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Quality Assurance in Laboratory Testing for IEM

ACDB Participant's Online Meeting
Friday 27th March 2026, 10:00 – 12:00 CET

Your microphone is automatically muted during this meeting to avoid background noise during the presentation

Please submit your questions using the 'chat' function, or by raising your hand during the question-and-answer session.

The meeting will be recorded, but we will not circulate the question-and-answer session

Scientific advisors

- ACDB London, UK
  Erin Emmett

- ACDB Rome, Italy
  Dr. Cristiano Rizzo

- ACDB Heidelberg, Germany
  Dr. Joachim Janda

Agenda

10.00 – Welcome and introductions

10.05 – Introduction to the ACDB schemes (JJ)

10.20 – Common samples (EE)

10.40 – ACDB Rome (Cristiano Rizzo)

11.00 – ACDB Heidelberg (Dr Joachim Janda)

11.20 – ACDB London (Erin Emmett)

11.40 – Questions

11.55 – Final remarks

ACDB scheme course

- For each ACDB centre, samples are distributed by CSCQ and analysed in two surveys
  - A, B, C in spring
  - D, E, F in early summer
- Results of analysis and their interpretation are reported via the CSCQ website and evaluated by the SA → interim reports
- One of the SAs from the other two centres conducts a second scoring, and results are discussed
- Final results, classification of educational samples and critical errors are discussed in a larger group at the SAB every autumn.

ACDB participant numbers

Registrations 2021 - 2025

ACDB Centre	Heidelberg	London	Rome	All Centres
2021	42	44	45	131
2022	47	43	49	139
2023	48	45	47	140
2024	44	45	45	134
2025	47	49	46	142

Analytical considerations I

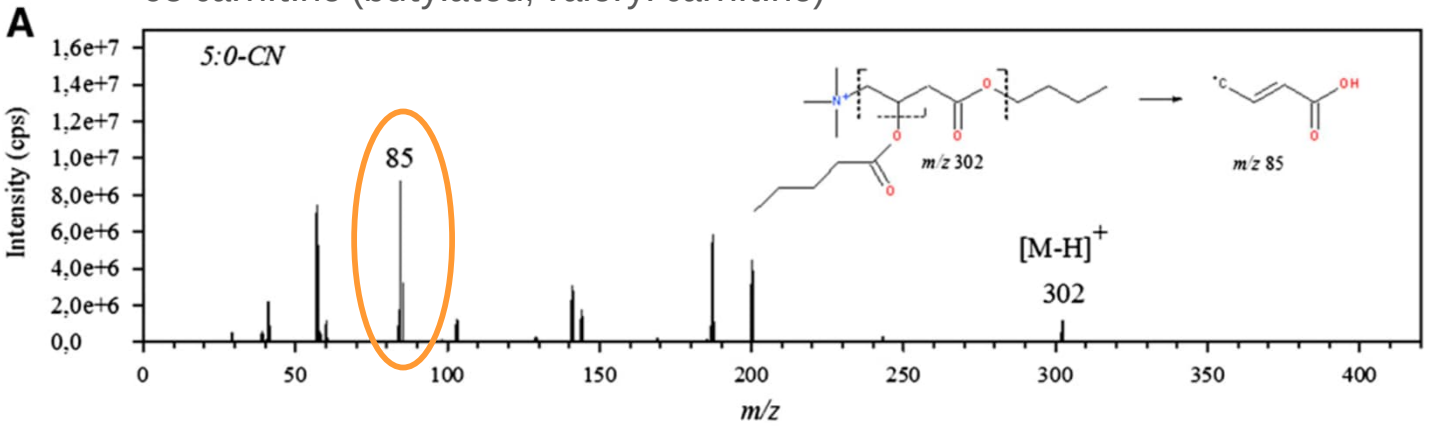
- Typical instrumental setup: FIA-ESI-MS/MS
  - Highly selective detector
  - Quick turnaround (screening capability)
- Well established analytical procedure:
  - Kits available (many manufacturers) and/or
  - ready-to-use components available, e.g. pre-mixed labelled standards for setting up in-house developed methods
- Reference ranges are typically lab-specific (method, cohort)

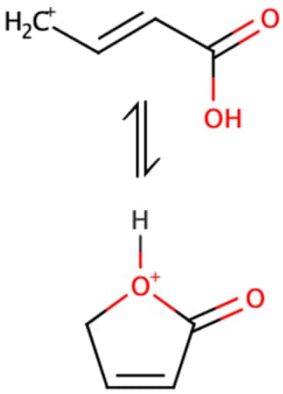
Analytical considerations I

- Principle:
  - Punching a DBS disc corresponding to certain sample volume
  - extraction with a solvent containing known amount of isotopically labelled AC standards
  - optional: derivatisation step (butylation / methylation)
  - instrumental analysis (MRM experiments, neutral loss)
  - software-based preparation of mass spectra and semi-quantitative evaluation (typically one-point calibration based on repose to an internal standard)

