not to inhale nitrous oxide ('whippets')

# Take home messages

- Homocysteine remethylation defects usually present in infancy with
  - progressive encephalopathy
  - accompanied by haematological & multisystem problems in cobalamin defects
- Neuropsychiatric problems are the commonest presentation for remethylation defects in adults
- Haemolytic uraemic syndrome can occur in cblC disease
- Anaemia (macrocytic or megaloblastic) is a frequent finding
- Plasma total homocysteine and urinary organic acids must be performed

# *Workshop organic acidemias*

# *Case 5*
# *2 y old with infection of upper airways and acute acidosis*

# *Clinical presentation –I –*

- 2 year old girl
- 2nd child of healthy consanguineous parents, healthy sibling
- Pregnancy, spontaneous birth at term
- no abnormality reported in newborn screening
- normal development
- At 2 years: infection of upper airways, suspicion of febrile bronchopneumonia
- Feeding difficulties, drowsiness, tachypnoea

# *Clinical presentation –II –*

- Hospitalization
- ↓ pH 7.08 (ref. 7.35-7.45)
- ↓ pCO2 11.7 mmHg (ref. 32-45)
- ↑ BE -22.2 mmol/l (ref. -3 - +3 )
- ↑ Anion gap 39 mmol/l (ref. 7-16)
- ✓ Ammonium, lactate, and glucose levels normal
- -> Pediatric ICU, intubated and ventilated
- i.v. antibiotic therapy (pneumonia), buffering, anabolization

  ->quick recovery

Which metabolic tests would you want to perform?

# *Metabolic investigations in parallel*

- Serum amino acids: unremarkable pattern
- Dried blood spots acylcarnitines:
  Free carnitine (C0) normal, C5:1 normal, C2, C4-OH, C5OH ↑
- Urinary organic acids: massive ketonuria:
  acetoacetate ↑↑↑, 3-hydroxybutyrate ↑↑↑, 3-hydroxy-2-methylbutyrate ↑↑↑, tiglylglycine ↑↑↑; 2-methylacetoacetate ↑
- Ratio FFA/ 3-hydroxybutyrate was low ↓

**ketolysis**

acetoacetyl-CoA

*2-methylacetoacetyl-CoA thiolase (MAT)*

acetyl-CoA  acetyl-CoA

**isoleucine catabolism**

isoleucine

alloisoleucine

2-ethylhydracrylic acid

2-keto-3-methylvaleric acid

*branched-chain 2-keto acid dehydrogenase complex* $CO_2$

2-methylbutyryl-CoA

*short branched-chain acyl-CoA dehydrogenase* FAD

tiglyl-CoA

tiglylglycine
tiglylcarnitine (C5:1)

*short-chain enoyl-CoA hydratase*

2-methyl-3-OH-butyryl-CoA

2-methyl-3-hydroxybutyric acid
2-methyl-3-hydroxybutyrylcarnitine (C5OH)

*2-methyl-3-hydroxybutyryl-CoA dehydrogenase* $NAD^+$

2-methylacetoacetyl-CoA

2-methylacetoacetic acid
2-butanone

*2-methylacetoacetyl-CoA thiolase (MAT)*

acetyl-CoA  propionyl-CoA

What is your diagnosis? How would you confirm?

Metabolite pattern strongly indicates **2-methylacetoacetyl-CoA thiolase (β-ketothiolase) deficiency**; OMIM #203750.

# Confirmatory Testing

- Sequencing *ACAT1* gene: homozygous *ACAT1* c.622C > T, p.(Arg208*)
- Enzyme activity assay (fibroblasts strongly preferred): ↓↓↓

# *Therapeutic approaches*

- Prevention: obviate ketogenesis to the extent possible
  (avoid prolonged fasting and meet increased energy requirements,
  avoid extreme fat/ protein intake)

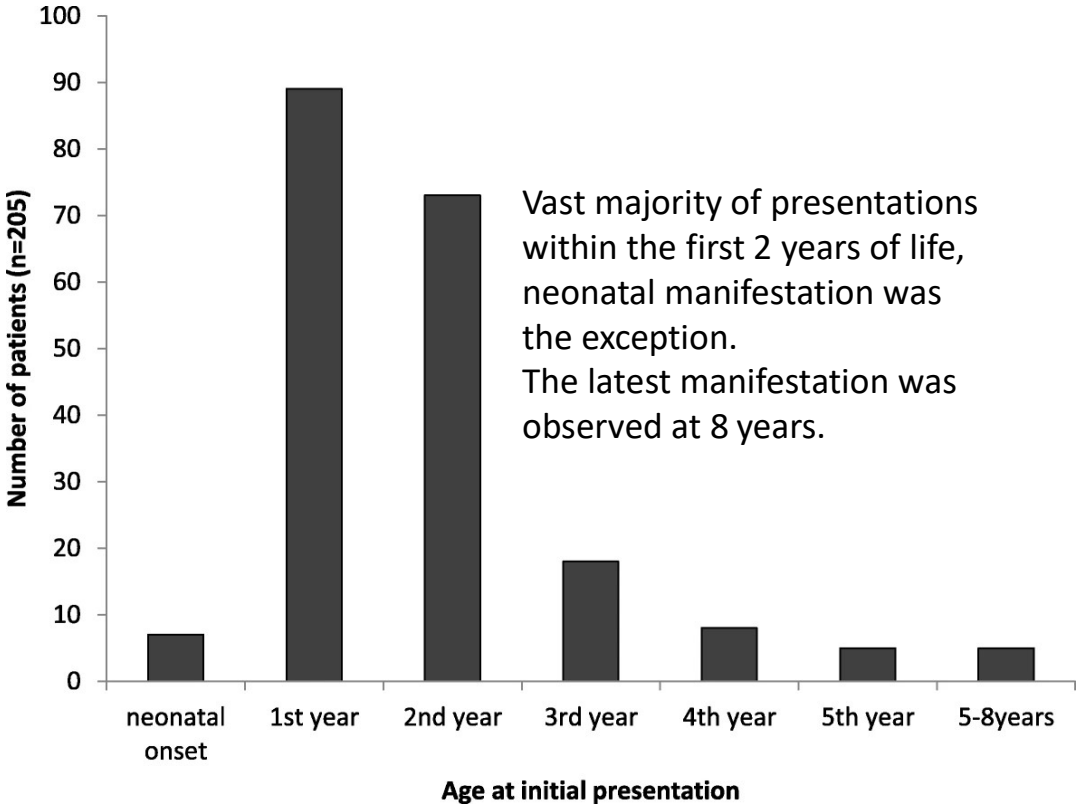
- Consider additional bedtime snack in young children and mild protein restriction.

- Compensate deficiency of L-carnitine, if present.

- Acute phase with ketoacidosis and dehydration:
  - glucose and fluid therapy

# Outcome

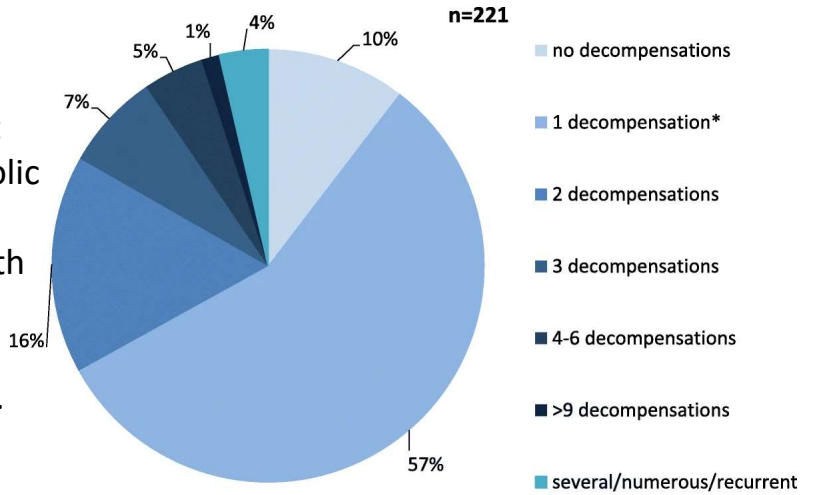
## *(based on 244 patients with MAT deficiency)*

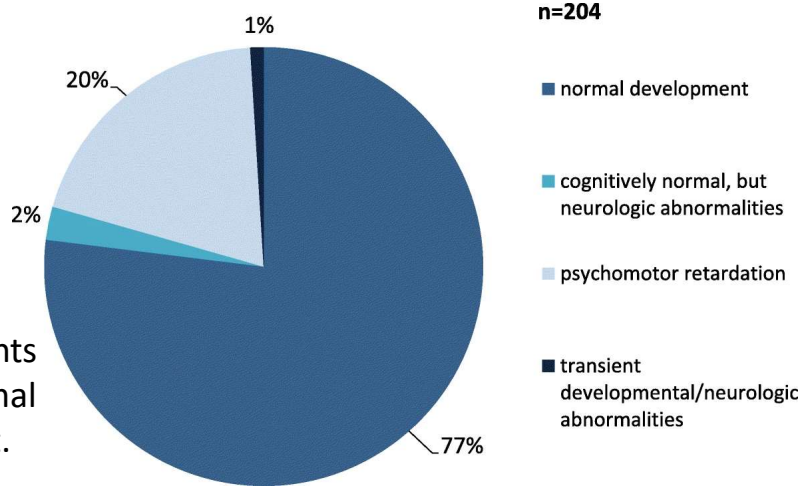



Vast majority of presentations within the first 2 years of life, neonatal manifestation was the exception.
The latest manifestation was observed at 8 years.

S.C. Grünert & J.O. Sass 2-methylacetoacetyl-coenzyme A thiolase (beta-ketothiolase) deficiency: one disease - two pathways. Orphanet J Rare Dis. 2020 Apr 28;15(1):106. doi: 10.1186/s13023-020-01357-0.

Most patients: at least one metabolic crisis.
Only minority with more than one reported acute decompensation.




77% of patients showed normal development.

# *Take home message*

- MATD often presents with a rather positive outcome, if survives first metabolic decompensation
- Awareness of the disease is important to minimize consequences of a (first) metabolic crises
- The mainstay of therapy in MAT is a rather preventive measure: minimizing the need for ketogenesis
- Organic acids in urine: consider instability of 2-methylacetoacetate not only during transport, but also during analysis (oximation recommended, if trimethylsilylation-based derivatization is performed)
- NBS not always reliable / available

# *Workshop organic acidemias*
# *Case 6*
# *Suspicion of stroke after collapse in the snow*

# *Clinical presentation*

- 7 years old girl
- collapsed when walking in snow
- impaired alertness, rigor, painful lower extremities with absent deep tendon reflexes

→ suspicion of stroke → brain MRI with normal results

→ admitted to intensive care unit

# *Clinical presentation*

On intensive care unit:

- severely reduced level of consciousness, intubated and ventilated
- 1st blood gas analysis: pH 6.87, $HCO_3$ 6.1 mmol/L, BE -24.5 mmol/L
- in blood: lactate 19.0 mmol/L, glucose 11.1 mmol/L

How would you proceed? Any metabolic tests?

