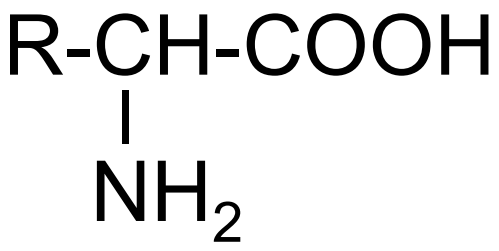
Organic acidurias

Jörn Oliver Sass / Diana Ballhausen

Selective Screening

- **Newborn screening** covers a general pattern of target diseases for every newborn.
- **Selective screening** searches only for selected diseases in individuals suspected to have a metabolic disease based on clinical, basic laboratory or family history data.
- **Analysis of urinary organic acids**:
  One of the most frequently applied tests in selective screening, often combined with acylcarnitines and amino acids, in particular, if an intoxication-type disease or energy deficiency is suspected.

Amino Acids



Chemically, amino acids are organic acids

What is the difference?

Amino Acids *versus* Organic Acids

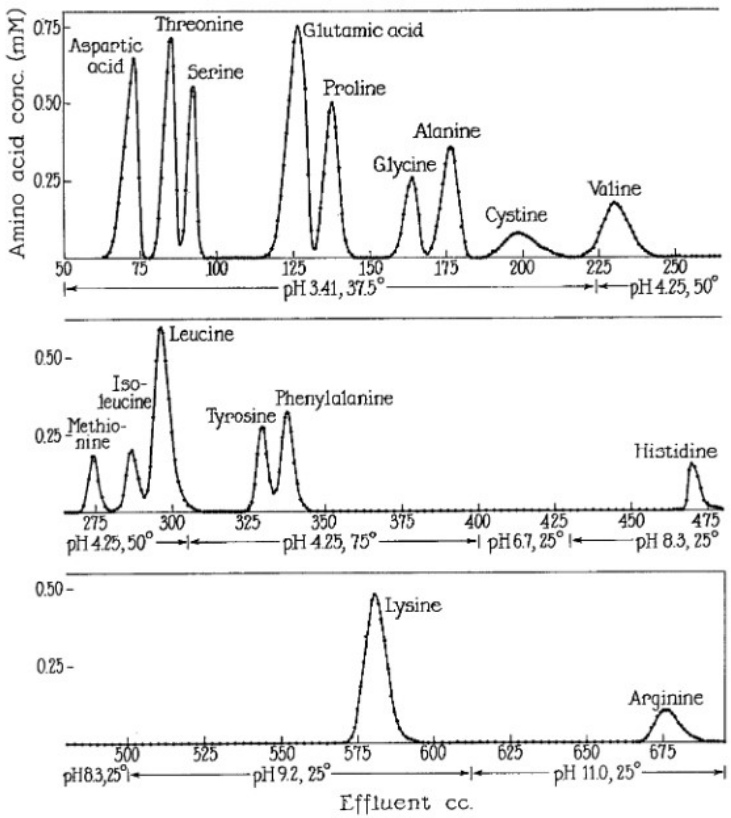
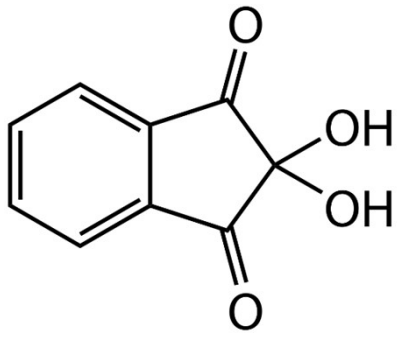


FIG. 1. Separation of amino acids from a synthetic mixture simulating the composition of a protein hydrolysate. The column of Dowex-50, 0.9 × 100 cm., was operated in the sodium form, with buffers of the pH and temperature indicated as eluants. A sample of about 6 mg. of amino acids (see Table II) was chromatographed. The position of an amino acid peak is reproducible, on the average, to better than 5 per cent.

Historic differentiation



Ninhydrin

CHROMATOGRAPHY OF AMINO ACIDS ON SULFONATED POLYSTYRENE RESINS

BY STANFORD MOORE AND WILLIAM H. STEIN

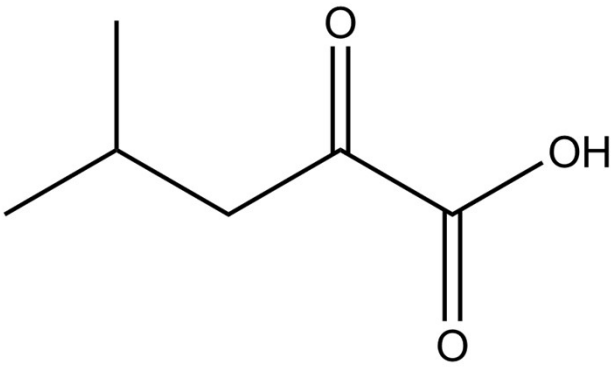
(From the Laboratories of The Rockefeller Institute for Medical Research, New York, New York)

(Received for publication, May 17, 1951)

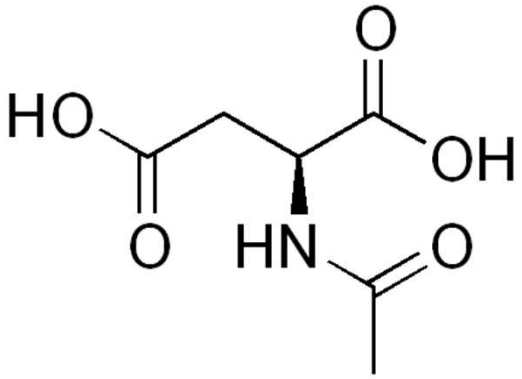
J Biol Chem. 1951;192:663-81.

Organic Acids

Amino acid metabolites after transamination or *N*-acylation



alpha-ketoisocaproic acid



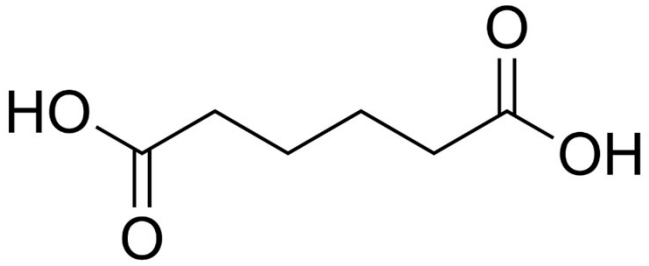
N-acetylaspartic acid

No primary amino group
⇒ no reaction with ninhydrin

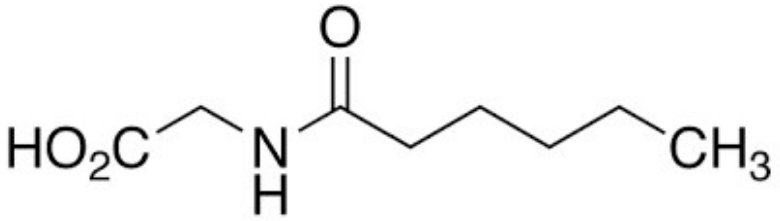
Organic Acids

Fatty acid metabolites

e.g., dicarboxylic acids and *N*-glycine conjugates



adipic acid



N-hexanoylglycine

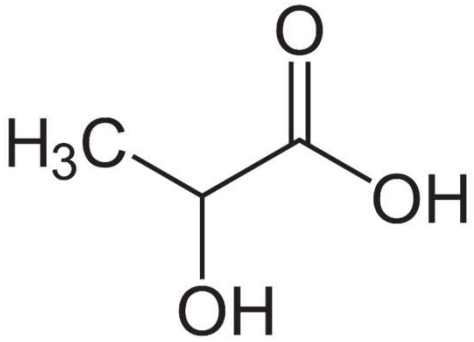
No primary amino group

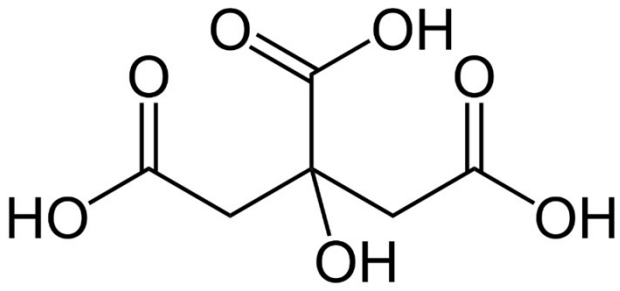
⇒ no reaction with ninhydrin

Organic Acids

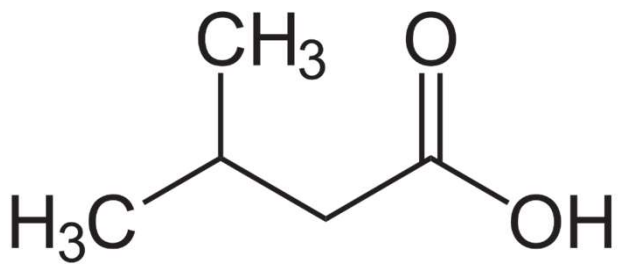
Lactate, TCA cycle metabolites, carbohydrate derivatives, somes purines/ pyrimidines

and many more compounds...





Early Organic Acid Analysis



Isovaleric Acid

*ISOVALERIC ACIDEMIA: A NEW GENETIC DEFECT OF LEUCINE METABOLISM**

By K. Tanaka, M. A. Budd, M. L. Efron, and K. J. Isselbacher

DEPARTMENTS OF MEDICINE AND NEUROLOGY, HARVARD MEDICAL SCHOOL AND MASSACHUSETTS GENERAL HOSPITAL, BOSTON

Communicated by Herman M. Kalckar, May 19, 1966

Proc Natl Acad Sci U S A. 1966;56:236-42

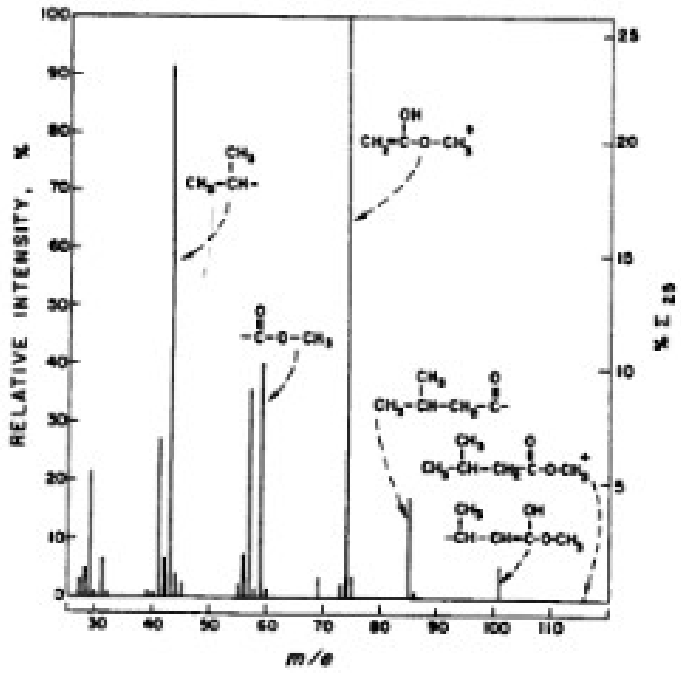


FIG. 2.—Mass spectrum of methy ester of the "unknown" peak from B. A.'s urine. Experimental conditions and the interpretation are described in the text.

Organic Acids in Urine

- If an organic aciduria is suspected in a critically ill patient, urine should be collected immediately (bag, consider catheter).

- Urine obtained during a metabolic crisis may be of particular value for organic acids analysis (sensitivity). Often therapy needs to be initiated while lab diagnostics are still running.

- For selective screening, the first morning urine sample is the best (high concentration). Very diluted urine may give incorrect results as results are nomalized to creatinine.