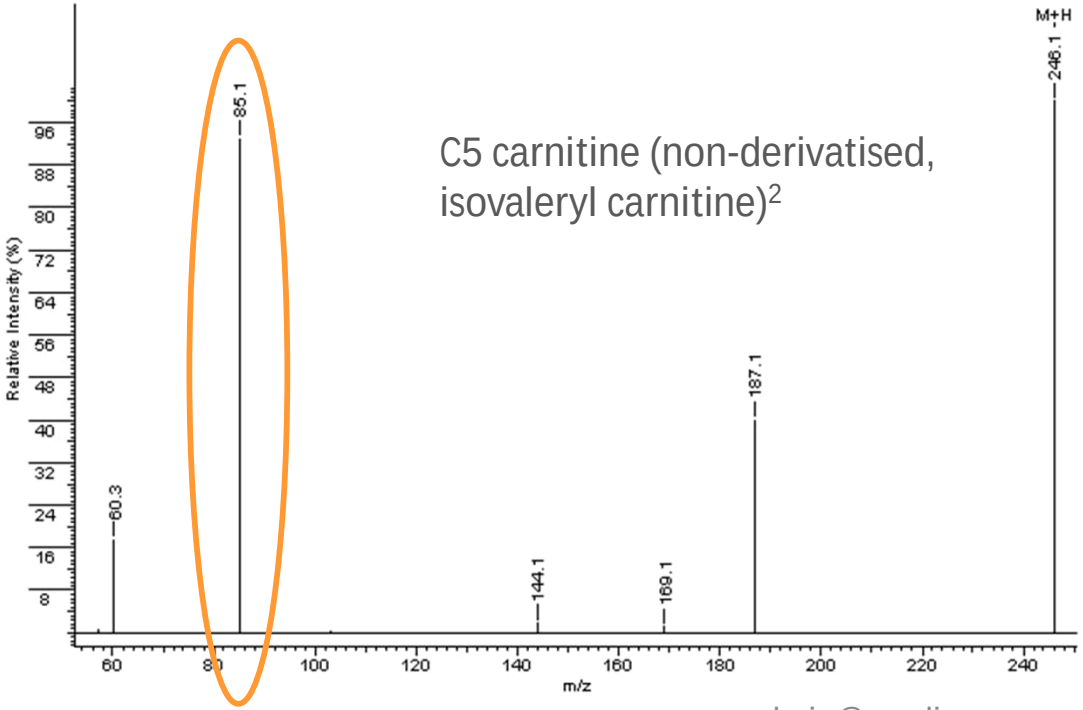
Acylcarnitines in Tandem MS

C5 carnitine (butylated, valeryl carnitine)[1]





C5 carnitine (non-derivatised, isovaleryl carnitine)[2]



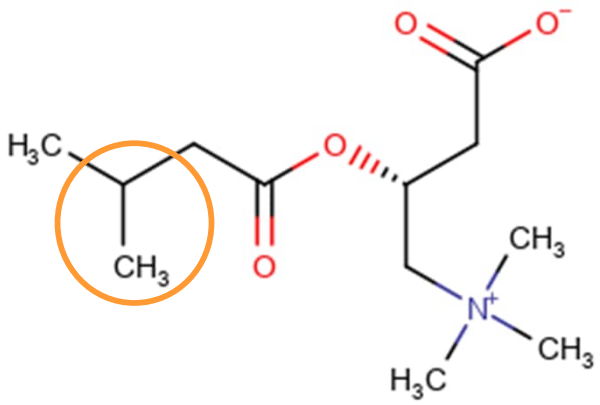
- Mostly used fragment ion $C_4H_5O_2^+$ (m/z 85) from carnitine moiety
- Typically analysed in precursor ion scan or multiple reaction monitoring (MRM)
- Methylated ACs show different MS fragments

1) Giesbertz et al. 2015, J. Lipid Res. 56, 2029ff
2) Wishart et al. 2009, Nucleic Acids Res. 37

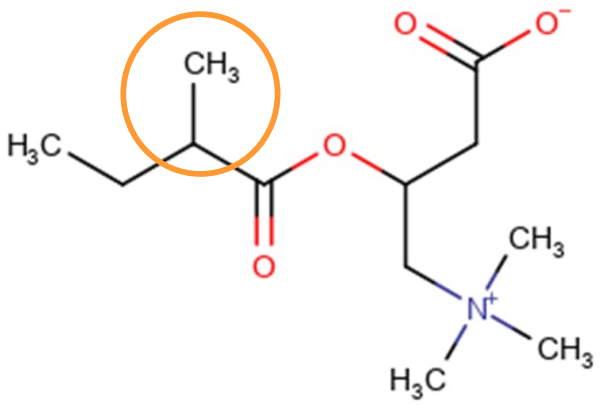
Analytical considerations II

Potential pitfalls

- Isobaric analytes can not be distinguished



isovaleryl-carnitine



2-methylbutyryl-carnitine

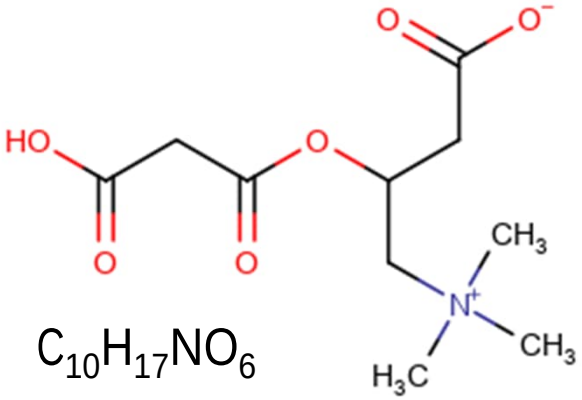
structural isomers of „C5 carnitine“, $C_{12}H_{23}NO_4$, M_I: 245.16 Da