# *Metabolic investigations*

- Review Newborn screening: normal amino acid and acylcarnitine profiles

Selective testing:

- Amino acids in plasma: no relevant abnormality
- Negative drug screening
- Acylcarnitines in blood: see next slide
- Organic acids in urine: see next slide

| Acylcarnitine im Blut | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| freies Carnitin (C0) | 11.3 - 43.9 | µmol/L | | **24.1** | | 31.0 | | 13.9 | | 21.9 | | |
| Acetylcarnitin (C2) | < 28.1 | µmol/L | | **61.9** | ↑ | 25.1 | | 25.5 | | 15.1 | | |
| Propionylcarnitin (C3) | < 3.19 | µmol/L | | **3.36** | ↑ | 1.97 | | 1.21 | | 1.05 | | |
| n-/iso-Butyrylcarnitin (C4) | < 0.43 | µmol/L | | **0.66** | ↑ | 0.19 | | 0.16 | | 0.12 | | |
| Tiglyl-/CH3-Crot.carn. (C5:1) | < 0.05 | µmol/L | | **0.10** | ↑ | 0.02 | | 0.02 | | 0.01 | | |
| n-/iso-Valerylcarnitin (C5) | < 0.31 | µmol/L | | **0.47** | ↑ | 0.24 | | 0.11 | | 0.11 | | |
| OH-But.+Mal.carn.(C4OH+C3DC) | < 0.14 | µmol/L | | **1.52** | ↑ | 0.07 | | 0.15 | ↑ | 0.04 | | |
| Hexanoylcarnitin (C6) | < 0.11 | µmol/L | | **0.49** | ↑ | 0.08 | | 0.07 | | 0.02 | | |
| C5OH+C4DC | < 0.38 | µmol/L | | **1.36** | ↑ | 0.67 | ↑ | 0.56 | ↑ | 0.45 | ↑ | |
| Glutarylcarnitin (C5DC+C6OH) | < 0.06 | µmol/L | | **0.12** | ↑ | 0.02 | | 0.06 | | 0.03 | | |
| Octenoylcarnitin (C8:1) | < 0.26 | µmol/L | | **0.10** | | 0.08 | | 0.07 | | 0.05 | | |
| Octanoylcarnitin (C8) | < 0.15 | µmol/L | | **0.09** | | 0.12 | | 0.07 | | 0.06 | | |
| Adipoyl- + CH3Glut.carn.(C6DC) | < 0.22 | µmol/L | | **0.05** | | 0.01 | | 0.01 | | <0.01 | | |
| Decenoylcarnitin (C10:1) | < 0.14 | µmol/L | | **0.07** | | 0.11 | | 0.06 | | 0.04 | | |
| Decanoylcarnitin (C10) | < 0.23 | µmol/L | | **0.16** | | 0.37 | ↑ | 0.17 | | 0.09 | | |
| Suberylcarnitin (C8DC) | < 0.05 | µmol/L | | **0.03** | | <0.01 | | 0.04 | | 0.01 | | |
| Laureoylcarnitin (C12:1) | < 0.09 | µmol/L | | **0.06** | | 0.04 | | 0.09 | | 0.02 | | |
| Lauroylcarnitin (C12) | < 0.10 | µmol/L | | **0.08** | | 0.07 | | 0.10 | | 0.02 | | |
| Myristdienoylcarnitin (C14:2) | < 0.05 | µmol/L | | **0.04** | | 0.05 | | 0.05 | | 0.02 | | |
| Myristeoylcarnitin (C14:1) | < 0.12 | µmol/L | | **0.10** | | 0.12 | | 0.19 | ↑ | 0.02 | | |
| Myristoylcarnitin (C14) | < 0.29 | µmol/L | | **0.10** | | 0.09 | | 0.11 | | 0.05 | | |
| OH-Myristeoylcarnitin(C14:1OH) | < 0.09 | µmol/L | | **0.02** | | 0.02 | | 0.04 | | 0.02 | | |
| OH-Myristoylcarnitin (C14OH) | < 0.04 | µmol/L | | **0.01** | | <0.01 | | 0.01 | | <0.01 | | |
| Palmiteoylcarnitin (C16:1) | < 0.25 | µmol/L | | **0.07** | | 0.04 | | 0.13 | | 0.05 | | |
| Palmitoylcarnitin (C16) | < 3.54 | µmol/L | | **0.75** | | 0.84 | | 1.07 | | 0.78 | | |
| OH-Palmiteoylcarnitin(C16:1OH) | < 0.13 | µmol/L | | **0.07** | | 0.04 | | 0.08 | | 0.04 | | |
| OH-Palmitoylcarn.(C16OH) | < 0.05 | µmol/L | | **0.01** | | <0.01 | | 0.02 | | <0.01 | | |
| Linoleoylcarnitin (C18:2) | < 0.63 | µmol/L | | **0.32** | | 0.51 | | 0.43 | | 0.44 | | |
| Oleoylcarnitin (C18:1) | < 2.57 | µmol/L | | **1.29** | | 1.76 | | 1.69 | | 1.25 | | |
| Stearoylcarnitin (C18) | < 1.30 | µmol/L | | **0.55** | | 0.65 | | 0.78 | | 0.49 | | |
| OH-Oleoylcarnitin (C18:1OH) | < 0.05 | µmol/L | | **0.03** | | 0.03 | | 0.04 | | 0.02 | | |
| OH-Stearoylcarnitin (C18OH) | < 0.03 | µmol/L | | **0.01** | | <0.01 | | 0.01 | | <0.01 | | |

| Organische Säuren im Urin | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Stoffwechsel der Fettsäuren und Ketonkörper** | | | | | | | |
| Acetoacetat (3-Keto-Butyrat) | <60 | mmol/mol | **49** | | <40 | n.d. | |
| 3-OH-n-Butyrat | <130 | mmol/mol | **323** | ↑ | 62 | n.d. | |
| Adipinsäure (C6-Dicarbons.) | <30 | mmol/mol | **<20** | | 28 | n.d. | |
| Suberinsäure (C8-Dicarbons.) | <20 | mmol/mol | **<10** | | 12 | n.d. | |
| Sebacinsäure (C10-Dicarbons.) | <20 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| 3-OH-Adipinsäure | <50 | mmol/mol | **<20** | | 21 | n.d. | |
| 3-OH-Suberinsäure | <10 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| 3-OH-Sebacinsäure | <30 | mmol/mol | **n.d.** | | 32 | ↑ | n.d. |
| 2-Ketoadipinsäure | <20 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| 2-OH-Sebacinsäure | <3 | mmol/mol | **n.d.** | | <3 | <3 | |
| N-Hexanoylglycin | <3 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| Ethylmalonsäure | <30 | mmol/mol | **<20** | | <20 | <20 | |
| Methylbernsteinsäure | <10 | mmol/mol | **<10** | | n.d. | n.d. | |
| Butyrylglycin | <3 | mmol/mol | **<3** | | | | |
| Glycerin | <50 | mmol/mol | **<20** | | | | |
| **Energiestoffwechsel** | | | | | | | |
| Milchsäure (Lactat) | <300 | mmol/mol | **10700** | ↑ | <50 | <50 | |
| Brenztraubensäure (Pyruvat) | <80 | mmol/mol | **655** | ↑ | <20 | 24 | |
| Citronensäure | <1500 | mmol/mol | **126** | | 300 | 164 | |
| 2-Ketoglutarsäure | <500 | mmol/mol | **144** | | 163 | 58 | |
| 2-OH-Butyrat | <20 | mmol/mol | **94** | ↑ | n.d. | n.d. | |
| Bernsteinsäure (Succinat) | <200 | mmol/mol | **<20** | | <20 | <20 | |
| Fumarsäure (tr.-Butendisäure) | <30 | mmol/mol | **<20** | | <20 | n.d. | |
| Äpfelsäure (Malat) | <60 | mmol/mol | **53** | | | | |
| **Stoffwechsel der verzweigtkettigen Aminosäuren** | | | | | | | |
| 2-OH-iso-Valeriansäure | <10 | mmol/mol | **<10** | | n.d. | n.d. | |
| 2-Keto-3-Methyl-Valeriansäure | <20 | mmol/mol | **15** | | <20 | n.d. | |
| 2-OH-3-Methyl-Valeriansäure | <20 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| 2-Keto-iso-Hexansäure | <10 | mmol/mol | **14** | ↑ | n.d. | n.d. | |
| 3-OH-iso-Valeriansäure | <50 | mmol/mol | **<20** | | 20 | <20 | |
| N-iso-Valerylglycin | <10 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| **Stoffwechsel der verzweigtkettigen Aminosäuren, Fortsetzung** | | | | | | | |
| 3-Methylglutarsäure | <3 | mmol/mol | **<3** | | n.d. | n.d. | |
| 3-OH-3-Methyl-Glutarsäure | <60 | mmol/mol | **<20** | | <10 | n.d. | |
| 4-OH-6-Me-2-Pyron | <3 | mmol/mol | **n.d.** | | | | |
| 2-Me-Butyrylglycin | <3 | mmol/mol | **n.d.** | | | | |
| N-Tiglylglycin | <10 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| 2-Methyl-3-OH-Butyrat | <20 | mmol/mol | **<10** | | <20 | <20 | |
| 2-Ethyl-3-OH-Propionsäure | <20 | mmol/mol | **<10** | | <50 | | |
| Isobutyrylglycin | <3 | mmol/mol | **n.d.** | | | | |
| 2,3-DiOH-2-Methylbuttersäure | <10 | mmol/mol | **<3** | | | | |
| 3-OH-iso-Butyrat | <100 | mmol/mol | **41** | | <100 | <100 | |
| N-Propionylglycin | <3 | mmol/mol | **n.d.** | | n.d. | n.d. | |
| 3-OH-Propionsäure | <50 | mmol/mol | **24** | | <20 | <20 | |
| Methylcitronensäure | <20 | mmol/mol | **<10** | | <20 | <20 | |
| Methylmalonsäure | <10 | mmol/mol | **54** | ↑ | <10 | 18 | ↑ |
| Malonsäure | <10 | mmol/mol | **<10** | | | | |
| **Lysinstoffwechsel** | | | | | | | |
| Glutarsäure (C5-Dicarbons.) | <20 | mmol/mol | **<10** | | n.d. | n.d. | |
| 3-OH-Glutarsäure | <10 | mmol/mol | **5** | | <10 | n.d. | |
| 2-OH-Glutarsäure | <50 | mmol/mol | **<20** | | <20 | <20 | |

***What is your interpretation so far?***

***Any other tests suggested?***

# Further course

- Within 24 hours, developed severe rhabdomyolysis with acute renal failure
- Maximum creatine kinase 200,000 U/L (< 147)
- Hemodiafiltration for 4 days

Differential diagnoses discussed at this point:

- Mitochondrial disease
- McArdle syndrome or other muscular glycogen storage disorder
- Myositis due to parainfluenza infection
- Genetic myopathy (eg. Lipin1 defect), aggravated by cold

# *Extended history*

- slight muscular hypotonia
- slight global delay
- similar but milder episode 2 years ago in winter