Organic Acids in Urine

- Consider temporary storage in refrigerator/ freezer (needs to be documented).
- Only in exceptional cases (e.g., if no other material available after death) organic acids will be determined in other body fluids than urine.
- Creatinine quantitation for normalization; dip-stick test for pre-assessment of the sample.

Analysis of Urinary Organic Acids (classical approach)

- GC-MS analysis of organic acids extracted from urine
- As organic acids are polar, thermally unstable, and have low volatility, it is necessary for GC analysis to convert them into nonpolar, volatile, and thermally stable derivatives and to separate them from all the inorganic salts etc. in urine
- First, internal standard(s) need(s) to be added
- Urine is acidified to obtain the organic acids in non-dissociated, rather non-polar form
- Extraction with liquid solvent(s), possible also using solid phase extraction
- Drying and (almost) evaporation of the organic phase

Analysis of Urinary Organic Acids (classical approach)

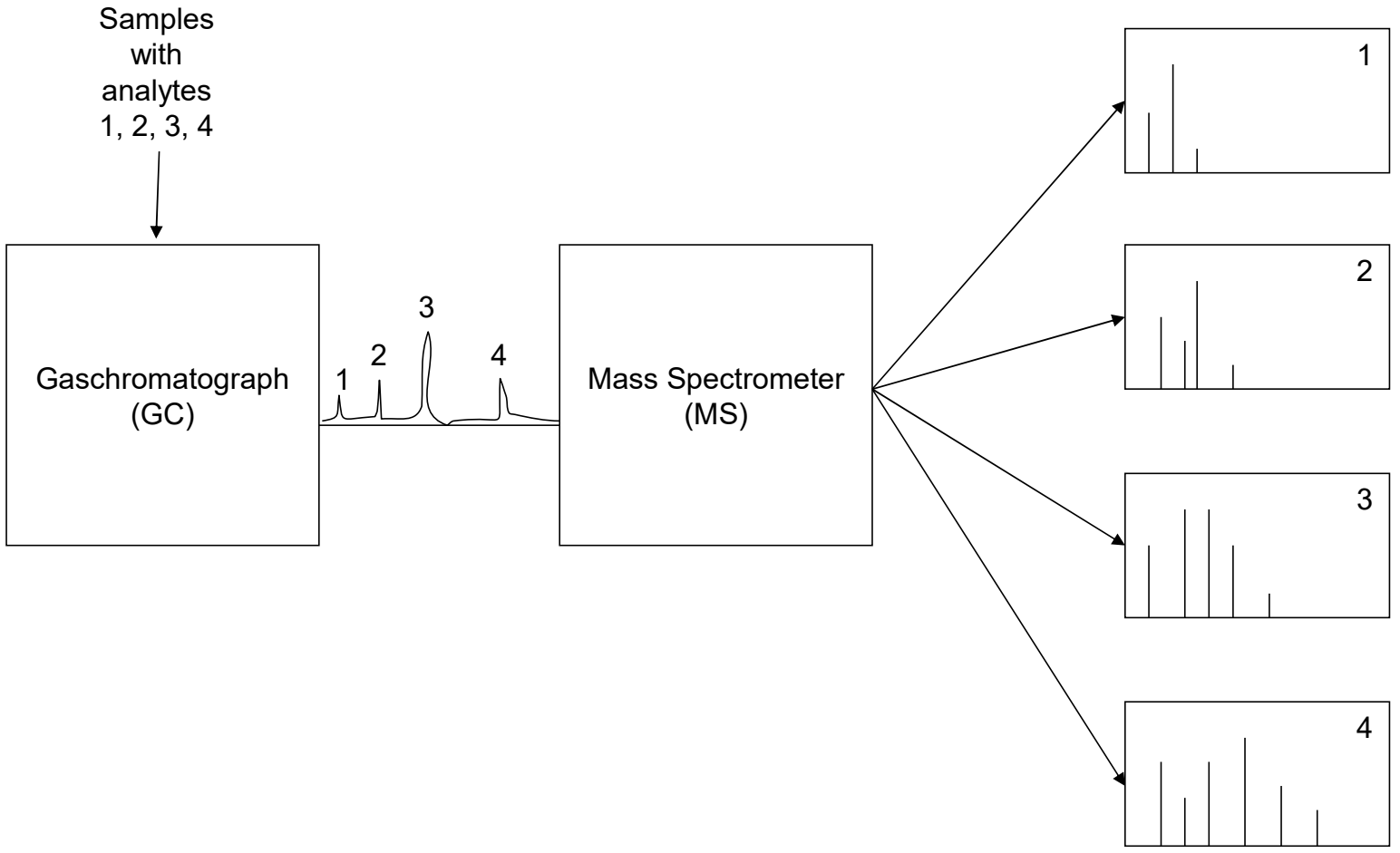
Esterification of the extracted organic acids:

- Derivatives can then be diluted and injected into the GC-MS instrument

  - mostly by trimethylsilylation with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane
  (often with prior stabilization of oxoacids by formation of an oxime [using hydroxylamine or a substituted derivative] prior to the esterification)
  - alternatively methylation with diazomethane is possible

- Derivatives can then be diluted and injected into the GC-MS instrument

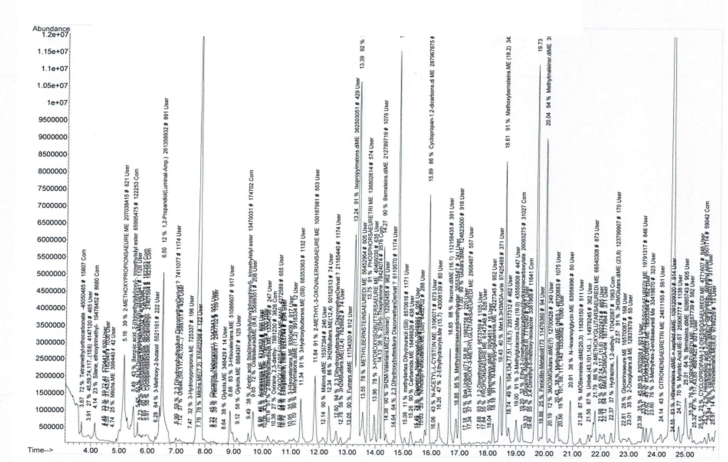
Organic Acid Analysis by GC-MS

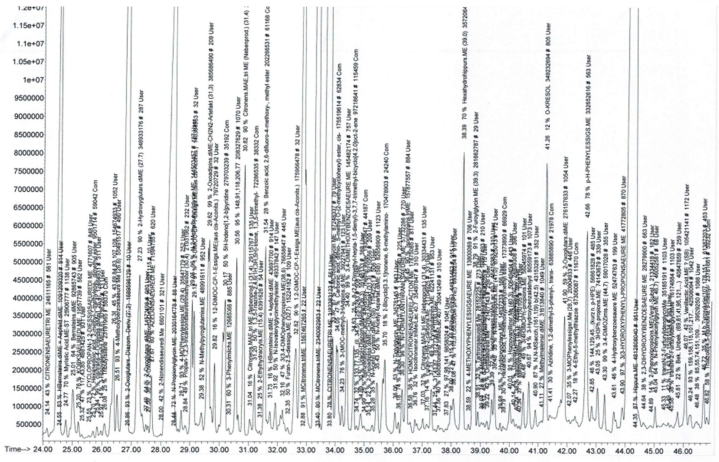


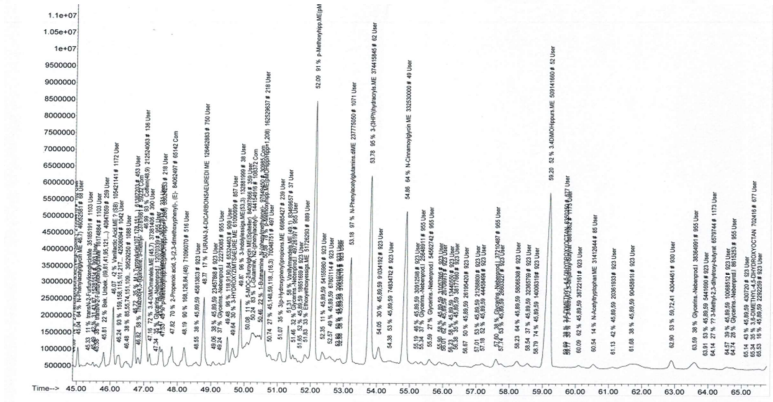
Adapted from Sass J Lab Med 2011;35:1–4

Mass spectra obtained after chromatographic separation of derivatized metabolites.

Urinary Organic Acids







MS-MS approaches have also been introduced.

Complex metabolite profiles, which may comprise with hundreds of signals, may be assessed with the help of mass spectra libraries.

Urinary Organic Acids: Pitfalls

- Bacterial degradation processes

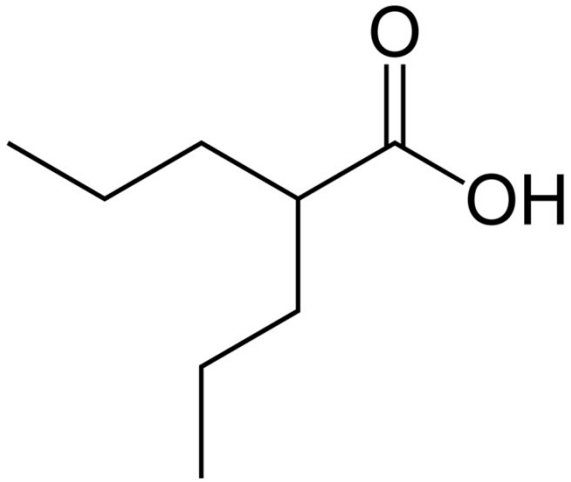
  e.g., Citrate ↓, Lactate/3-OH-Propionate/Succinate/Uracil ↑; 2-OH-Glutarate ↑

- Dietary effects

  e.g., aromatic compounds ↑ or epoxidicarboxylic acids ↑

- Impairment by putative preservatives such thymol, acetic acid, boric acid

Urinary Organic Acids: influence of drugs

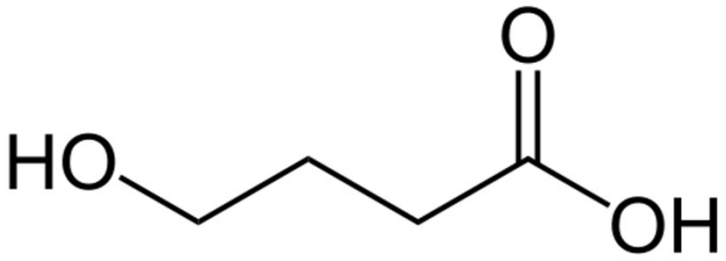


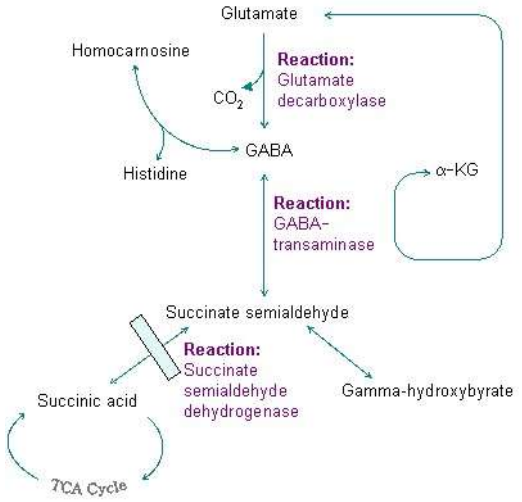
Valproate (Dipropylpentanoate)
$\Rightarrow$ many interfering signals

Urinary Organic Acids: influence of drugs

4-hydroxy-butyrate (GHB)

Sedativum and illegal drug





Pearl et al. Gene Reviews 2016;
https://www.ncbi.nlm.nih.gov/books/NBK1195/

⇒ May mimic
succinic semialdehyde dehydrogenase deficiency

Urinary Organic Acids: influence of drugs

- Chloral hydrate
- Ampicillin, Penicillamine
- (Acetyl-)Salicylic acid
- Paracetamol (acetaminophen)
- Phenobarbital (phenobarbitone)
- and many other drugs/ xenobiotics

This does not necessarily make organic acid interpretation impossible, but may make it more difficult.
It is very important to **communicate medication, dietary particularities and basic clinical information to the laboratory.**

Urinary Organic Acids analysis: Conclusions

Analyses of organic acids, amino acids and acylcarnitines complement each other.