- Same molecular ion
- Same (or very similar) fragmentation behaviour

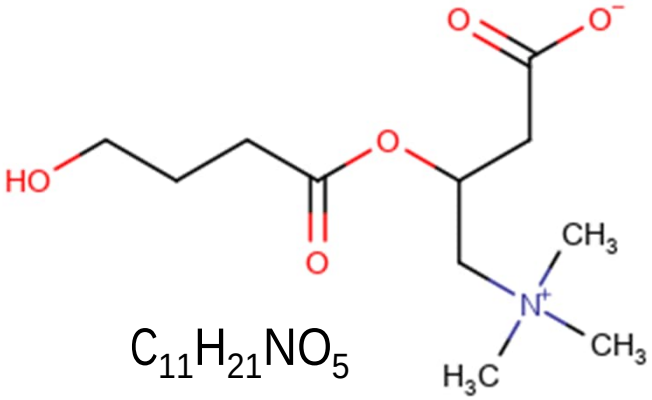
Analytical considerations III

Potential pitfalls

- Further isobaric „complexity“ depending on analytical strategy (lab specific!):
  - non-derivatized: C3DC $[M+H]^+$ 248.106 Da
    C4OH $[M+H]^+$ 248.142 Da



$C_{10}H_{17}NO_6$



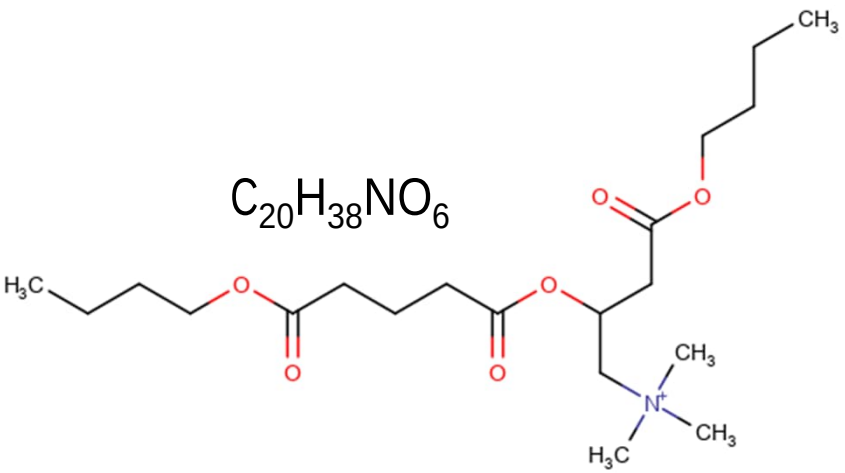
$C_{11}H_{21}NO_5$

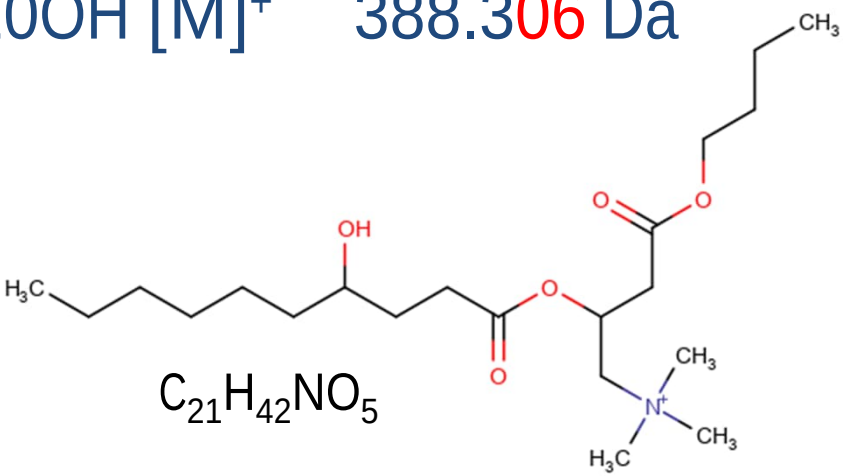
MS/MS unit resolution insufficient to distinguish

Analytical considerations III

Potential pitfalls

- Further isobaric „complexity“ depending on analytical strategy (lab specific!):
  - methylated: C3DC [M]+ 276.144 Da
    C5OH [M]+ 276.181 Da
  - butylated: C5DC [M]+ 388.269 Da
    C10OH [M]+ 388.306 Da