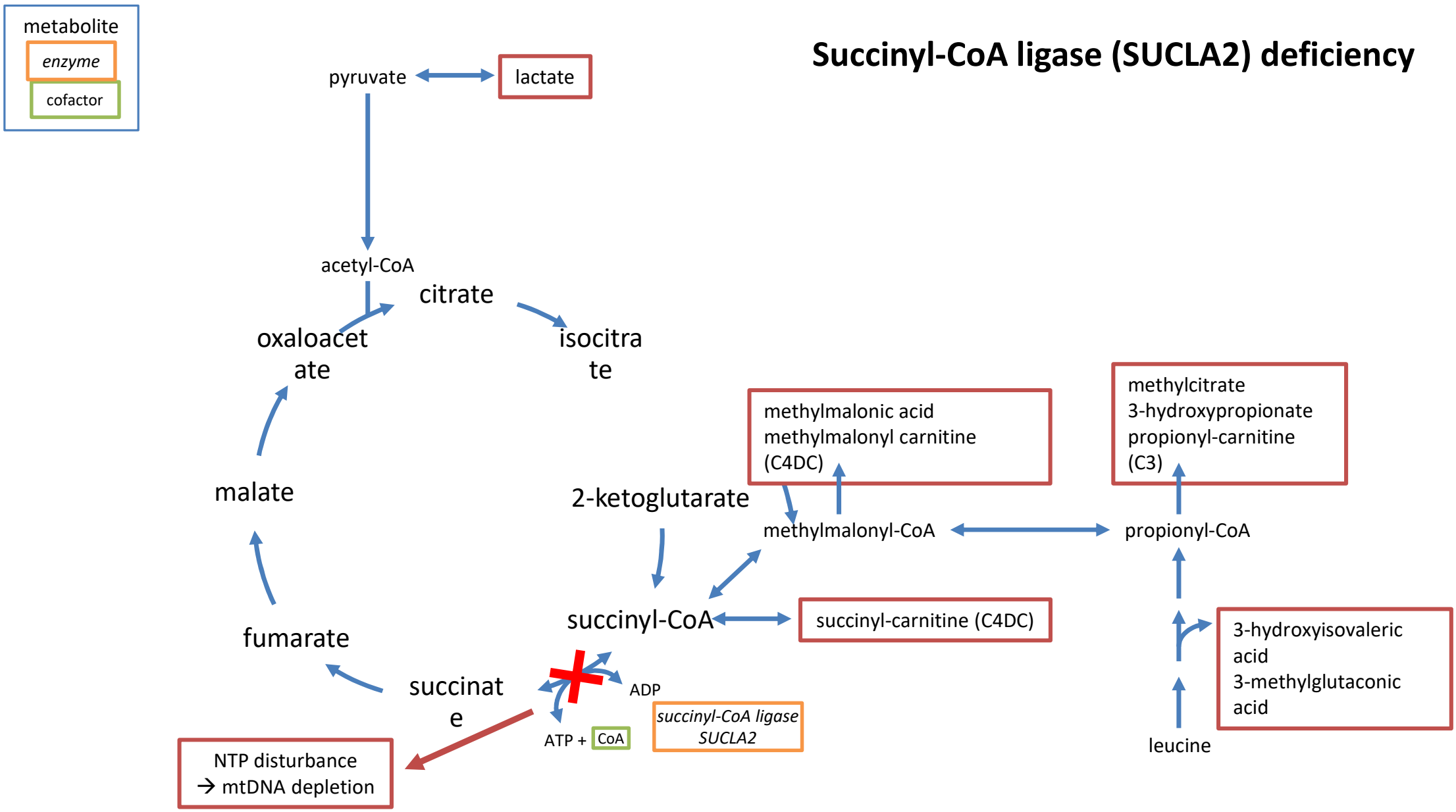
What test(s) would you order now?

# Genetic testing

Trio exome:

- compound-heterozygous for *SUCLA2* c.812T>G, p.Met271Arg & c.1043T>G, p.Val348Gly

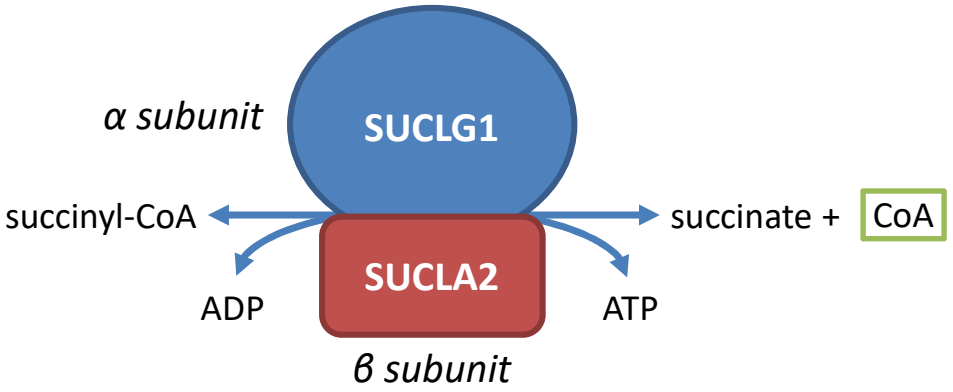
→ SUCLA2 defect

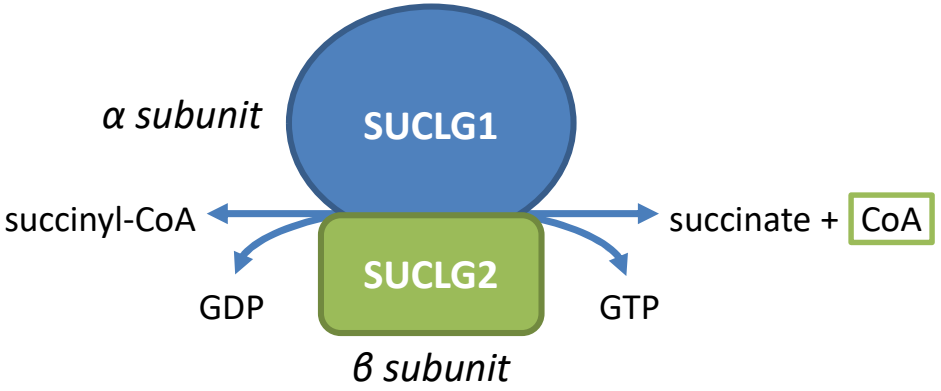

metabolite
enzyme
cofactor
Succinyl-CoA ligase (SUCLA2) deficiency
pyruvate
lactate
acetyl-CoA
citrate
oxaloacetate
isocitrate
malate
fumarate
succinate
2-ketoglutarate
succinyl-CoA
methylmalonic acid
methylmalonyl carnitine
(C4DC)
methylmalonyl-CoA
succinyl-carnitine (C4DC)
methylcitrate
3-hydroxypropionate
propionyl-carnitine
(C3)
propionyl-CoA
3-hydroxyisovaleric
acid
3-methylglutaconic
acid
leucine
ADP
ATP + CoA
succinyl-CoA ligase
SUCLA2
NTP disturbance
→ mtDNA depletion

- Succinyl-CoA ligase / synthetase
  - **SUCLG1**
    - **α subunit** of complex
  - **SUCLA2**
    - **β subunit** gives specificity for ADP
  - **SUCLG2**
    - **β subunit** gives specificity for GDP

- SUCL forms a complex with nucleoside diphosphate kinase (NDK)
- NDK is needed for mitochondrial NTP homeostasis and thus mtDNA replication
- Deficiency of the SUCL complex leads to disturbance of NTP homeostasis and mtDNA depletion
- SUCL deficiencies also are categorized as mtDNA depletion syndromes

**Succinyl-CoA ligase (SUCL) complex**

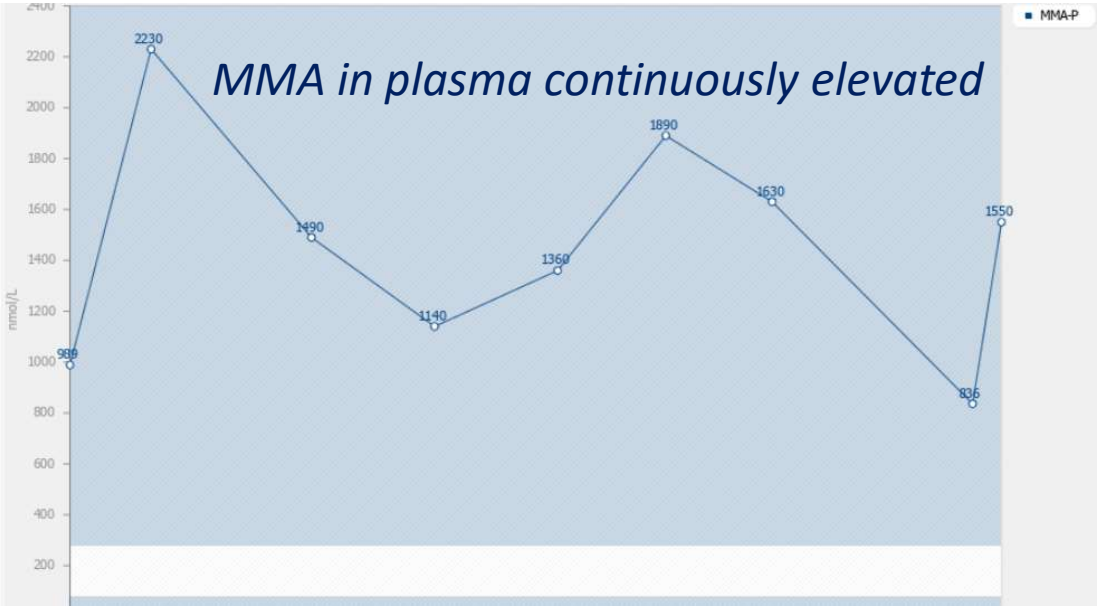


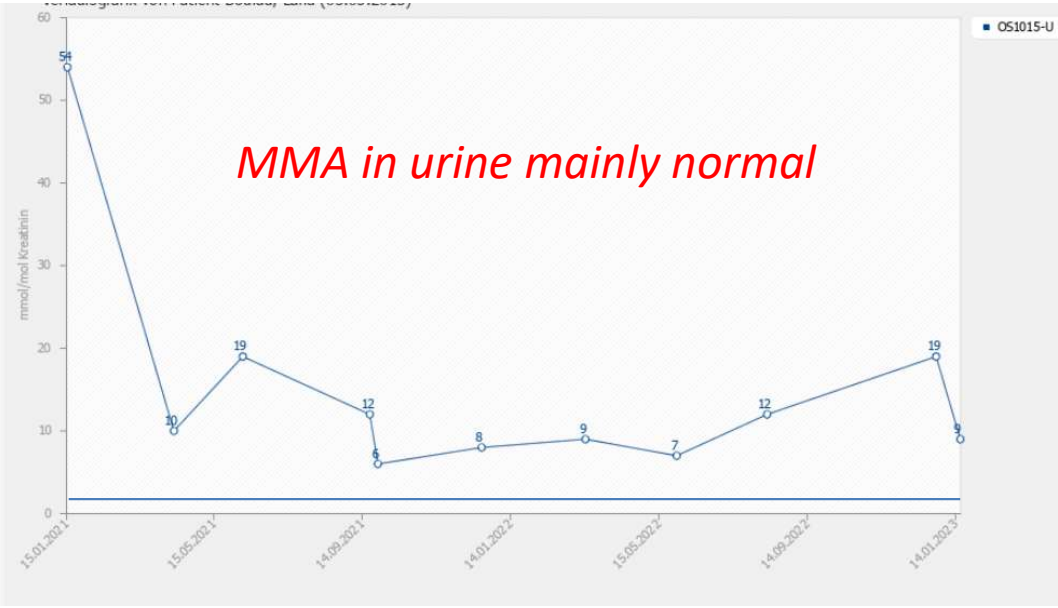



# Outcome

- required 5 months of rehabilitation for her motor deficits
- “therapy” started to avoid catabolism
- 6 months after the episode almost back to situation before crisis
- 2 years after the episode: stable, no further crises, but parents avoid strenuous exercise or cold

# Biochemical course



# Take home messages

- SUCLA2 defects can present late and with mainly muscular phenotype
- Elevations of MMA in SUCLA2 defects are moderate
- Plasma MMA may be more informative than urine MMA
- Succinylcarnitine cannot be differentiated from methylmalonylcarnitine using FIA-MS/MS
- Acylcarnitine assay without derivatization cannot differentiate C4DC from C5OH!