- several aminoacidopathies (e.g. PKU, MSUD) can also be identified by the organic acids pattern

- But: many downstream metabolites of amino acids can only be identified in the organic acids pattern

- acylcarnitine analysis reveals only those organic acidurias that are associated with alterations in coenzyme A patterns, while organic acid analysis may also identify others.

Presentation of Organic Acidurias

- Most become apparent by acute decompensation during newborn period or early infancy (early onset)
- Newborns: life-threatening episode of metabolic acidosis after initial period of well-being (free interval), might be mistaken for sepsis
- Risk for increased mortality and morbidity, if not recognized
- Milder forms: presentation in childhood, adolescence, adulthood or not at all (late onset)
- Chronic manifestations without decompensation

Triggers for Decompensation

- **Catabolism** due to: infections, poor feeding (postnatal), prolonged fasting, vomiting, diarrhea, fever, prolonged or intense physical exercise, psychological stress, surgery and/or general anesthesia
- **Excessive protein intake**
- **Medication**: e.g., chemotherapy, high dose glucocorticoids

Lab Findings in Decompensation

- Metabolic acidosis with high anion gap
- Lactic acidemia and/or lactic aciduria
- Ketosis and ketonuria
- Other possible findings:
  - hypoglycemia
  - hyperammonemia
  - electrolyte alterations
  - anemia, neutropenia, thrombocytopenia

Initial Treatment in Decompensation

- Stop protein intake (24h - 48h)
- Start high-dose iv glucose (+insulin)
- Treat hyperammonemia (Carbaglu)
- Administer:
  - 100 mg/kg L-carnitine slowly iv
  - 1 mg/d hydroxocobalamin iv/im
  - 10 mg biotin iv/po
- Collect samples for diagnostics

Diagnosis within the first 24-48 h is vital and can prevent chronic sequels!

Classification of Organic Acidurias

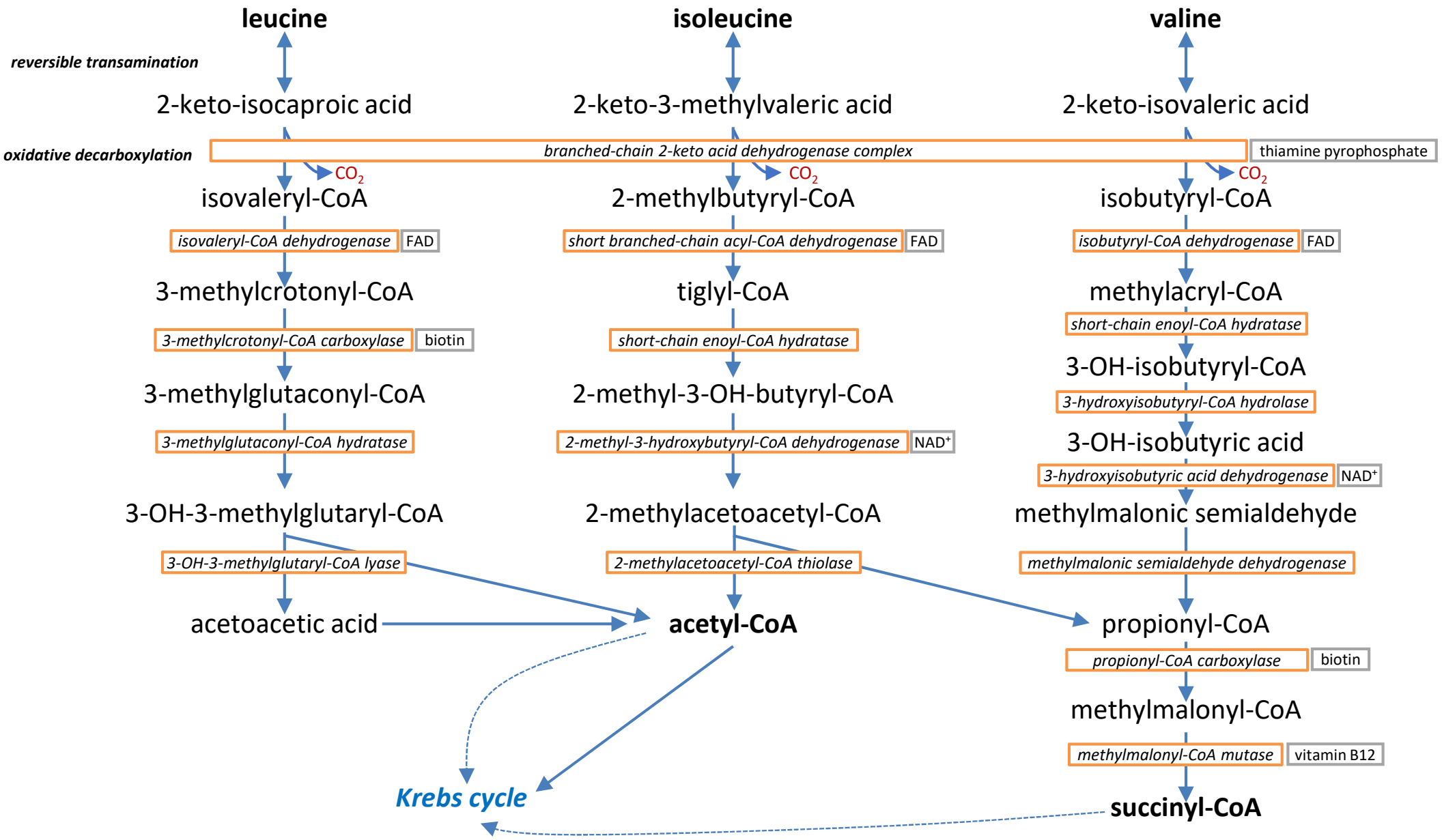
Classical organic acidurias

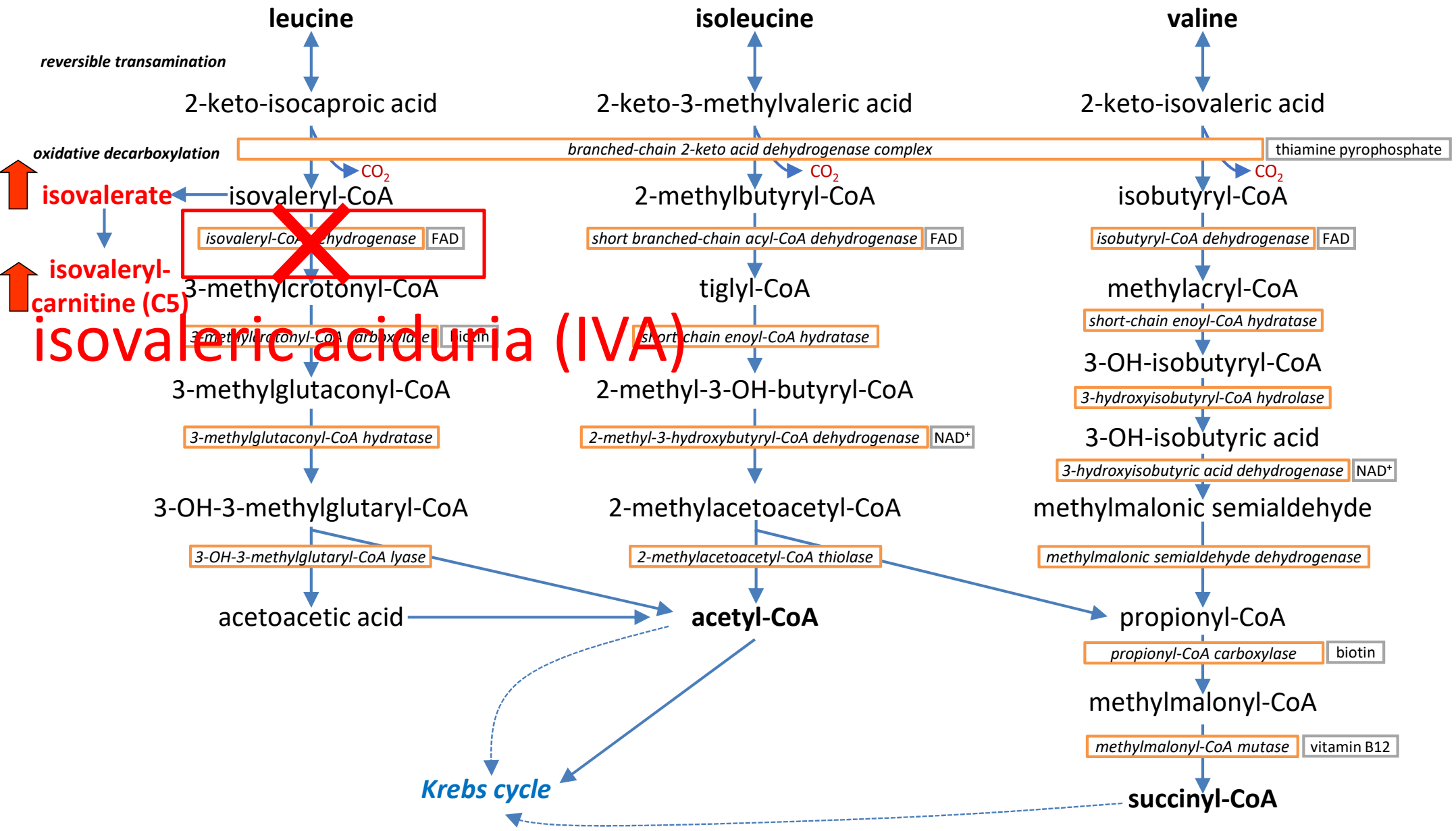
MMA, PA, (IVA)