MS/MS unit resolution insufficient to distinguish

Analytical considerations IV

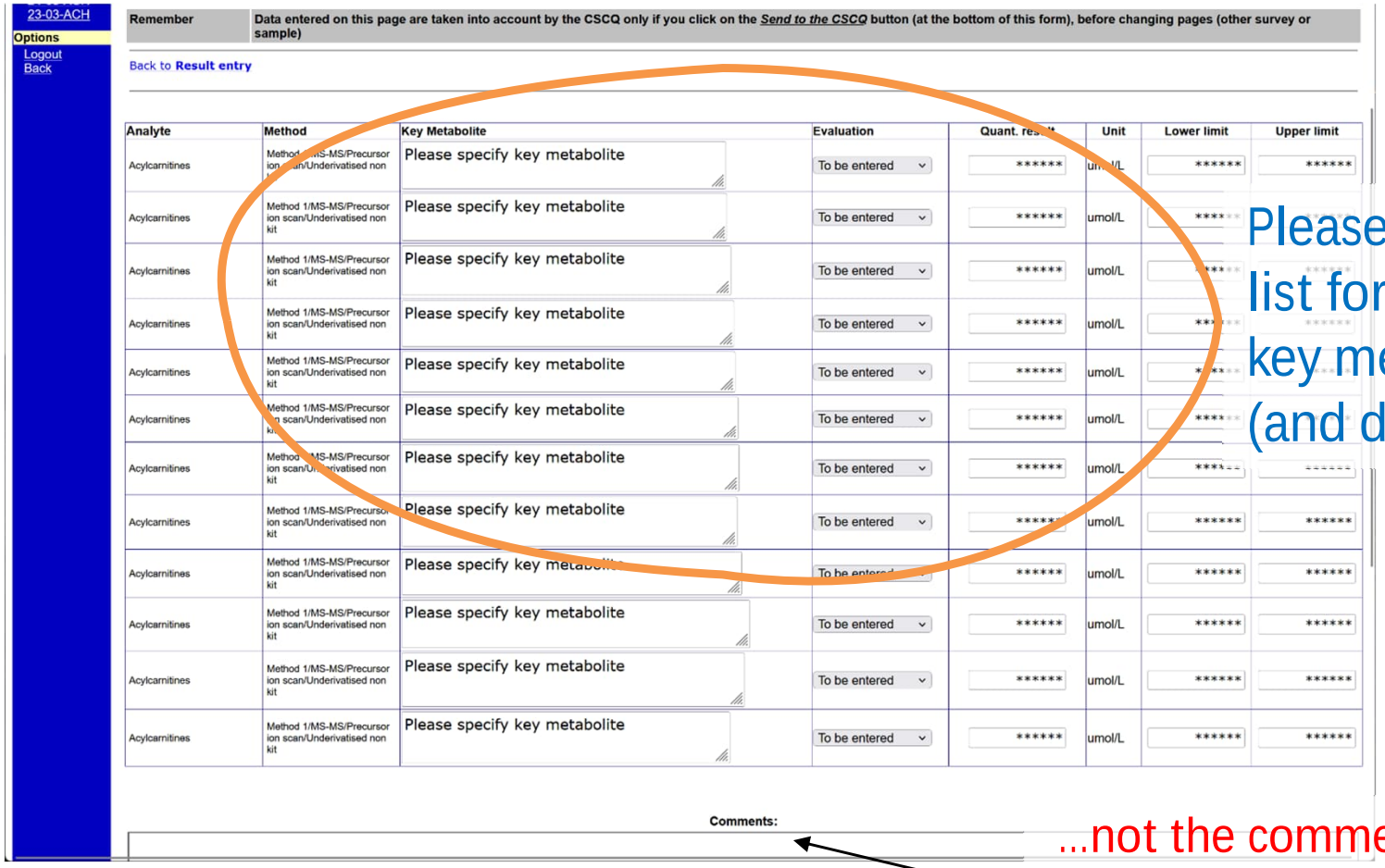
Evaluation is based on principal acylcarnitine results, i.e.

- acylcarnitine concentrations
- diagnostic acylcarnitine ratios

Not considered in evaluation:

- Results of other analyte groups, e.g., obtained from multiplexing (amino acids, guanidino compounds or long-chain lysosomal markers)
- Sole confirmatory / differential analysis results (can be additionally reported if performed)

Reporting analytical results



Please use the analyte list for reporting key metabolites (and diagnostic ratios)

...not the comment field (not considered in evaluation)

Reporting analytical results

Analyte	Method	Key Metabolite	Evaluation	Quant. result	Unit	Lower limit	Upper limit
Acylcarnitines	Method 1/MS-MS/Precursor ion scan/Underivatised non kit	C3 carnitine	Elevated	6	umol/L	******	******
Acylcarnitines	Method 1/MS-MS/Precursor ion scan/Underivatised non kit	Please specify key metabolite	To be entered	******	umol/L	******	******
Acylcarnitines	Method 1/MS-MS/Precursor ion scan/Underivatised non kit	Please specify key metabolite	To be entered	******	umol/L	******	******

Most important:

- Key metabolite
- Qualitative evaluation relative to lab reference range

Quantitative results are welcome but not essential

Interpretive considerations I

Even if the analytical procedure works well, the result may be ambiguous

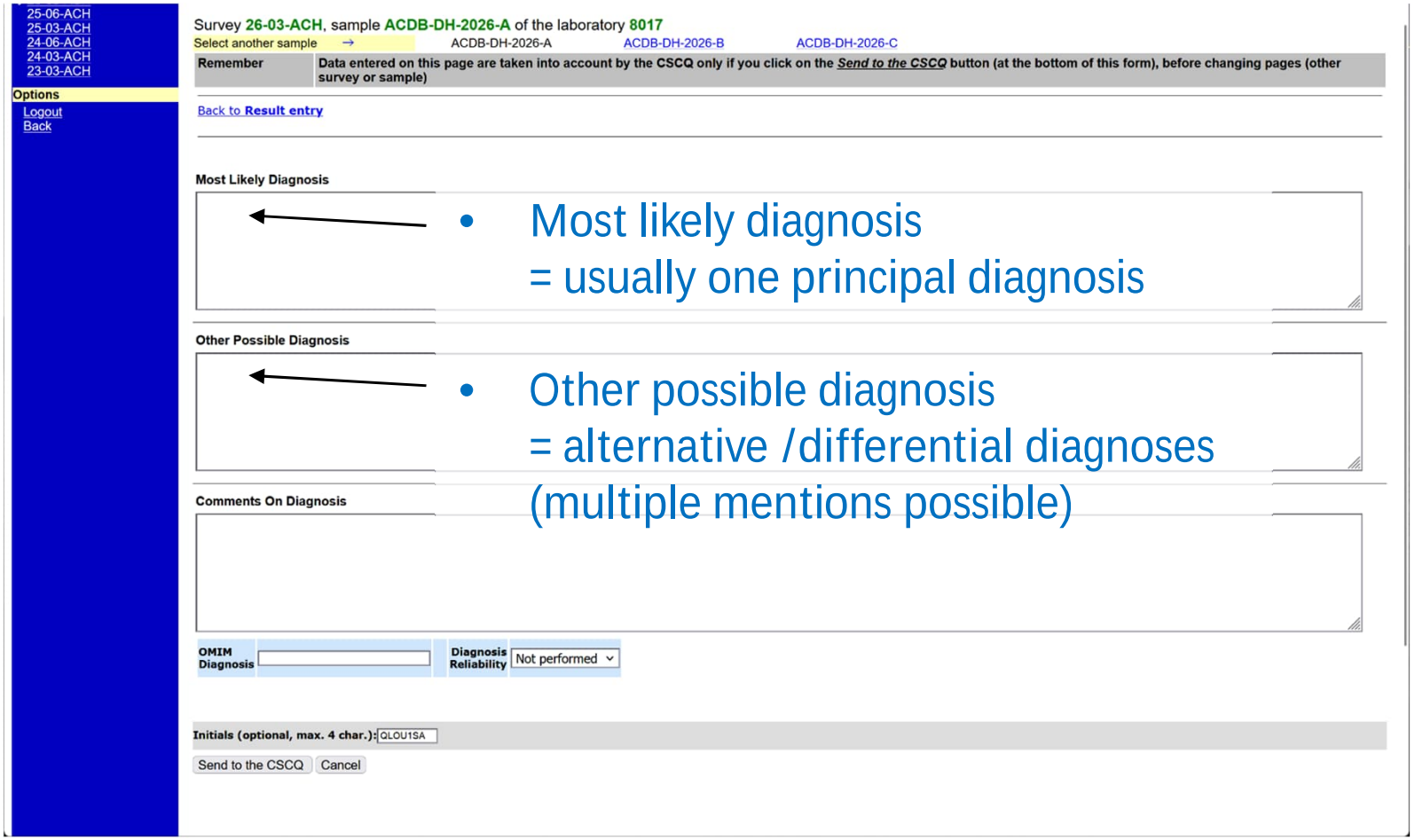
- … due to analytes that cannot be distinguished
- … due to profiles that are not specific
- … due to other interferences
  (e. g. drug artefacts, nutritional, …)

Table 5.1 Clinically relevant acylcarnitine species (as butylesters) included in a typical acylcarnitine analysis and their relevance when abnormally elevated (unless otherwise noted)

Acylcarnitine species		Disorder
C0	Free carnitine	Carnitine supplementation (deficiency if low)
C2	Acetyl-	Carnitine supplementation or ketosis (deficiency if low)
C3	Propionyl-	PA, MCD, MMA, SUCLA2 (Carrozzo et al. 2007), treatment with heptanoic acid
m/z 287	FIGLU (Malvagia et al. 2006)	Glutamate formimino-transferase deficiency
C4	Butyrl-/isobutyryl-	SCAD, IBDH, MADD, EE
C5:1	Tiglyl-	MAT, MCC, MHBD, MCD
C5	Isovaleryl-/2-methylbutyryl-/Pivaloyl-	IVA, SBCAD, MADD, EE, antibiotics derived artifact (Abdenur et al. 1998), treatment with heptanoic acid