# A boy with hypoglycaemia

# Case background

- 2 year-old boy, fit and well
- No family history of note, parents unrelated
- Found unresponsive at home, emergency services called
- Blood glucose < 1 mmol/L (point of care test)
- Glucose administered by paramedic
- Transferred to Children's hospital emergency department
- *What tests would you request to investigate the cause of low glucose?*

# Hypoglycaemia screen

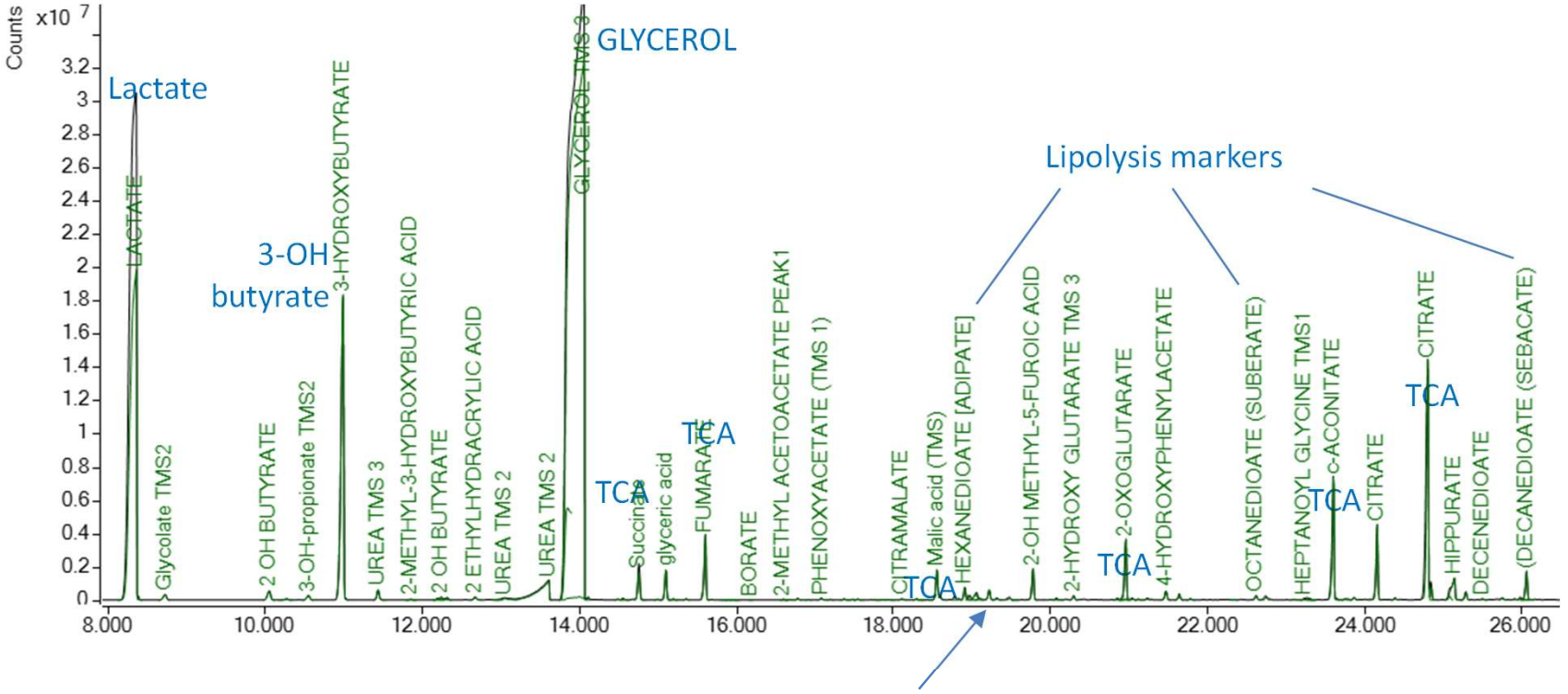
| Test (plasma, serum) | Result (reference range) |
|---|---|
| Lab glucose | 6.6 mmol/L |
| Lactate | 4.2 mmol/L (0.6 – 2.5) |
| Cortisol | 733 nmol/L (<410) |
| Insulin | 133 pmol/L (12 – 150) |
| Free fatty acids | 0.6 mmol/L (< 0.9 non-fasting) |
| 3-OH butyrate | 0.8 mmol/L (<0.2 non-fasting) |
| Amino acids | Within normal ranges |
| Acylcarnitines | Increased C4OH, C12, C12:1, C14, C14:1 |

*How do you interpret these results?*
*What further tests are required?*

# Hypoglycaemia screen

| Test (plasma, serum) | Result (reference range) | Interpretation |
|---|---|---|
| Lab glucose | 6.6 mmol/L | Not hypoglycaemic |
| Lactate | 4.2 mmol/L (0.6 – 2.5) | Lactic acidosis |
| Cortisol | 733 nmol/L (<410) | Stress response |
| Insulin | 133 pmol/L (12 – 150) | Difficult to interpret when glucose is normal |
| Free fatty acids | 0.6 mmol/L (< 0.9 non-fasting) | |
| 3-OH butyrate | 0.8 mmol/L (<0.2 non-fasting) | Ketosis |
| Amino acids | Within normal ranges | No evidence of a primary amino acid disorder |
| Acylcarnitines | Increased C4OH, C12, C12:1, C14, C14:1 | Ketotic pattern |

# Urine organic acids




*What are the abnormalities? What causes high urine glycerol?*

Internal standard
(very low compared with other compounds)

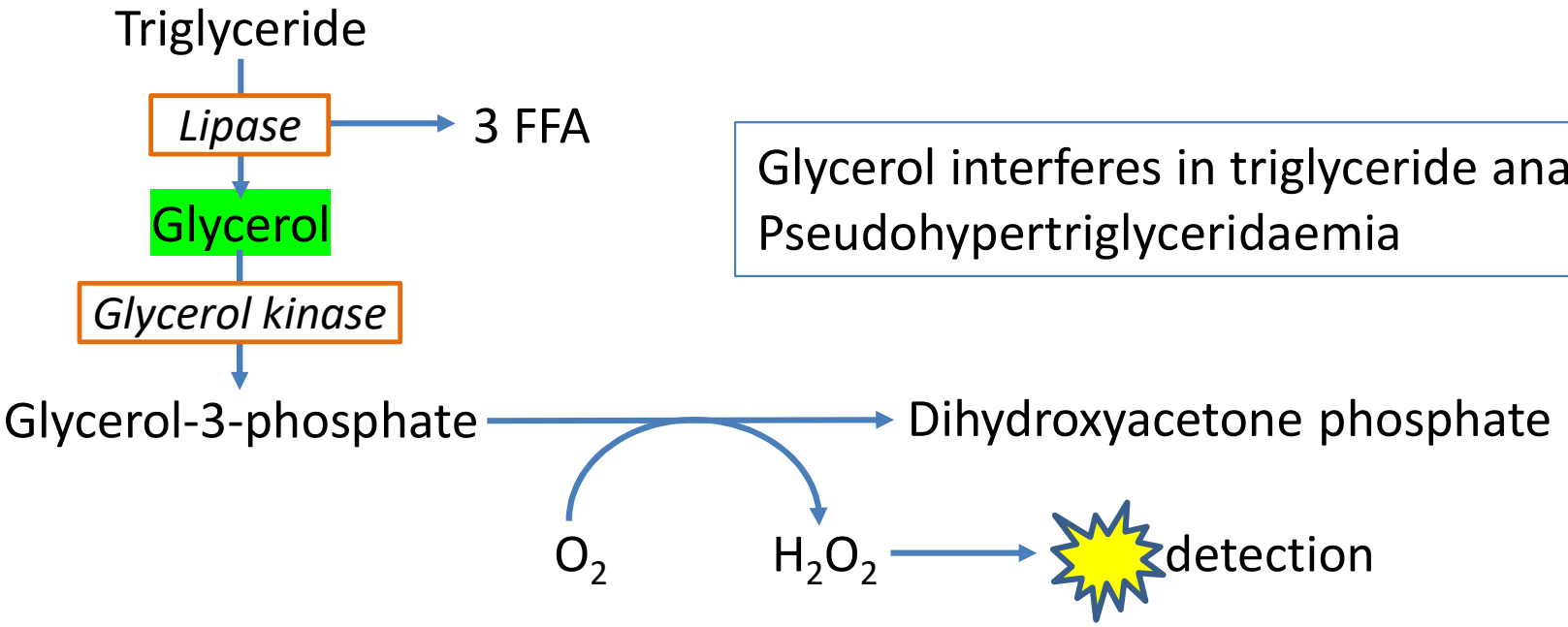
# Urine glycerol

| Causes of increased urine glycerol |
| --- |
| Contamination (e.g. emollients) |
| Ingestion (e.g. soap, slush puppy, medications) |
| Inherited metabolic disorders:<br>•Glycerol kinase deficiency<br>•Glycerol 3-phosphate dehydrogenase 1 deficiency<br>•Fructose-1,6-bisphosphatase deficiency |

*What is the most likely cause in this case?*
*How do you exclude glycerol contamination as a cause?*

# Glycerol: endogenous or contamination?

- Plasma triglyceride 22.7 mmol/L (reference range 0.5 – 2.5)
- Sample not lipaemic (clear plasma, normal lipaemic index)




Glycerol interferes in triglyceride analysis
Pseudohypertriglyceridaemia

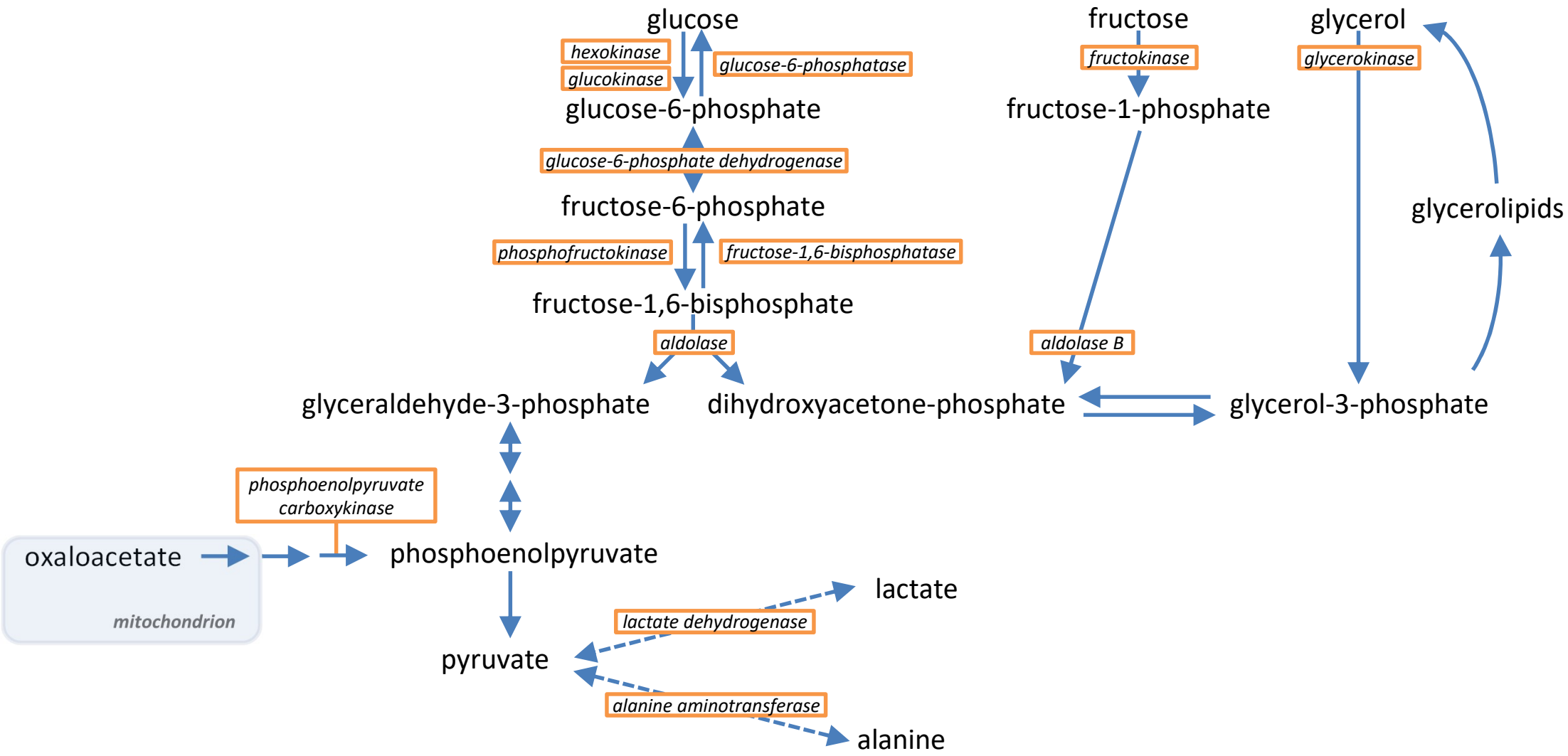
# Other methods

- Reference method for triglycerides analysis
  - Glycerol analysis by isotope dilution GC-MS ± hydrolysis to cleave glycerol from glycerides