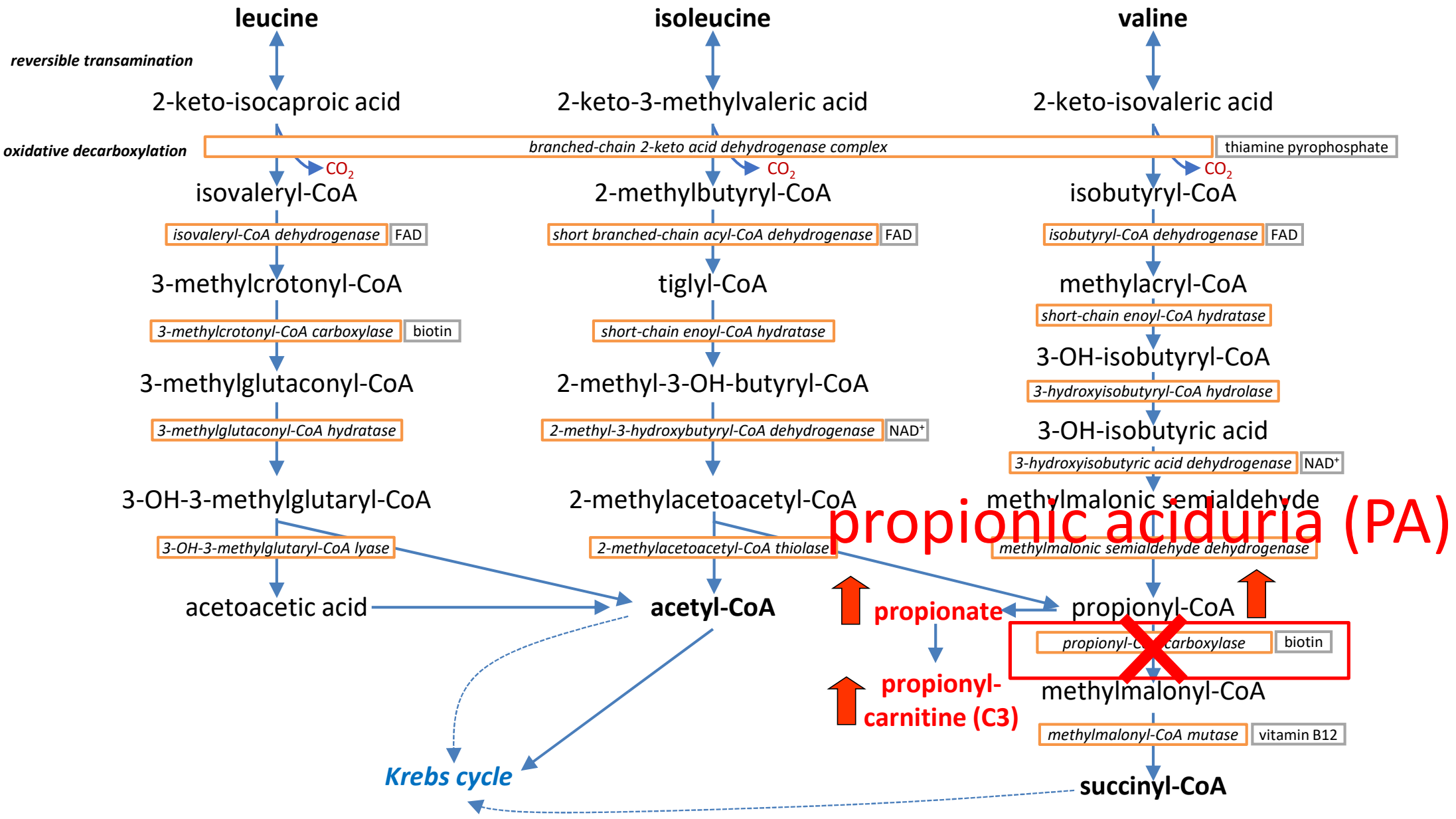
“Cerebral” organic acidurias

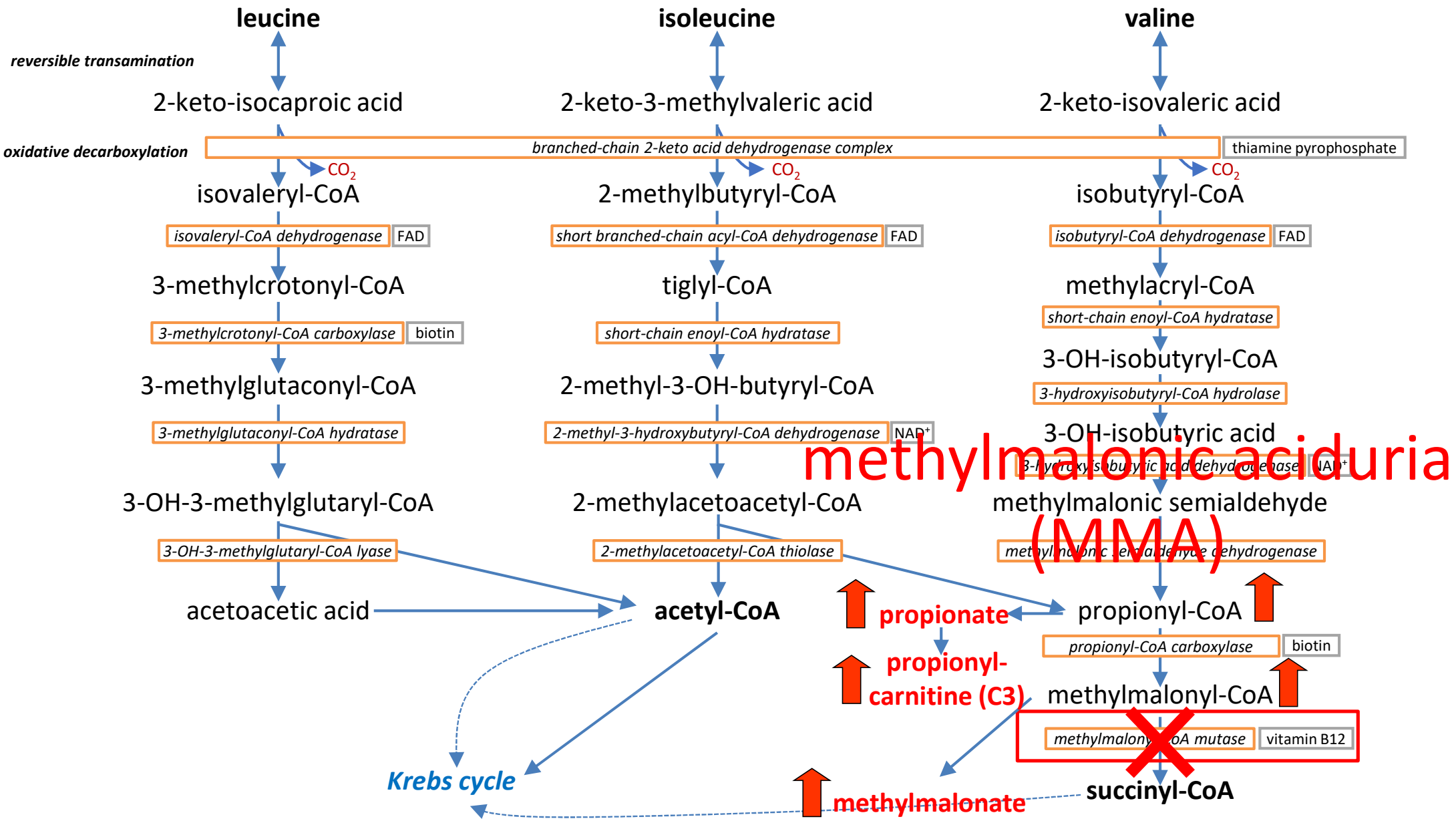
- GA 1
- Canavan disease,
  D- and L-2-hydroxyglutaric acidurias,
  4-hydroxybutyric aciduria etc.

Ketone body synthesis & utilization defects

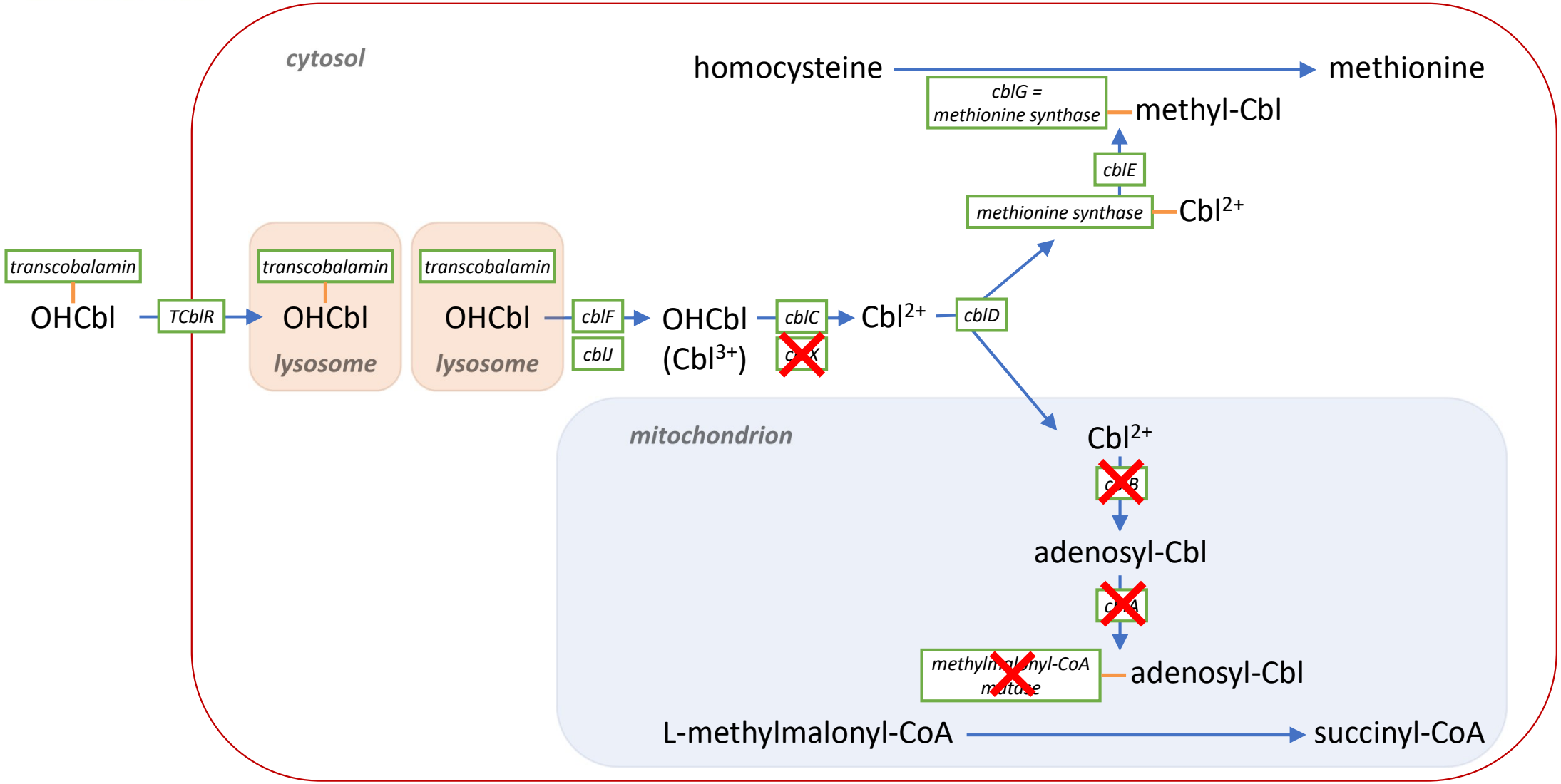

leucine
isoleucine
valine
reversible transamination
2-keto-isocaproic acid
2-keto-3-methylvaleric acid
2-keto-isovaleric acid
oxidative decarboxylation
branched-chain 2-keto acid dehydrogenase complex
thiamine pyrophosphate
CO2
CO2
CO2
isovaleryl-CoA
2-methylbutyryl-CoA
isobutyryl-CoA
isovaleryl-CoA dehydrogenase
FAD
short branched-chain acyl-CoA dehydrogenase
FAD
isobutyryl-CoA dehydrogenase
FAD
3-methylcrotonyl-CoA
tiglyl-CoA
methylacryl-CoA
3-methylcrotonyl-CoA carboxylase
biotin
short-chain enoyl-CoA hydratase
short-chain enoyl-CoA hydratase
3-OH-isobutyryl-CoA
3-methylglutaconyl-CoA
2-methyl-3-OH-butyryl-CoA
3-hydroxyisobutyryl-CoA hydrolase
3-methylglutaconyl-CoA hydratase
2-methyl-3-hydroxybutyryl-CoA dehydrogenase
NAD+
3-OH-isobutyric acid
3-hydroxyisobutyric acid dehydrogenase
NAD+
3-OH-3-methylglutaryl-CoA
2-methylacetoacetyl-CoA
methylmalonic semialdehyde
3-OH-3-methylglutaryl-CoA lyase
2-methylacetoacetyl-CoA thiolase
methylmalonic semialdehyde dehydrogenase
acetoacetic acid
acetyl-CoA
propionyl-CoA
propionyl-CoA carboxylase
biotin
methylmalonyl-CoA
methylmalonyl-CoA mutase
vitamin B12
Krebs cycle
succinyl-CoA