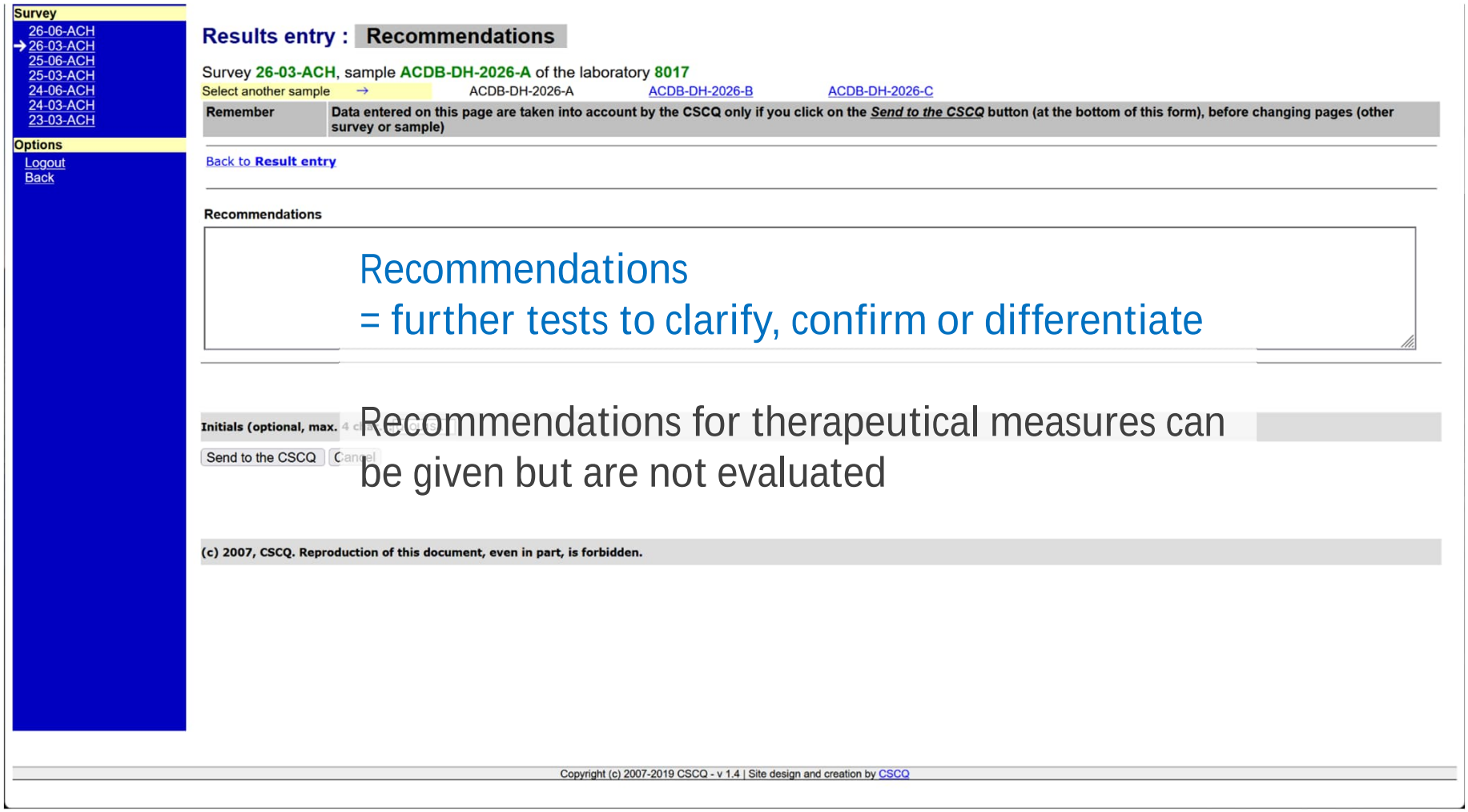
excerpt from “Physician’s Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases” (2022)

Reporting interpretive results



- Most likely diagnosis
  = usually one principal diagnosis
- Other possible diagnosis
  = alternative /differential diagnoses
  (multiple mentions possible)