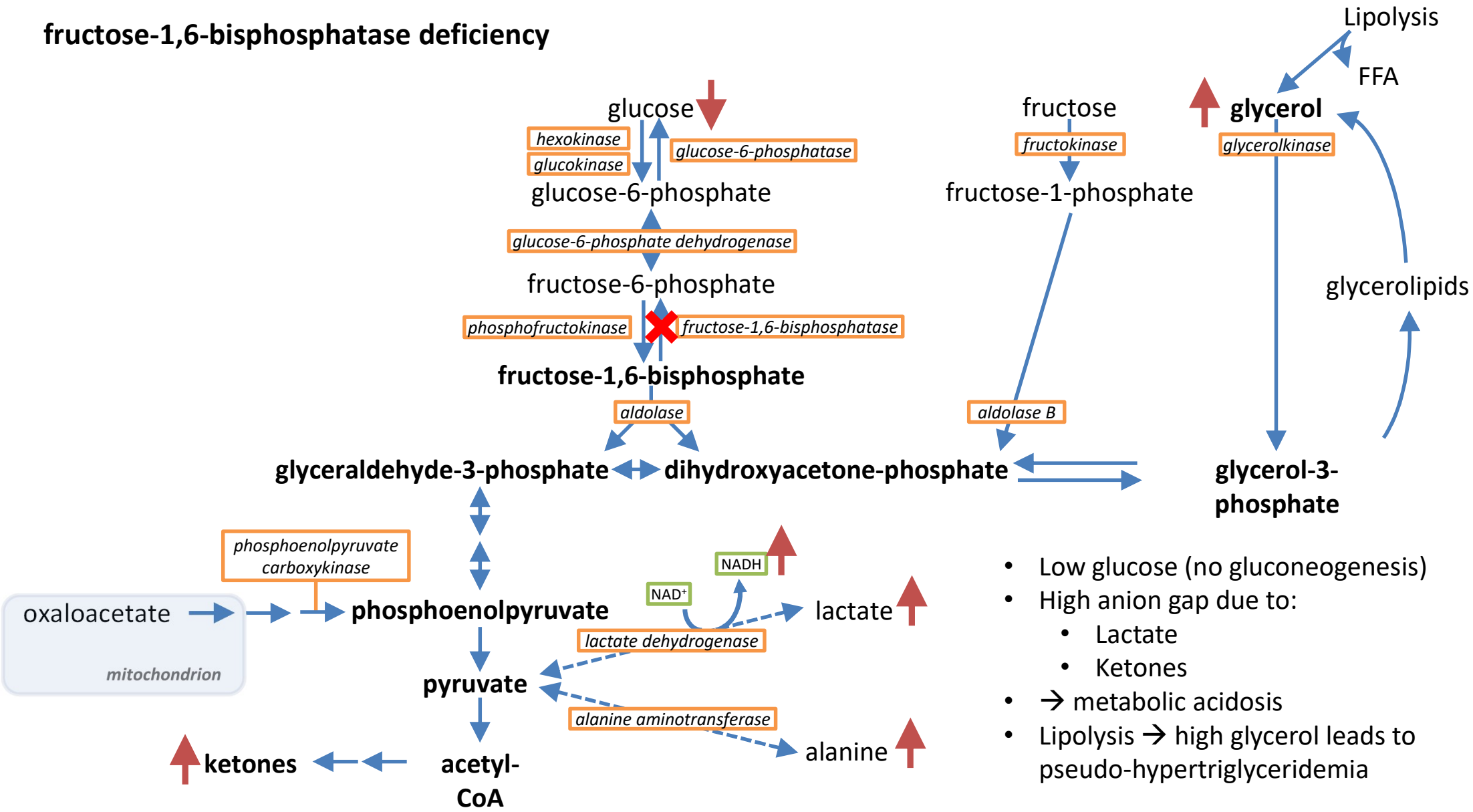
| | |
|---|---|
| Total glycerides | 23.25 mmol/L |
| Free glycerol | 22.12 mmol/L |
| Triglyceride | 1.13 mmol/L |

- Glycerol enzymatic method (not done in this case)

*What is the likely diagnosis? Any further tests?*


glucose/fructose/glycerol catabolism
glucose
hexokinase
glucokinase
glucose-6-phosphatase
glucose-6-phosphate
glucose-6-phosphate dehydrogenase
fructose-6-phosphate
phosphofructokinase
fructose-1,6-bisphosphatase
fructose-1,6-bisphosphate
aldolase
fructose
fructokinase
fructose-1-phosphate
aldolase B
glycerol
glycerokinase
glycerolipids
glyceraldehyde-3-phosphate
dihydroxyacetone-phosphate
glycerol-3-phosphate
phosphoenolpyruvate carboxykinase
oxaloacetate
mitochondrion
phosphoenolpyruvate
lactate
lactate dehydrogenase
pyruvate
alanine aminotransferase
alanine

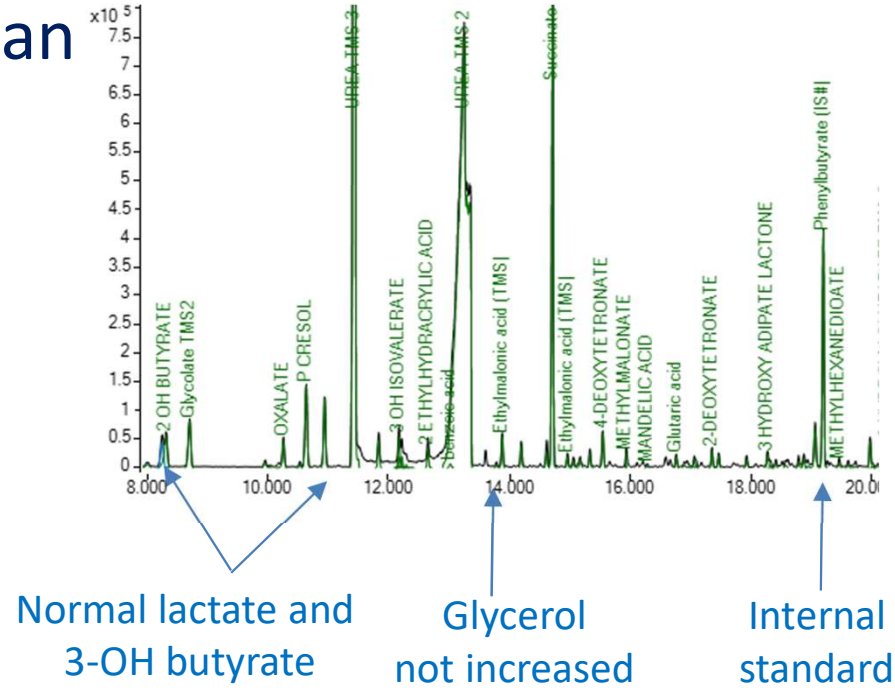
## fructose-1,6-bisphosphatase deficiency




- Low glucose (no gluconeogenesis)
- High anion gap due to:
  - Lactate
  - Ketones
- → metabolic acidosis
- Lipolysis → high glycerol leads to pseudo-hypertriglyceridemia

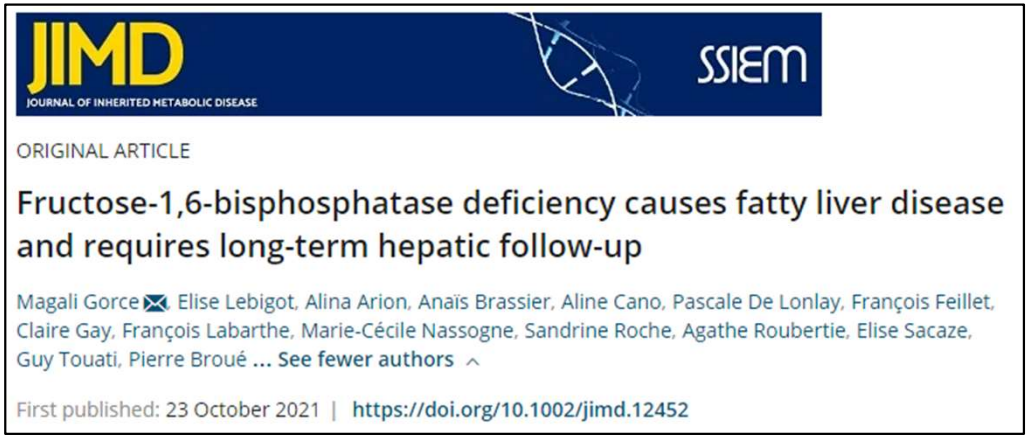
# Follow-up

- Hypoglycaemia resolved
- Discharged home with emergency plan
- Organic acids 12 days later normal
- Genetic testing: no *FBP1* variants
- Leucocyte enzyme analysis
  FBPase activity undetectable
- Review of presenting organic acids
  no glycerol-3-phosphate
- Further genetic testing ongoing



# FBPase deficiency

- May present with ketotic hypoglycaemia and lactic acidosis
- Many cases have considerable delay and repeat presentations before diagnosis
- Patients are well between episodes
- Normal development
- Hepatic follow-up required
- Some patients have no *FBP1* variants identified


JIMD JOURNAL OF INHERITED METABOLIC DISEASE SSIEM

ORIGINAL ARTICLE

Fructose-1,6-bisphosphatase deficiency causes fatty liver disease and requires long-term hepatic follow-up

Magali Gorce, Elise Lebigot, Alina Arion, Anaïs Brassier, Aline Cano, Pascale De Lonlay, François Feillet, Claire Gay, François Labarthe, Marie-Cécile Nassogne, Sandrine Roche, Agathe Roubertie, Elise Sacaze, Guy Touati, Pierre Broué ... See fewer authors

First published: 23 October 2021 | https://doi.org/10.1002/jimd.12452

# Take home messages

- FPBase deficiency is a cause of ketotic hypoglycaemia with lactic acidosis
- Increased glycerol is found on urine organic acids analysis during hypoglycaemia but not when patient is well
- Glycerol-3-phosphate is increased but may not be detected on organic acids (method dependent)
- Pseudo-hypertriglyceridaemia can confirm endogenous glycerol
- Timing of samples is critical for biochemical diagnosis
- Adding plasma triglyceride to hypoglycaemia screen may help identify cases

# F-1,6-BP deficiency - adults

## Pregnancy

Risk of maternal hypoglycemia and lactic acidosis - consider the metabolic demands of pregnancy:

- Regular carbohydrate-rich meals and snacks
- Nocturnal uncooked cornstarch
- Prompt control of nausea and vomiting
- Intravenous dextrose during labour and delivery

| | Pre-conception | 1st trimester | 2nd trimester | 3rd trimester |
|---|---|---|---|---|
| Calories | | + 100 kcal/day | + 300 kcal/day | + 300 kcal/day |
| Protein | | First half: 0.8-1.0 g/kg/day; Second half: 1.1 g/kg | | |
| CHO | Unchanged: 50-60% of energy (< 5% as sugar) | | | |
| Fat | Unchanged: 30% of energy | | | |