leucine
isoleucine
valine
reversible transamination
2-keto-isocaproic acid
2-keto-3-methylvaleric acid
2-keto-isovaleric acid
oxidative decarboxylation
branched-chain 2-keto acid dehydrogenase complex
thiamine pyrophosphate
CO2
CO2
CO2
isovalerate
isovaleryl-carnitine (C5)
isovaleryl-CoA
isovaleryl-CoA dehydrogenase
FAD
2-methylbutyryl-CoA
short branched-chain acyl-CoA dehydrogenase
FAD
isobutyryl-CoA
isobutyryl-CoA dehydrogenase
FAD
3-methylcrotonyl-CoA
tiglyl-CoA
methylacryl-CoA
short-chain enoyl-CoA hydratase
3-OH-isobutyryl-CoA
3-hydroxyisobutyryl-CoA hydrolase
3-OH-isobutyric acid
3-hydroxyisobutyric acid dehydrogenase
NAD+
isovaleric aciduria (IVA)
3-methylglutaconyl-CoA
2-methyl-3-OH-butyryl-CoA
3-methylglutaconyl-CoA hydratase
2-methyl-3-hydroxybutyryl-CoA dehydrogenase
NAD+
3-OH-3-methylglutaryl-CoA
2-methylacetoacetyl-CoA
methylmalonic semialdehyde
3-OH-3-methylglutaryl-CoA lyase
2-methylacetoacetyl-CoA thiolase
methylmalonic semialdehyde dehydrogenase
acetoacetic acid
acetyl-CoA
propionyl-CoA
propionyl-CoA carboxylase
biotin
methylmalonyl-CoA
methylmalonyl-CoA mutase
vitamin B12
Krebs cycle
succinyl-CoA

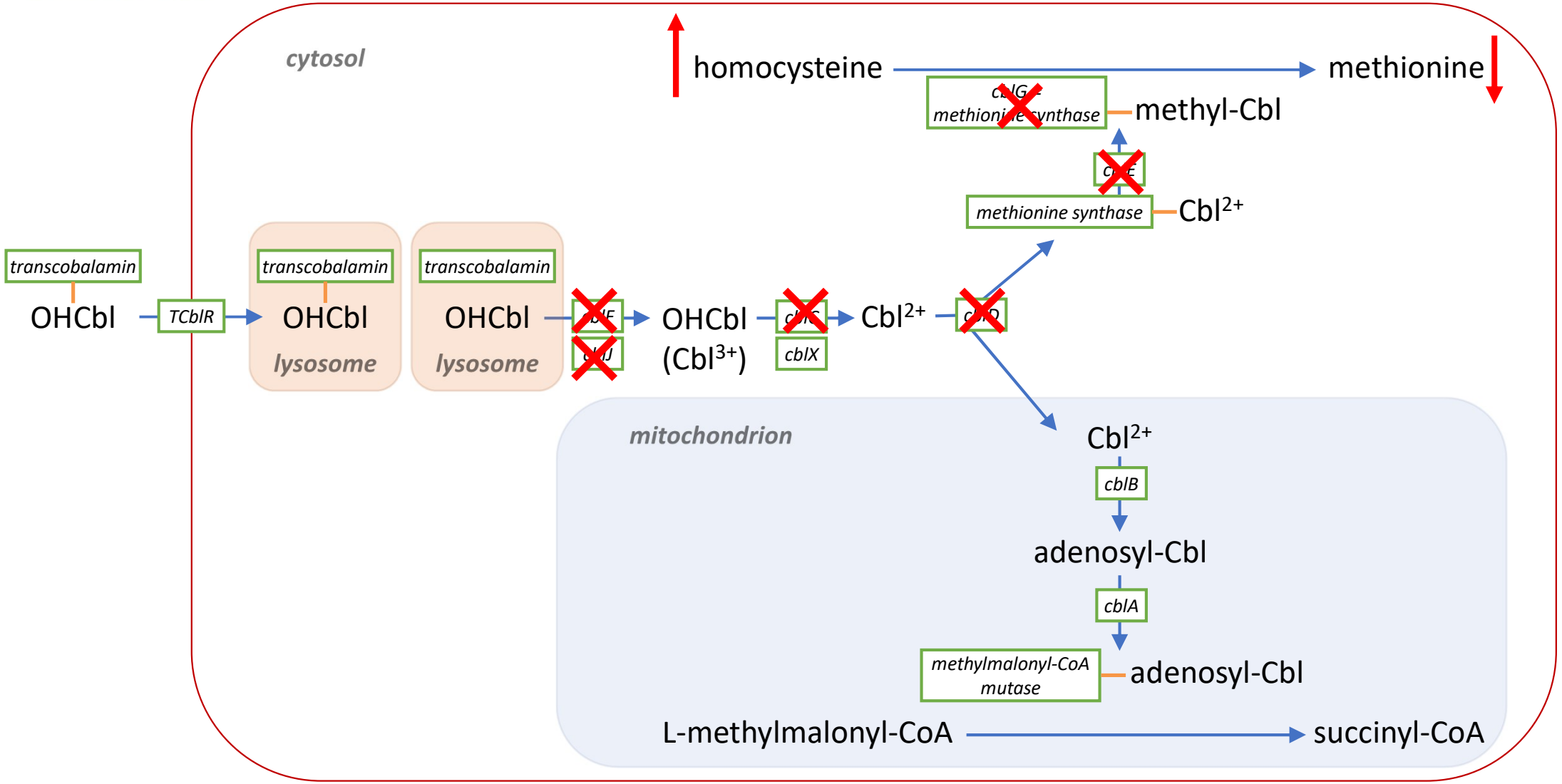

leucine
isoleucine
valine
reversible transamination
2-keto-isocaproic acid
2-keto-3-methylvaleric acid
2-keto-isovaleric acid
oxidative decarboxylation
branched-chain 2-keto acid dehydrogenase complex
thiamine pyrophosphate
CO2
CO2
CO2
isovaleryl-CoA
2-methylbutyryl-CoA
isobutyryl-CoA
isovaleryl-CoA dehydrogenase
FAD
short branched-chain acyl-CoA dehydrogenase
FAD
isobutyryl-CoA dehydrogenase
FAD
3-methylcrotonyl-CoA
tiglyl-CoA
methylacryl-CoA
3-methylcrotonyl-CoA carboxylase
biotin
short-chain enoyl-CoA hydratase
short-chain enoyl-CoA hydratase
3-OH-isobutyryl-CoA
3-methylglutaconyl-CoA
2-methyl-3-OH-butyryl-CoA
3-hydroxyisobutyryl-CoA hydrolase
3-methylglutaconyl-CoA hydratase
2-methyl-3-hydroxybutyryl-CoA dehydrogenase
NAD+
3-OH-isobutyric acid
3-hydroxyisobutyric acid dehydrogenase
NAD+
3-OH-3-methylglutaryl-CoA
2-methylacetoacetyl-CoA
methylmalonic semialdehyde
propionic aciduria (PA)
3-OH-3-methylglutaryl-CoA lyase
2-methylacetoacetyl-CoA thiolase
methylmalonic semialdehyde dehydrogenase
acetoacetic acid
acetyl-CoA
propionate
propionyl-CoA
propionyl-CoA carboxylase
biotin
propionyl-carnitine (C3)
methylmalonyl-CoA
methylmalonyl-CoA mutase
vitamin B12
Krebs cycle
succinyl-CoA


leucine
isoleucine
valine
reversible transamination
2-keto-isocaproic acid
2-keto-3-methylvaleric acid
2-keto-isovaleric acid
oxidative decarboxylation
branched-chain 2-keto acid dehydrogenase complex
thiamine pyrophosphate
CO2
CO2
CO2
isovaleryl-CoA
2-methylbutyryl-CoA
isobutyryl-CoA
isovaleryl-CoA dehydrogenase
FAD
short branched-chain acyl-CoA dehydrogenase
FAD
isobutyryl-CoA dehydrogenase
FAD
3-methylcrotonyl-CoA
tiglyl-CoA
methylacryl-CoA
3-methylcrotonyl-CoA carboxylase
biotin
short-chain enoyl-CoA hydratase
short-chain enoyl-CoA hydratase
3-OH-isobutyryl-CoA
3-methylglutaconyl-CoA
2-methyl-3-OH-butyryl-CoA
3-hydroxyisobutyryl-CoA hydrolase
3-methylglutaconyl-CoA hydratase
2-methyl-3-hydroxybutyryl-CoA dehydrogenase
NAD+
3-OH-isobutyric acid
3-hydroxyisobutyric acid dehydrogenase
NAD+
3-OH-3-methylglutaryl-CoA
2-methylacetoacetyl-CoA
methylmalonic semialdehyde
3-OH-3-methylglutaryl-CoA lyase
2-methylacetoacetyl-CoA thiolase
methylmalonic semialdehyde dehydrogenase
acetoacetic acid
acetyl-CoA
propionate
propionyl-CoA
propionyl-CoA carboxylase
biotin
propionyl-carnitine (C3)
methylmalonyl-CoA
methylmalonyl-CoA mutase
vitamin B12
Krebs cycle
methylmalonate
succinyl-CoA
methylmalonic aciduria (MMA)

MMAuria without hyperhomocysteinemia



MMAuria with hyperhomocysteinemia



Neonatal Onset: History

- Poor feeding, vomiting, decreasing consciousness
- Deterioration after initial period of health