Reporting recommendations



Recommendations
= further tests to clarify, confirm or differentiate

Recommendations for therapeutical measures can be given but are not evaluated

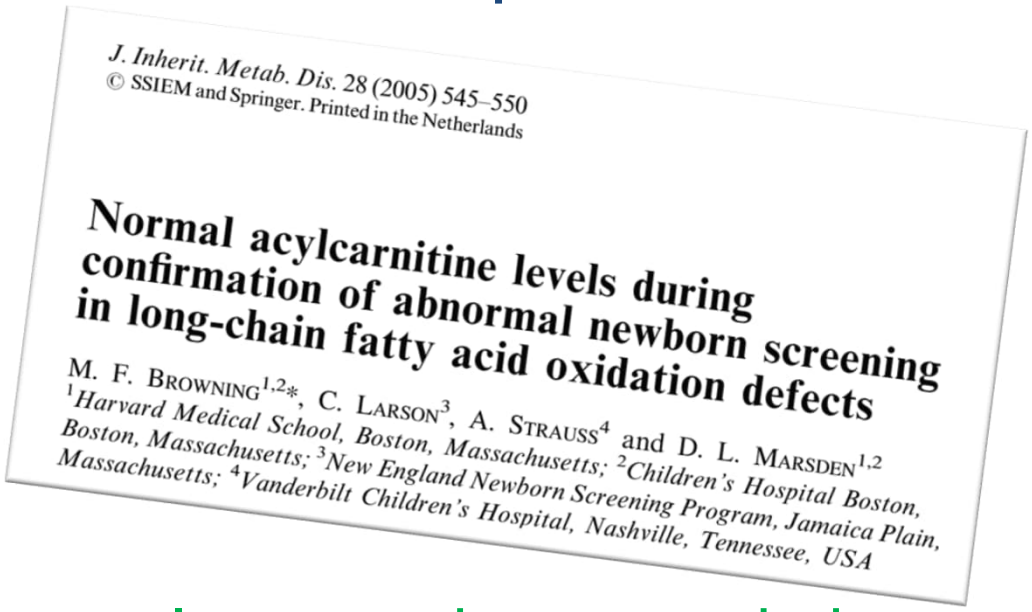
Evaluation of results

- Based on criteria defined by SA/team and may be adjusted according to difficulty (e. g. clarity of the AC profile)
- Critical errors:
  - Discussed in SAB meeting
  - Typical criteria
    - Missing a diagnosis when proficiency for that EQA sample is >95%.
    - Drawing misleading conclusions (e.g., missing a diagnosis or, even worse,
    - making an incorrect diagnosis).
    - Identifying a normal sample as having an IEM, in specific cases,
    - depends on the impact.

Interpretive considerations II

„Acylcarnitine profiles are dependent on the clinical status of the patient at the time of sample collection …"

→ Obtaining suitable samples is not an easy task.



J. Inherit. Metab. Dis. 28 (2005) 545–550
© SSIEM and Springer. Printed in the Netherlands

Normal acylcarnitine levels during confirmation of abnormal newborn screening in long-chain fatty acid oxidation defects

M. F. BROWNING[1,2*], C. LARSON[3], A. STRAUSS[4] and D. L. MARSDEN[1,2]
[1]*Harvard Medical School, Boston, Massachusetts;* [2]*Children's Hospital Boston, Boston, Massachusetts;* [3]*New England Newborn Screening Program, Jamaica Plain, Massachusetts;* [4]*Vanderbilt Children's Hospital, Nashville, Tennessee, USA*

Sample donations are always welcome and are awarded with a discount in participation costs (if useful and included in a scheme).

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Quality Assurance in Laboratory Testing for IEM