Successful pregnancies reported (Sugita, JIMD Rep 2014; Krishnamurthy, JIMD 2007)

# *Workshop organic acidemias*
# *Case 8*
# *Learning difficulties and ataxia*

# *Clinical presentation*

- 7 years old girl, third child of non-related parents
- “Hypotonic infant” (muscular hypotonia), walked at 2 years of age
- Global developmental delay, no regression
- Ataxia of trunc and extremities
- Joint laxity (generalized)
- Hyperopia & congenital strabism

# *Clinical presentation*

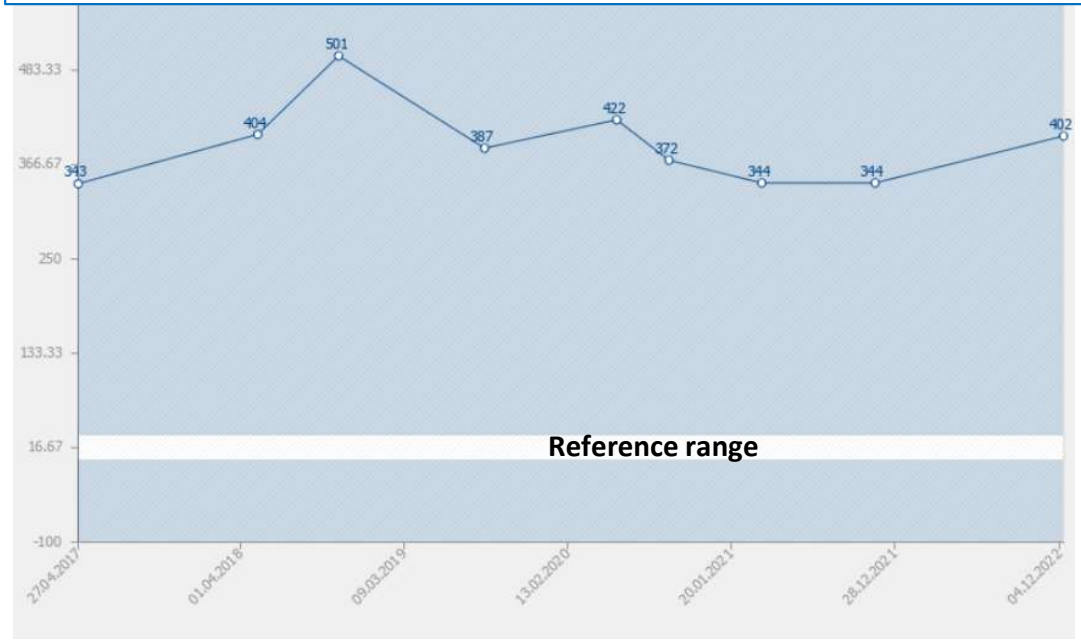
- Since age 4 years monitored by neurologists
- Normal results: blood count, ammonia, lactate, creatine kinase, thyroid hormones, amino acids (plasma & urine), transferrin isoelectric-focusing, EEG, brain MRI
- Genetic testing (array-CGH) planned, but moved to Hong Kong

If you were involved in Hong Kong, what tests would you consider?

# *Further metabolic work-up*

Urine organic acids: repeatedly increased 2-hydroxyglutaric acid

2-hydroxyglutaric acid in urine (mmol/mol creatinine)




What is your diagnosis?
What would you look additionally?
How would you proceed?

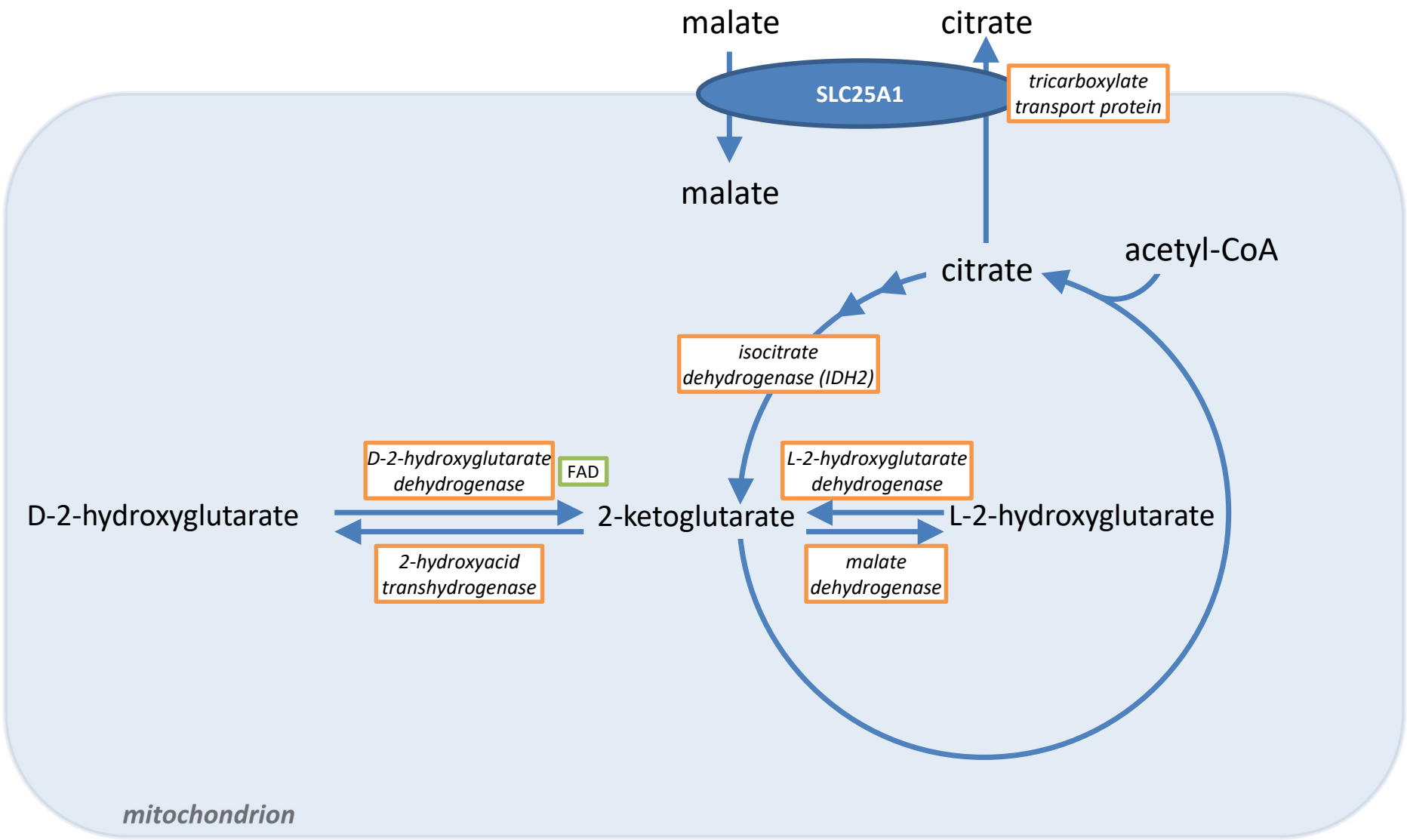
# Additional tests

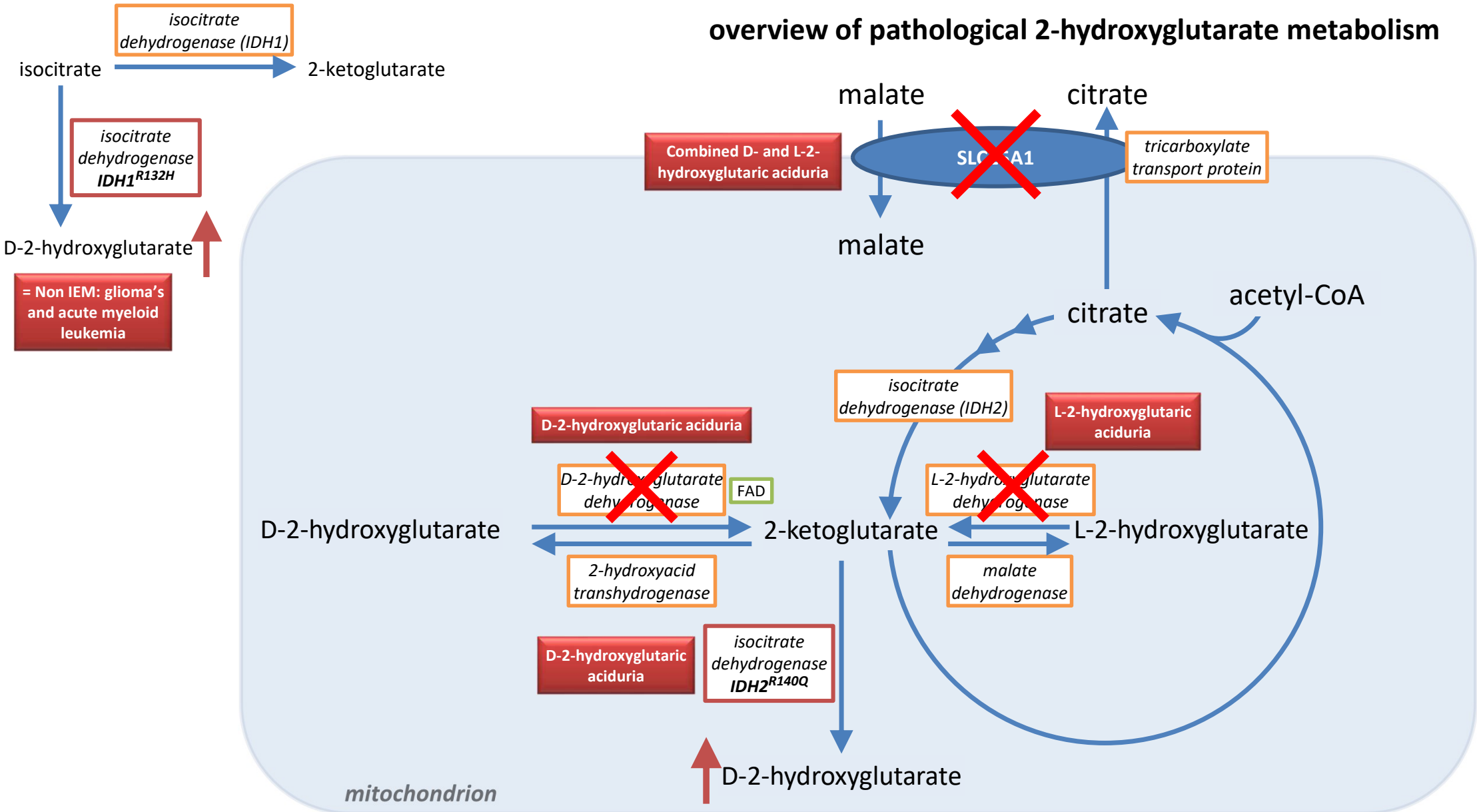
Family returned to Europe, and the following tests were done:

- Targeted NGS-exome (*IDH2* and *D2HGDH* genes): de novo *IDH2*-variant c.419G>A, p.Arg140Gln in heterozygous state (known from literature to be pathological variant causing D-2-hydroxyglutaric aciduria, type II (Kranendijk et al., 2010)

- Enantiomer analysis: signal of 2-hydroxyglutaric acid almost entirely from D-enantiomer with only traces of L-2-hydroxyglutaric acid