  8-36 hrs in MMA, PA, (IVA)
- Family history / Parental consanguinity

Neonatal Onset: Examination

- Reduced consciousness
- Abnormal movements/tone
- Kussmaul's respiration

± Hepatomegaly

± Dehydration

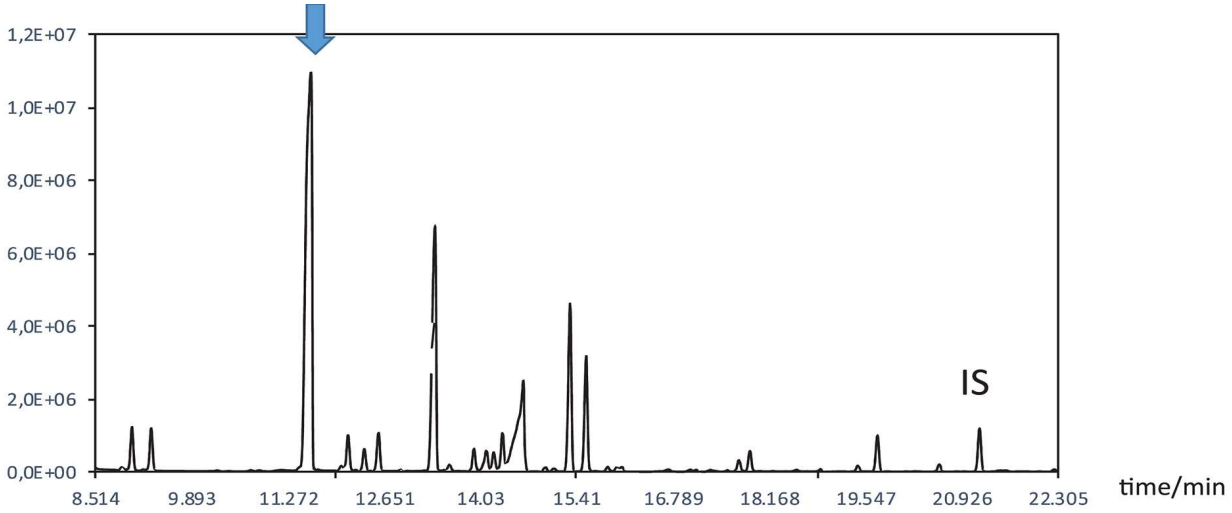
± Temperature instability

± Epileptic seizures

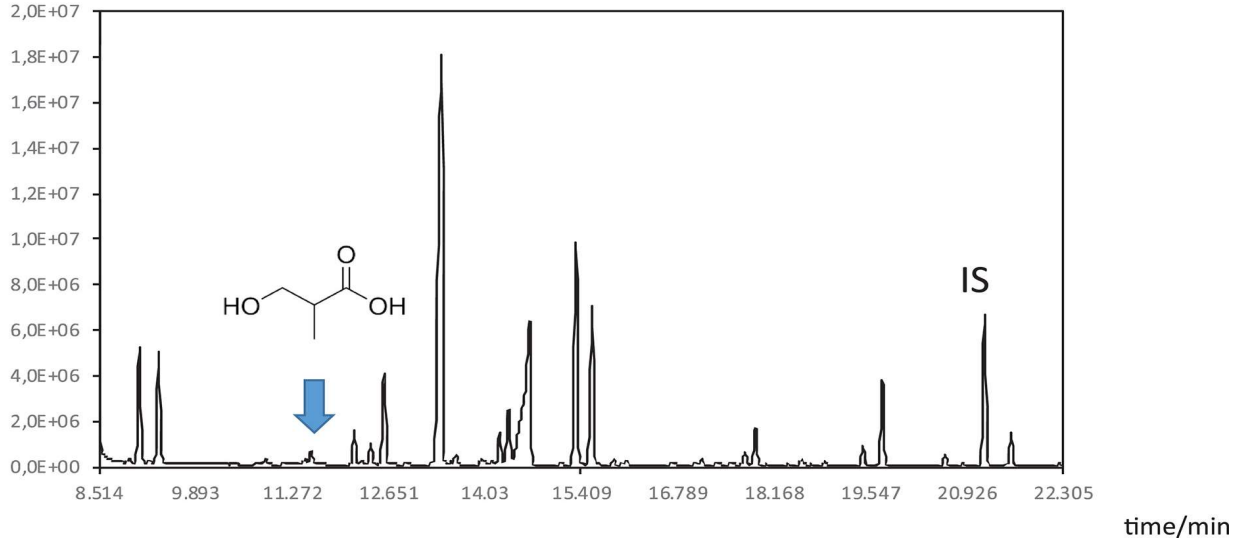
± Cardiomyopathy

± Odour: sweaty feet in IVA

patient



control



Recently discovered organic aciduria

Meyer *et al.* J Inherit Metab Dis 2021;22:1323-9

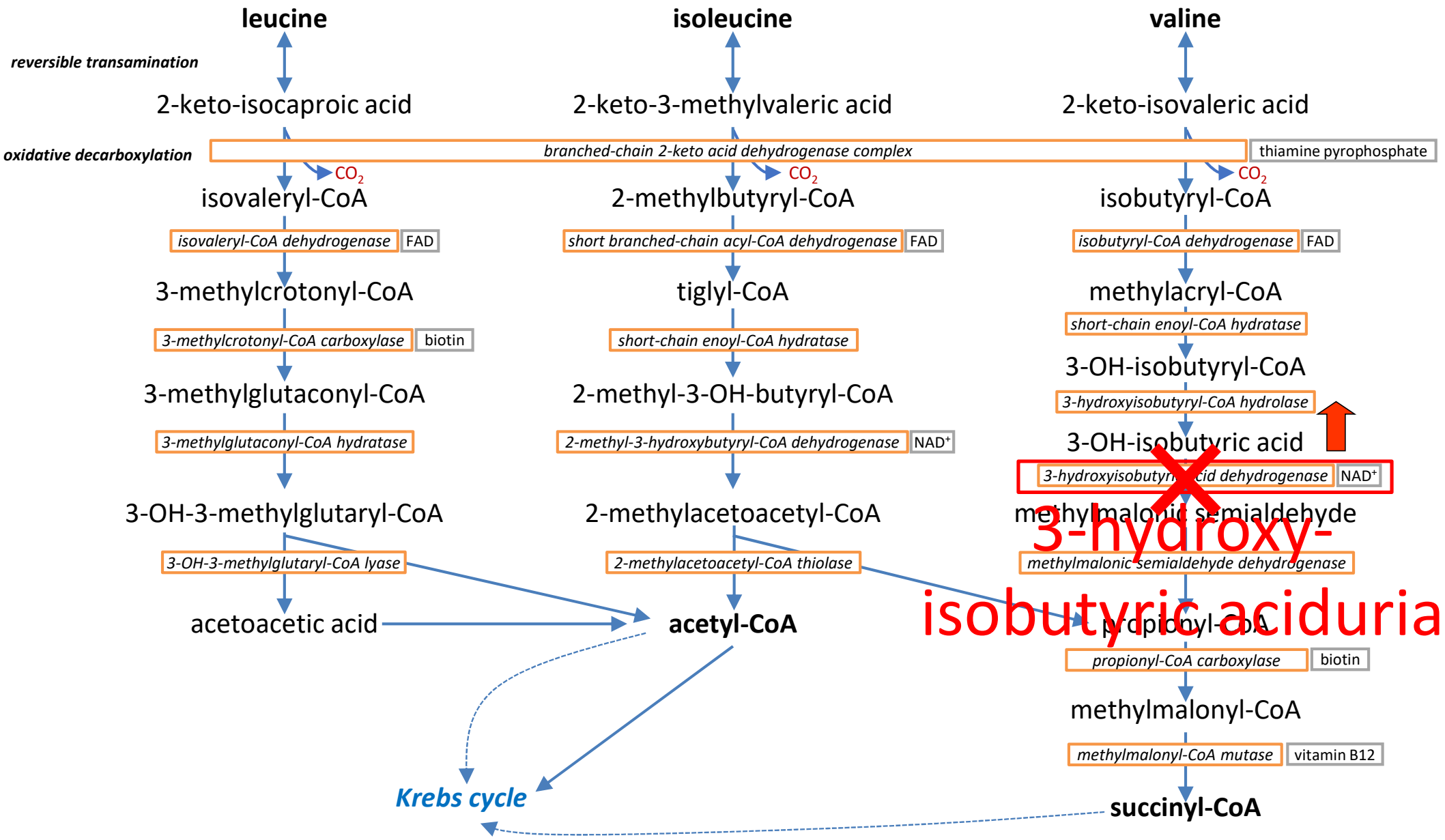

leucine
isoleucine
valine
reversible transamination
2-keto-isocaproic acid
2-keto-3-methylvaleric acid
2-keto-isovaleric acid
oxidative decarboxylation
branched-chain 2-keto acid dehydrogenase complex
thiamine pyrophosphate
CO2
CO2
CO2
isovaleryl-CoA
2-methylbutyryl-CoA
isobutyryl-CoA
isovaleryl-CoA dehydrogenase
FAD
short branched-chain acyl-CoA dehydrogenase
FAD
isobutyryl-CoA dehydrogenase
FAD
3-methylcrotonyl-CoA
tiglyl-CoA
methylacryl-CoA
3-methylcrotonyl-CoA carboxylase
biotin
short-chain enoyl-CoA hydratase
short-chain enoyl-CoA hydratase
3-OH-isobutyryl-CoA
3-methylglutaconyl-CoA
2-methyl-3-OH-butyryl-CoA
3-hydroxyisobutyryl-CoA hydrolase
3-methylglutaconyl-CoA hydratase
2-methyl-3-hydroxybutyryl-CoA dehydrogenase
NAD+
3-OH-isobutyric acid
3-hydroxyisobutyrate dehydrogenase
NAD+
3-OH-3-methylglutaryl-CoA
2-methylacetoacetyl-CoA
methylmalonic semialdehyde
3-OH-3-methylglutaryl-CoA lyase
2-methylacetoacetyl-CoA thiolase
methylmalonic semialdehyde dehydrogenase
acetoacetic acid
acetyl-CoA
propionyl-CoA
propionyl-CoA carboxylase
biotin
methylmalonyl-CoA
methylmalonyl-CoA mutase
vitamin B12
Krebs cycle
succinyl-CoA
3-hydroxy-
isobutyric aciduria

TABLE 1 Summary of findings in HIBADH deficiency

	Patient 1	Patient 2	Patient 3	Patient 4 (Meyer et al.[2])	Patient 5 (Meyer et al.[2])
Family history					
Ethnicity	Persian	Italian	Italian (Sibling of Patient 2)	Syrian	Syrian (Sibling of Patient 4)
Consanguinity	Yes	No[a]	No[a]	Yes	Yes
Clinical findings					
Feeding	Poor suck, feeding difficulties in infancy	Poor suck, vomiting and diarrhea in infancy	–	–	–
Growth	Failure to thrive in infancy	Failure to thrive in infancy	–	–	–
Development	Delayed speech, developmental coordination disorder	Delayed speech; normal intellect	Motor delay; normal intellect	Psychomotor delay; low IQ; speech unclear and muffled, clumsy movements	–
Other	Behavioral insomnia, astigmatism	Recurrent respiratory infections in infancy; alopecia (resolved)	Headaches, bicuspid aortic valve	Hypomimia	Asymptomatic

Sasarman *et al.* J Inherit Metab Dis 2022; 45: 445-55

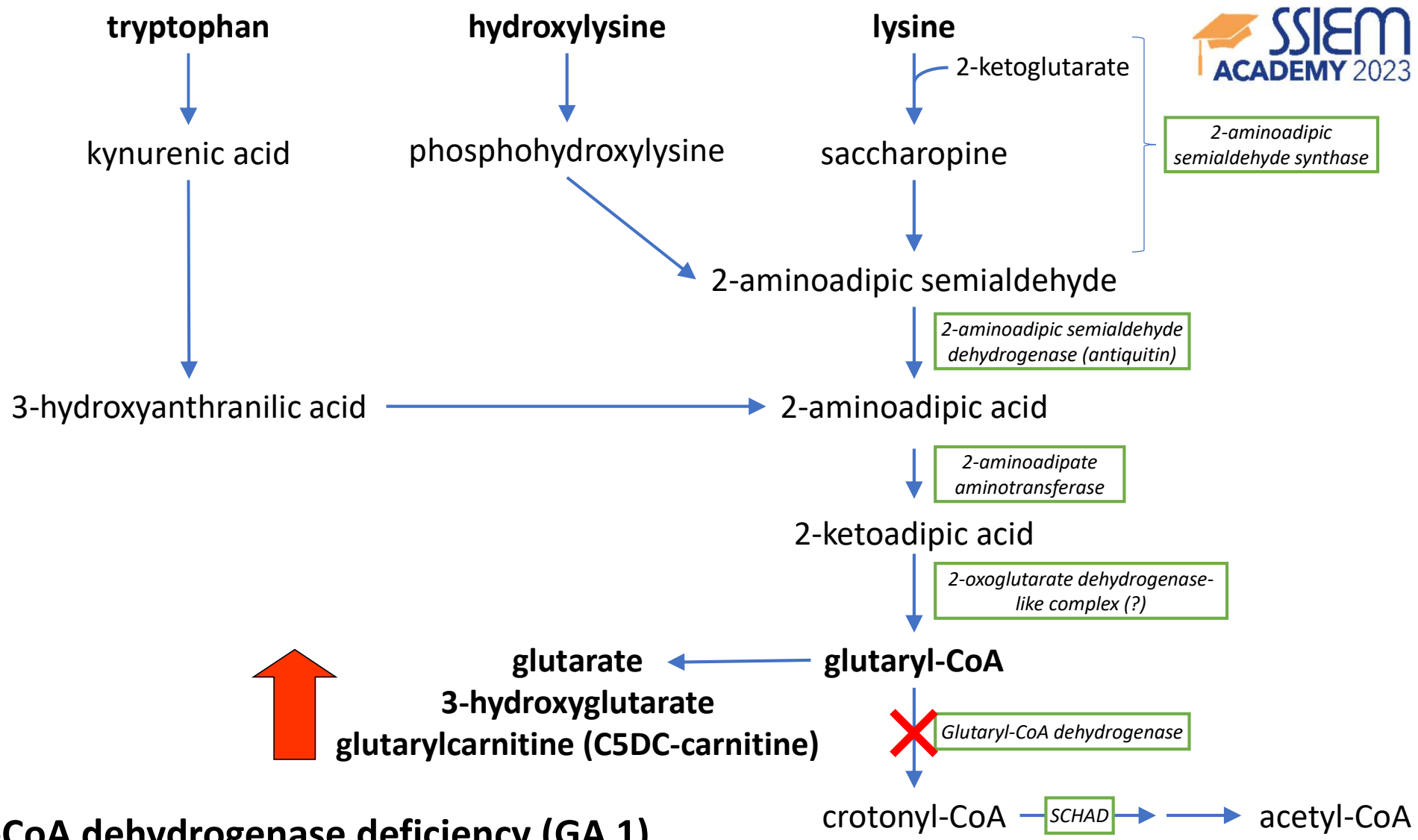
Classification of Organic Acidurias

Classical organic acidurias MMA, PA (,IVA)

"Cerebral" organic acidurias

- GA 1
- Canavan disease, D&L-2-Hydroxyglutaric acidurias, 4-hydroxybutyric aciduria etc.

Ketone body synthesis & utilization defects



Glutaryl-CoA dehydrogenase deficiency (GA 1)

Manifestations of GA 1

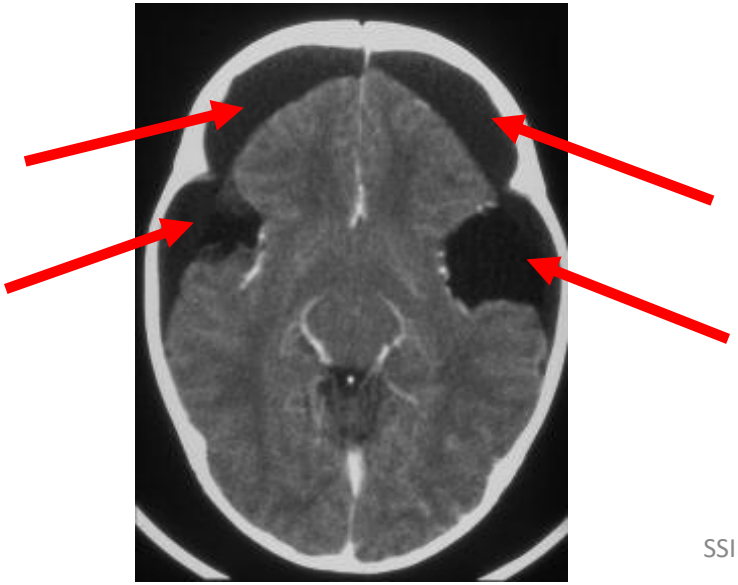
Acute presentation

- Acute encephalopathy with hypotonia ± seizures
- Triggered by minor illness (3 m to 2.5 y)
- Subsequent severe extrapyramidal movement disorder
- IQ relatively preserved but communication difficult
- Tube feeding often required

Other forms

- Chronic progressive dystonia (insidious onset), adult-onset & asymptomatic

Cerebral Imaging in GA 1



- Macrocephaly
- Frontotemporal atrophy (highly suggestive) ± subdural and/or retinal bleeds (DD shaken baby)
- White matter changes
- Basal ganglia damage after acute encephalopathy

False-negative NBS results for GA 1

International Journal of *Neonatal Screening*

MDPI

Article

Glutaric Aciduria Type I Missed by Newborn Screening: Report of Four Cases from Three Families

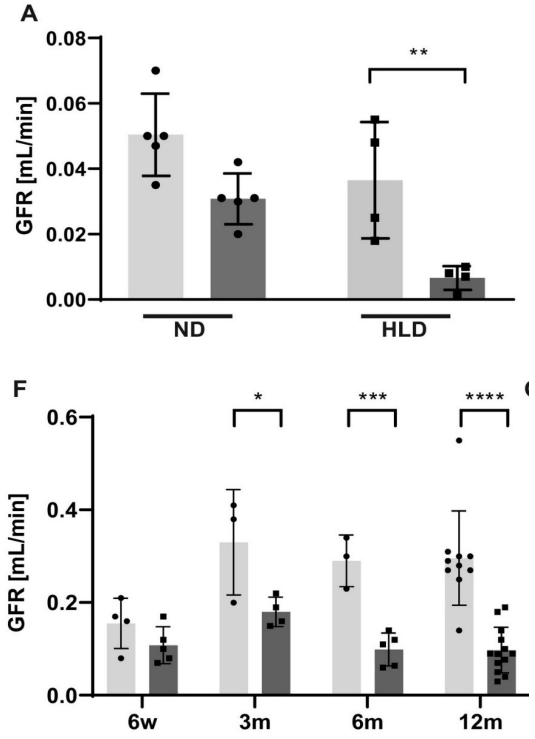
Johannes Spenger [1], Esther M. Maier [2], Katharina Wechselberger [3], Florian Bauder [3], Melanie Kocher [4], Wolfgang Sperl [1], Martin Preisel [1], Katharina A. Schiergens [2], Vassiliki Konstantopoulou [5], Wulf Röschinger [6], Johannes Häberle [7], Thomas Schmitt-Mechelke [3], Saskia B. Wortmann [1] and Ralph Fingerhut [7,8,*,†]

Int. J. Neonatal Screen. **2021**, *7*(2), 32; https://doi.org/10.3390/ijns7020032

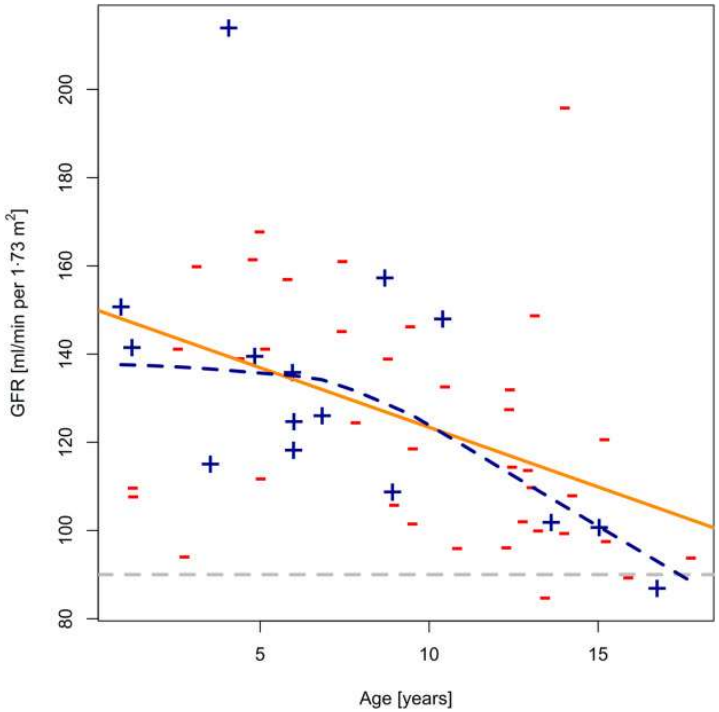
1 patient with acute crisis, 3 with insidious onset
Not a technical problem!

GA 1, a cerebral organic aciduria?

Decline of Renal Function in GA 1

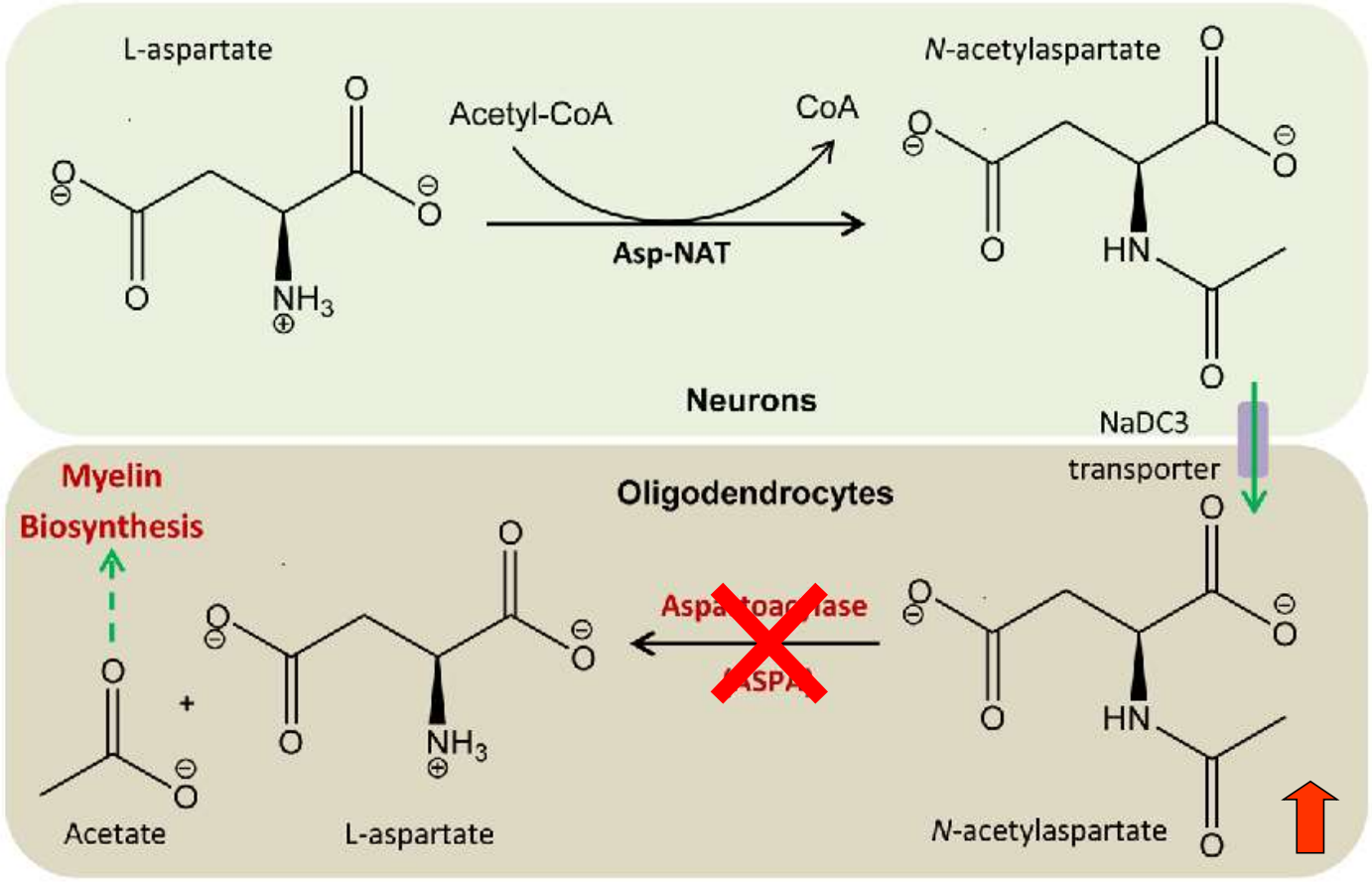


Decreased GFR in 6-week-old *Gcdh*$^{ki/ki}$ rats under HLD and progressive GFR decline in aging *Gcdh*$^{ki/ki}$ rats under ND
(Gonzalez Melo *et al.* Mol Genet Metab 2021;34: 287-300)