# 2-hydroxyglutarate metabolism




overview of pathological 2-hydroxyglutarate metabolism
isocitrate
isocitrate dehydrogenase (IDH1)
2-ketoglutarate
isocitrate dehydrogenase IDH1R132H
D-2-hydroxyglutarate
= Non IEM: glioma's and acute myeloid leukemia
malate
citrate
Combined D- and L-2-hydroxyglutaric aciduria
SLC25A1
tricarboxylate transport protein
malate
citrate
acetyl-CoA
isocitrate dehydrogenase (IDH2)
D-2-hydroxyglutaric aciduria
L-2-hydroxyglutaric aciduria
D-2-hydroxyglutarate dehydrogenase
FAD
L-2-hydroxyglutarate dehydrogenase
D-2-hydroxyglutarate
2-ketoglutarate
L-2-hydroxyglutarate
2-hydroxyacid transhydrogenase
malate dehydrogenase
D-2-hydroxyglutaric aciduria
isocitrate dehydrogenase IDH2R140Q
D-2-hydroxyglutarate
mitochondrion

# 2-hydroxyglutaric aciduria

Different types with predominant neurological symptoms

- L-2-hydroxyglutaric aciduria (*L2HGDH* gene): L-2-hydroxyglutarate dehydrogenase deficiency (metabolite repair enzyme), abnormal MRI
- D-2-hydroxyglutaric aciduria type I (*D2HGDH* gene): D-2-hydroxyglutarate dehydrogenase deficiency
- D-2-hydroxyglutaric aciduria type II
  - ✓ heterozygous variants *IDH2* gene (mitochondrial isocitrate dehydrogenase): often severe encephalopathy, cardiomyopathy
  - ✓ heterozygous variants *IDH1* gene (cytosolic isocitrate dehydrogenase): metaphyseal chondromatosis
  - ✓ *IDH1/2: de novo* variants in the majority of patients
- D/L-2-hydroxyglutaric aciduria (*SCL25A1* gene)

# 2-hydroxyglutaric aciduria

- Heterozygous variants in *IDH1* or *IDH2* genes*:* create a neoform enzyme (gain of function) that produces D-2-hydroxyglutaric acid from 2-ketoglutaric acid
- Other possible causes of elevation of 2-hydroxyglutaric acid
  - ✓ Adult form of multiple acyl-CoA dehydrogenase (riboflavin responsive): can be the only elevated metabolite
  - ✓ Nonspecific finding in mitochondrial diseases
  - ✓ Bacterial degradation of 2-ketoglutaric acid

# Further course

At 10 years

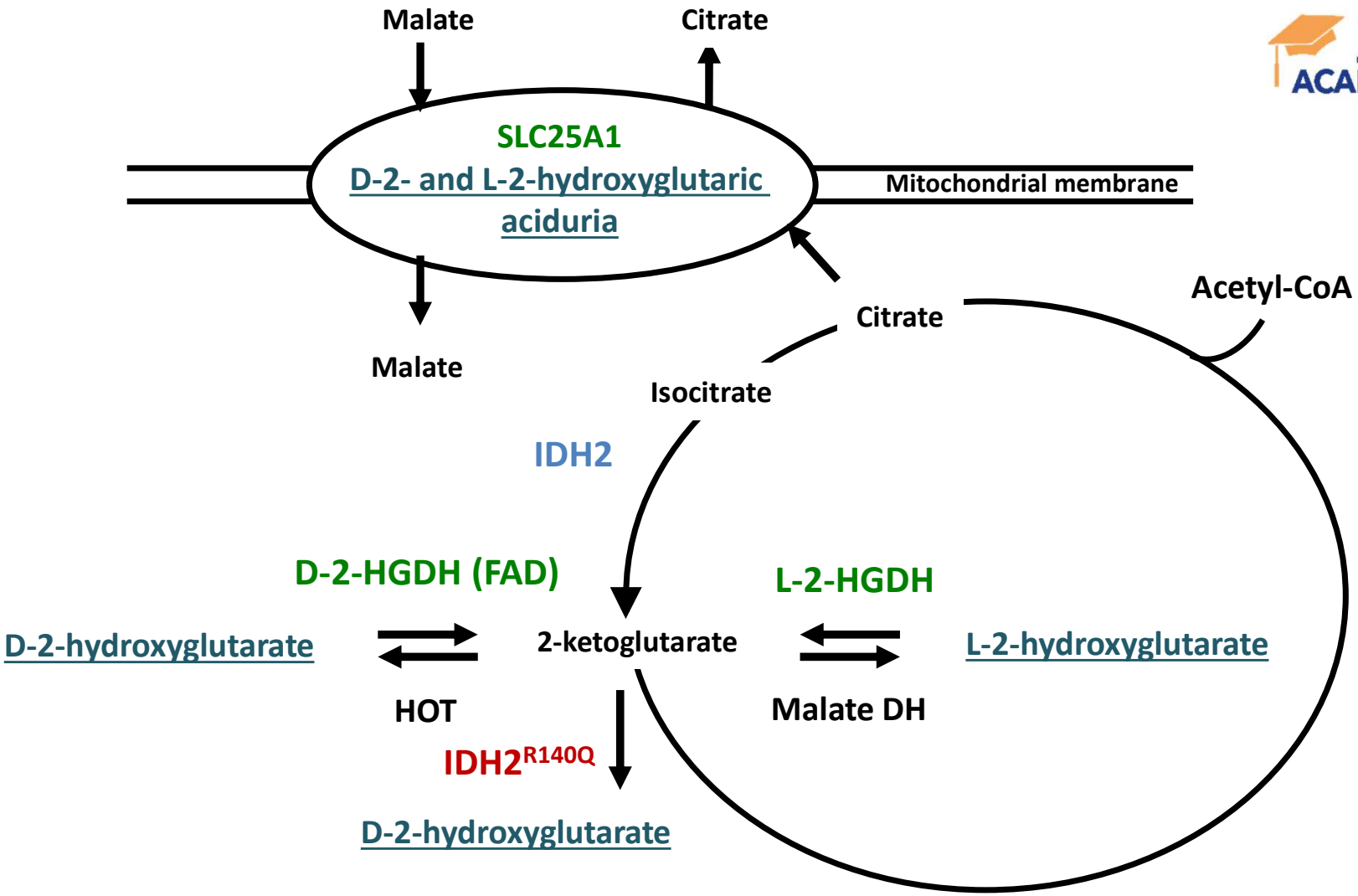
- Clinical situation stable, main problem: learning difficulties
- No diet or medication
- On physiotherapy, speech therapy and ergotherapy
- No progress of symptoms, but still delay, ataxia and hypotonia
- Brain MRI still without signs of leukencephalopathy
- Cardiac echogram: normal, no cardiomyopathy

# *Therapy considerations*

- Possibility of treatment with enasidenib
- Enasidenib has demonstrated striking efficacy in adults with somatic *IDH2* mutations and leukemia
- Possible adverse effect: hyperbilirubinemia related to UGT1 (uridine diphosphate-glucuronosyltransferase 1) inhibition by enasidenib

# Take home message

- Metabolic screening tests are still useful
- D-2-hydroxyglutaric aciduria has wide clinical variability, even with the same mutation




HOT: 2-hydroxyacid transhydrogenase

# Workshop organic acidemias

## *Case 9*

## Developmental delay with characteristic neuroimaging

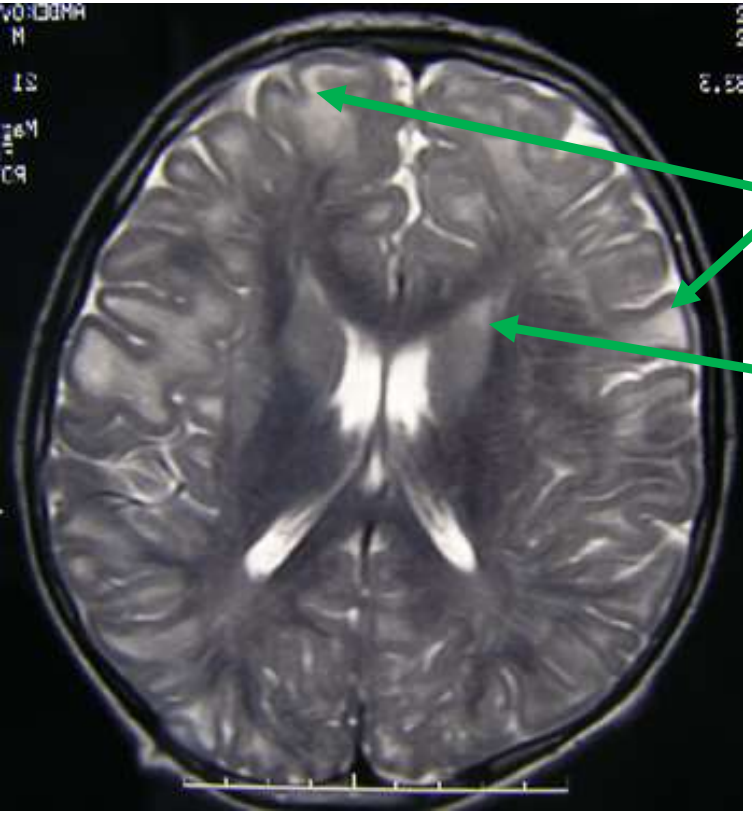
# Safa

- 1st child of consanguineous Pakistani parents

Presentation

- Developmental delay & ataxia
- First words at 18 months
- Sat at 9 months, walked at 20 months (poor balance)
- Normal fine motor skills
- Febrile seizure aged 11 months with occasional subsequent focal afebrile seizures
- Normal head circumference

# Neuroimaging aged 2 years

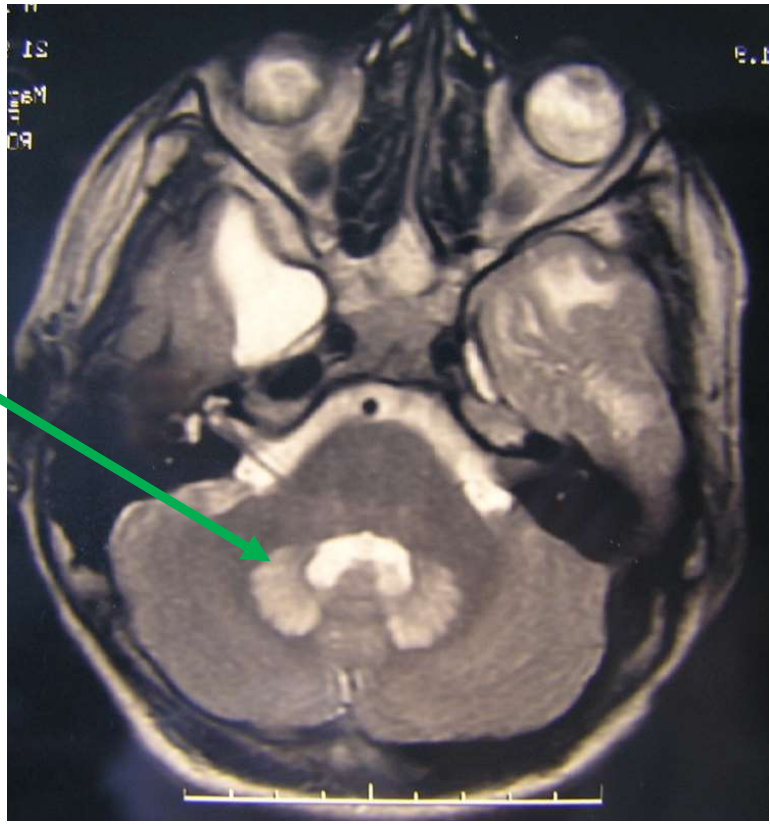


T2 weighted images

Foci of high signal in subcortical white matter, becoming confluent

Increased signal in dentate nucleus & caudate/globus pallidus/putamen

Cerebellar atrophy in some cases



# What are your differential diagnoses?

## White matter abnormalities

- Congenital CMV
- Acute disseminated encephalomyelitis /
- Periventricular leukomalacia