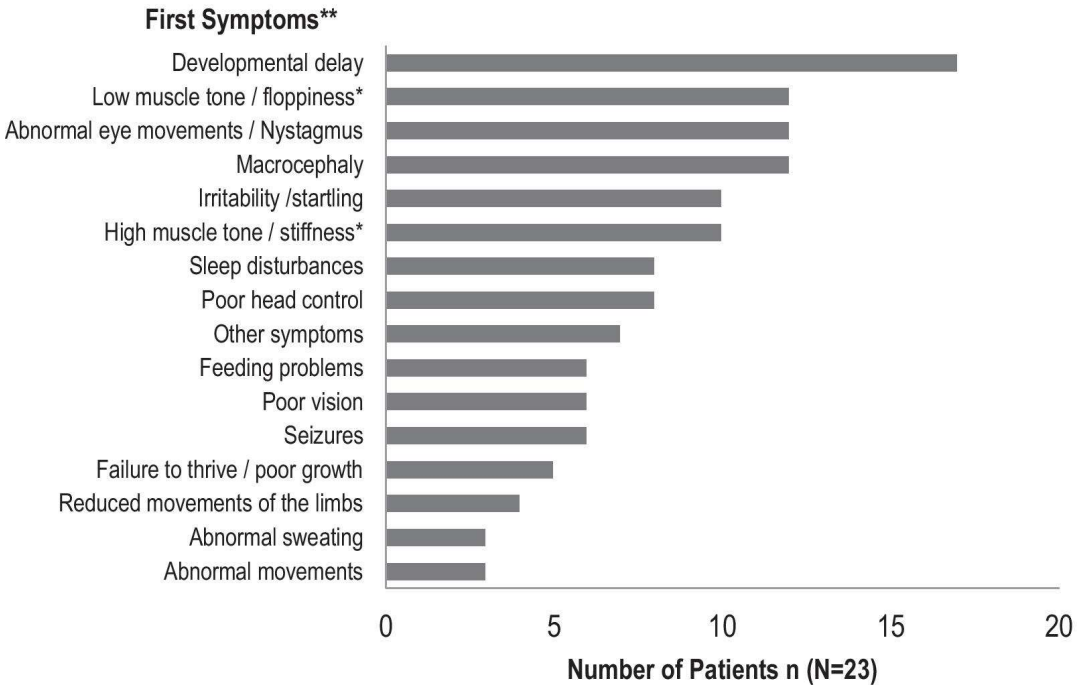
GFR estimated by Schwartz formula of 54 GA 1 patients from the German cohort at last visit
(Boy *et al.* Ann Neurol 2018, 83:970-9)

Canavan Disease

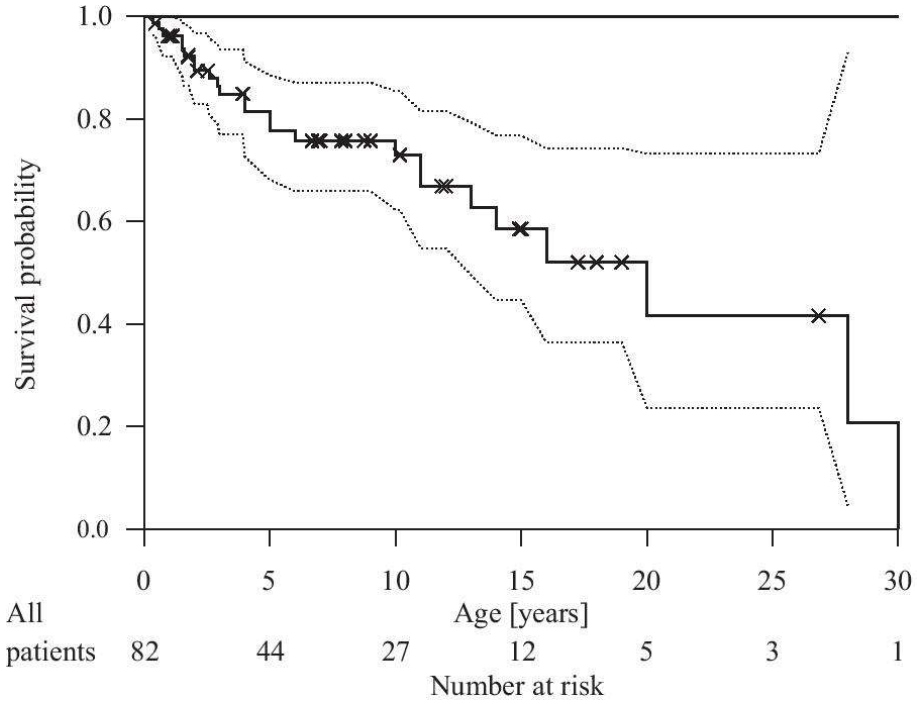


Modified from Wijayasinghe *et al.*, Biochemistry 2014:53:4970-8

Canavan Disease



All patients showed symptoms within the first 6 months of life



About 75% of patients reach the age of 10 years

Bley *et al.* Orphanet J Rare Dis 2021:16;227

Canavan Disease: Diagnosis

- OA: *N*-acetylaspartate (NAA)
- MRI: leukodystrophy
- MRS: increased NAA peak
- Enzymatics: Aspartoacylase Activity
- Genetics: gene *ASPA*

Canavan Disease: Treatment

- Only symptomatic treatment
- Muscle relaxants & tube feeding can help

CAVEAT

- Canavan disease
- ACY1 deficiency
- 2-OH-glutaric acidurias
- 4-hydroxybutyric aciduria

can only be diagnosed by organic acid analysis!

Classification of Organic Acidurias

Classical organic acidurias (COA)

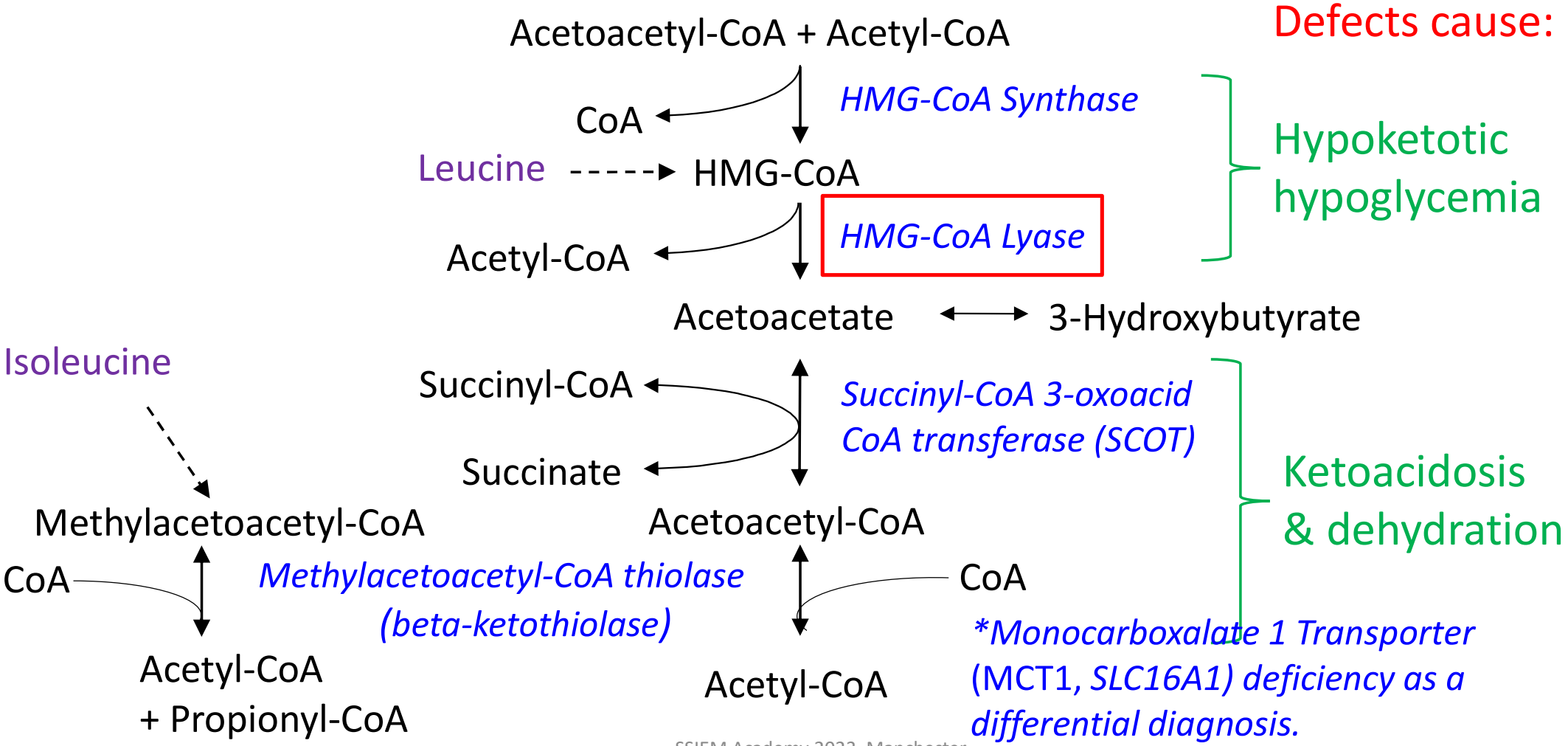
- MMA, PA, (IVA)

“Cerebral” organic acidurias

- GA 1
- Canavan disease, D&L-2-Hydroxyglutaric acidurias, 4-hydroxybutyric aciduria etc.

Ketone body synthesis & utilization defects

Ketone Body Synthesis and Utilization



HMG-CoA Lyase Deficiency

- Poor feeding, vomiting, decreasing consciousness
- 42 % in neonatal period after free interval
- More than 80% during 1st year, often triggered by an infection, about 16 % deceased
- Hypoglycemia, often moderate hyperammonemia
- Lactic acidosis, no or low ketosis (caveat: aceto-acetate as artefact following methylation)
- OA: 3-hydroxy-3-methylglutaric, 3-methylglutaric, and 3-hydroxyisovaleric acids

Grünert & Sass Orphanet J Rare Dis 2020;15:48

HMG-CoA Lyase Deficiency

- Poor feeding, vomiting, decreasing consciousness
- 42 % in neonatal period after free interval
- More than 80% during 1st year, often triggered by an infection, about 16 % deceased
- Hypoglycemia, often moderate hyperammonemia
- Lactic acidosis, no or low ketosis (caveat: aceto-acetate as artefact following methylation)
- OA: 3-hydroxy-3-methylglutaric, 3-methylglutaric, and 3-hydroxyisovaleric acids

Grünert & Sass Orphanet J Rare Dis 2020;15:48

HMG-CoA Lyase Deficiency: Complications

- Neurological sequelae, pancreatitis, cardiomyopathy
- Abnormal cerebral white matter (asymptomatic)
- High risk pregnancies

Organic acidurias: Conclusions

- A still evolving heterogeneous group of diseases
- A majority manifests early in life, but late onset and possibly asymptomatic forms exist
- Most (but not all) show acute presentations, in several presymptomatic treatment might substantially improve the prognosis
- Check which ones are included in the newborn screening program of your country (CAVE: false negative results might exist)
- The historical classification is amended by the discovery of new manifestations in longer surviving patients

Bibliography

P. Forny *et al.*: Guidelines for the diagnosis and management of methylmalonic acidaemia and propionic acidaemia. J Inherit Metab Dis 2021; 44:566-92.
doi: 10.1002/jimd.12370.

N. Boy *et al.*: Recommendations for diagnosing and managing individuals with glutaric aciduria type 1: third revision. J Inherit Metab Dis 2022;
doi: 10.1002/jimd.12566

A. Bley *et al.*: The natural history of Canavan disease: 23 new cases and comparison with patients from literature. Orphanet Journal of Rare Diseases 2021;16;227.
doi: 10.1186/s13023-020-01659-3.

J.O. Sass *et al.*: Inborn Errors of Ketone Body Metabolism and Transport: An Update for the Clinic and for Clinical Laboratories. Journal of Inborn Errors of Metabolism and Screening. 2018;6.
doi:10.1177/2326409818771101