An MRI-based approach to the diagnosis of white matter disorders

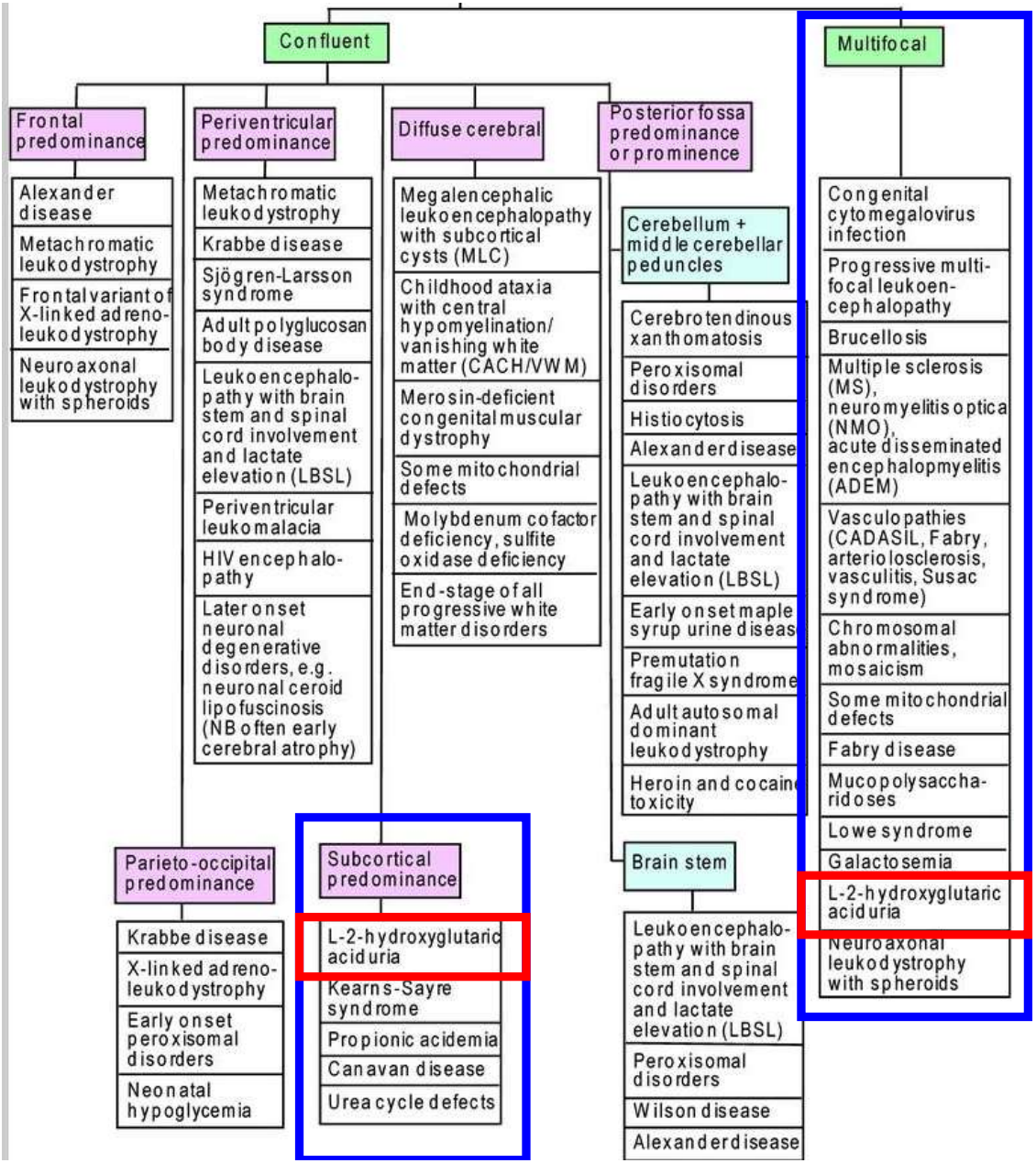
Raphael Schiffmann, MD
Marjo S. van der Knaap, MD, PhD

Neurology 2009; 72: 750-9.

ABSTRACT

**Background:** There are many different white matter disorders, both inherited and acquired, and consequently the diagnostic process is difficult. Establishing a specific diagnosis is often delayed at great emotional and financial costs. The pattern of brain structures involved, as visualized by MRI, has proven to often have a high diagnostic specificity.

- Lysosomal disorders e.g. MLD, Krabbe, MPS
- Peroxisomal disorders e.g. X-ALD, biogenesis disorders
- Mitochondrial disorders e.g. Kearns-Sayre, DARS2, EARS & AARS2 defects
- Cerebral organic acidurias (Canavan, L-2-hydroxyglutaric aciduria)
- Vanishing white matter disease
- Megaloencephalic leukoencephalopathy with subcortical cysts
- Alexander disease….

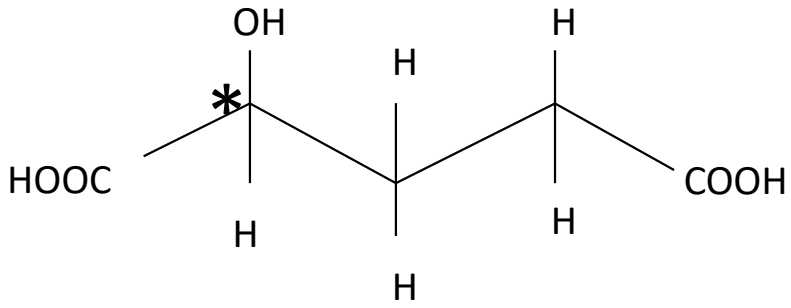



## Dentate ± basal ganglia lesions

- Neurodegeneration with brain iron accumulation (*reduced* signal on T2)
- Friedreich ataxia
- Mitochondrial (Leigh syndrome)
- Cerebral organic acidurias (Canavan, GA1, L-2-hydroxyglutaric aciduria)

## Diagnosis

- Organic acids: 2-hydroxyglutaric acid




The 2 enantiomers, can be distinguished in specialist labs

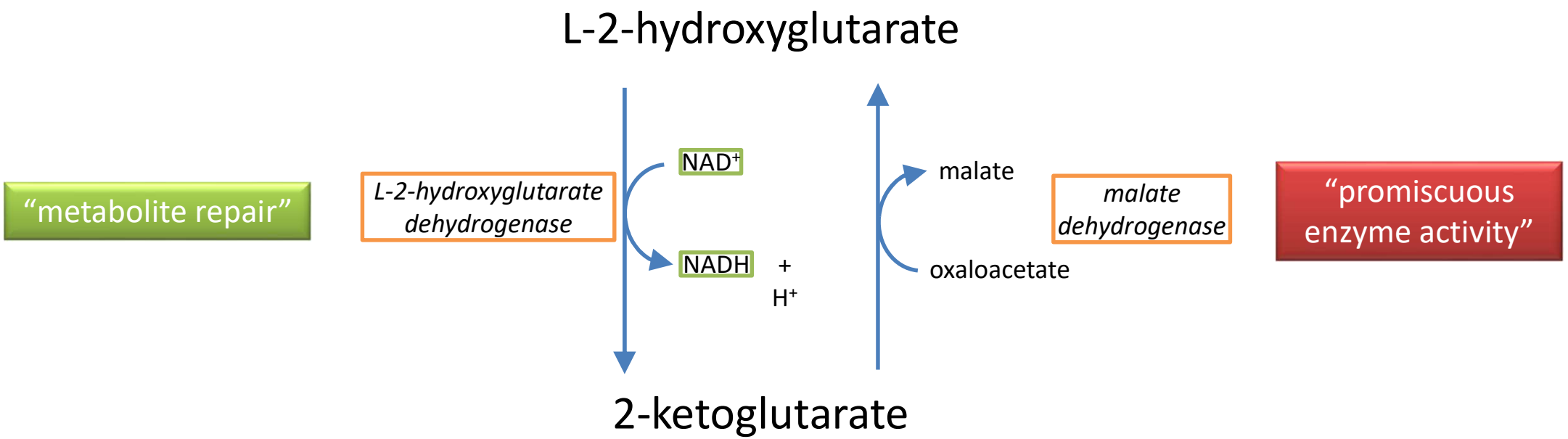
- Derivatisation with a chiral reagent or chromatography with chiral stationary phase
- later confirmed to be L-2-hydroxyglutaric acid
- Homozygous c.1316delC in *L2HGDH*

## Treatment & Progress

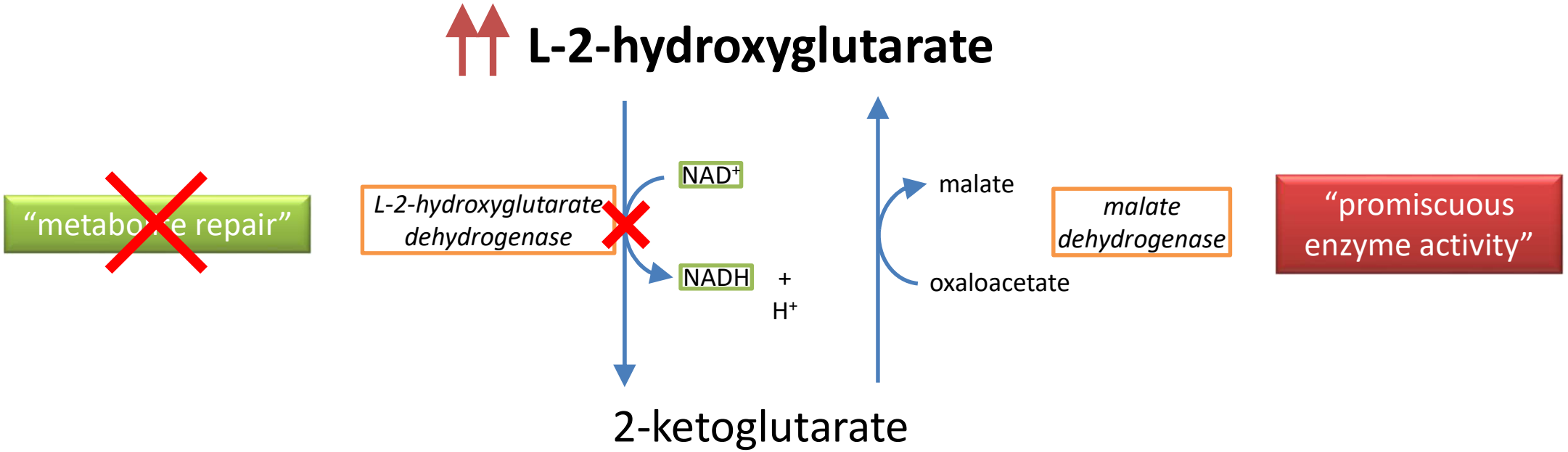
- No response to riboflavin
- Carbamazepine controlled seizures
- Continued slow development
- Special schooling

Why does the body make L-2-hydroxyglutaric acid?

**L-2-hydroxyglutarate dehydrogenase**



# L-2-hydroxyglutarate dehydrogenase deficiency



# L-2-hydroxyglutaric aciduria

- Delayed development
- Febrile & afebrile seizures
- Learning difficulties & intellectual decline in mid-childhood
- Ataxia, nystagmus, dysarthria, tremor

± Dystonia, myoclonus

± Macrocephaly

- Increased risk of CNS malignancies

# Messages

- Cerebral Organic Acidaemias, such as L-2-hydroxyglutaric aciduria & GA1, cause movement disorders, neurodevelopmental problems or seizures ± macrocephaly with little or no systemic disturbance

- L-2-hydroxyglutaric aciduria is a metabolite repair disorder

- Special techniques are needed to distinguish L & D enantiomers