ACDB Participant's meeting 2026
Common samples

Erin Emmett

Common sample 2024

Source: Rome

Participants: 126

Diagnosis: CPT1

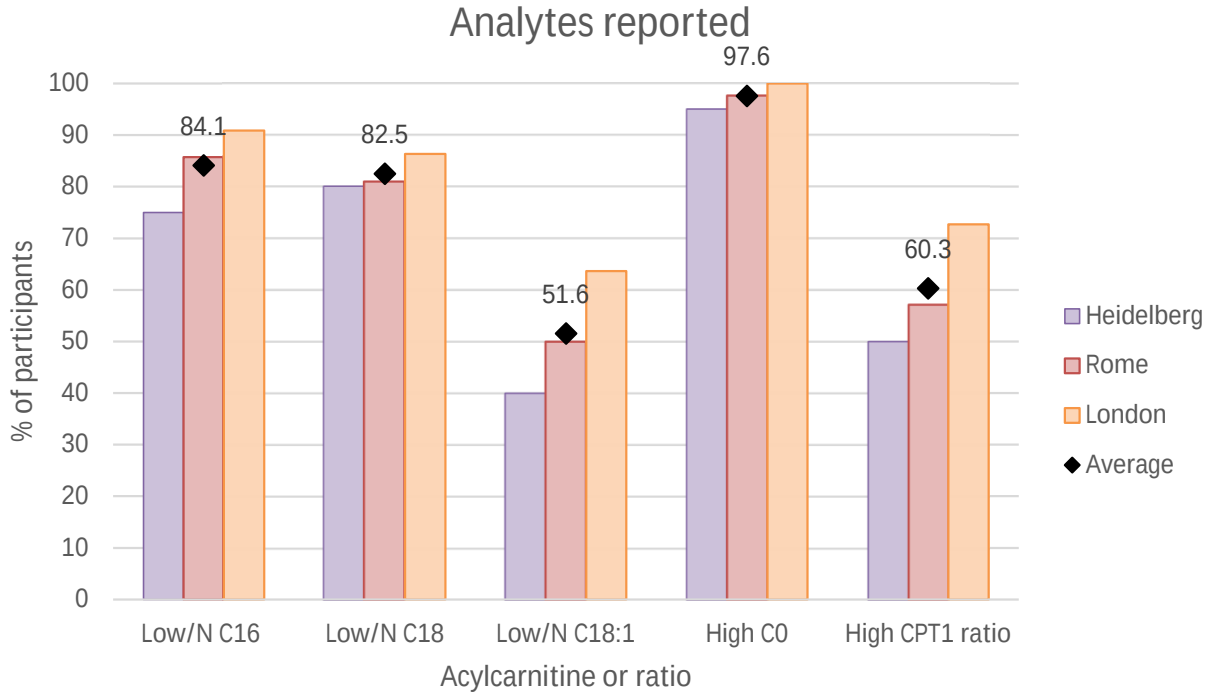
Patient details

Sample from a 12 year old male

Presented at six months old with hypoglycaemia

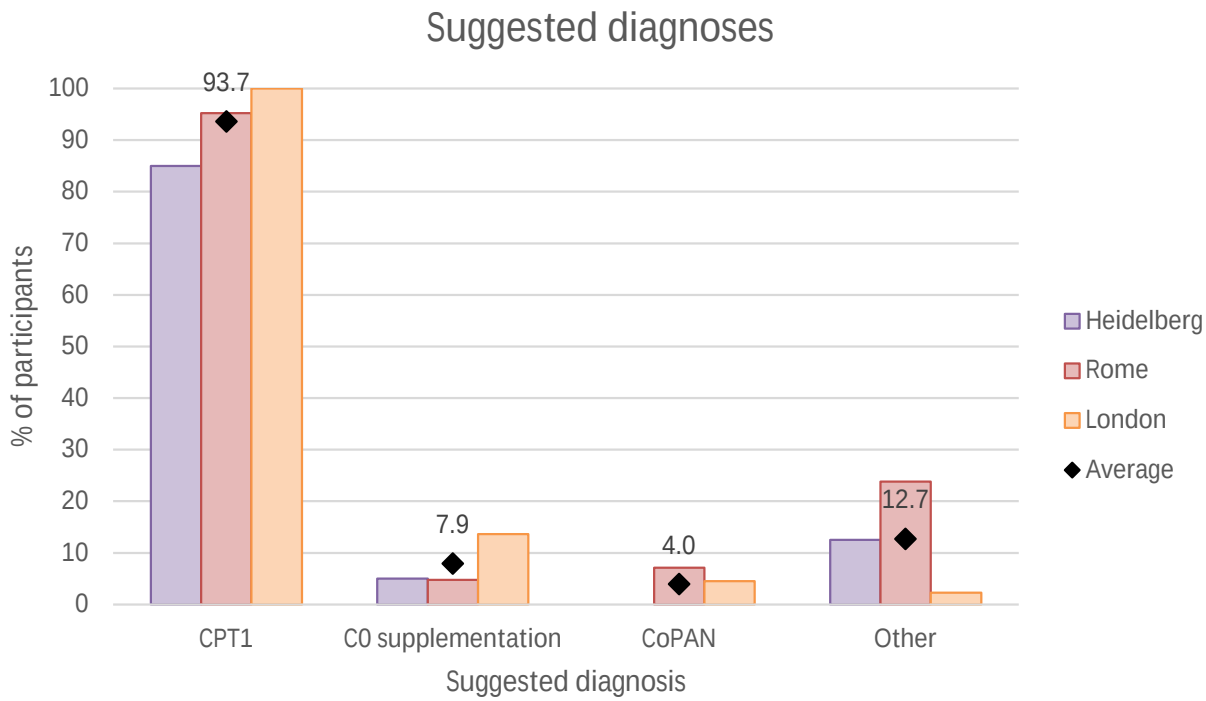
Currently has language and learning difficulties

Analytical findings



- CPT1 ratio: C0 / (C16 + C18)
- Agreed scoring across all three centres:
  - 1 point increased C0
  - 1 point decreased long chains / high CPT1 ratio

Interpretation

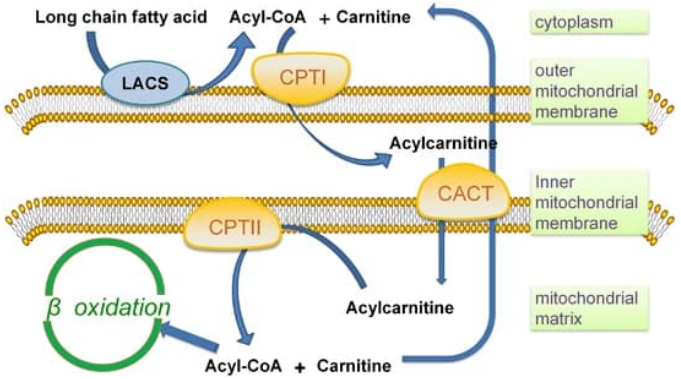


- Other diagnoses included other FAOD (MCADD, LCHADD, MTP), GA1, HMG-CoA lyase, HMG-CoA synthase, F-1,6-BP, hyperinsulinism
- Agreed scoring across all three centres:
  - 2 points CPT1
  - 1 point for CPT1 as alternative diagnosis and 1 point appropriate recommendations

CPT1A

- ✓ Carnitine palmitoyltransferase 1A (CPT1A) deficiency is an autosomal recessive disorder of the carnitine cycle that results in impaired mitochondrial long-chain fatty acid oxidation. CPT1 is found at the outer mitochondrial membrane and catalyzes the conversion of long-chain (C16 and C18) acyl-CoA species to acylcarnitines, which can be directed into the mitochondrial matrix.
- ✓ CPT1A is present in liver, kidney, and fibroblasts.
- ✓ CPT1A deficiency is primarily hepatic, and presentation results from failure of ketogenesis, although renal tubular acidosis may also be present.
- ✓ CPT1A deficiency does not present with skeletal muscle or cardiac disease.
- ✓ CPT1A deficiency can be diagnosed in the newborn period by acylcarnitine analysis in DBS. The ratio of free carnitine to the sum of palmitoylcarnitine and stearoylcarnitine [C0/(C16 + C18)] is highly specific for CPT-I deficiency

- Hepatic carnitine palmitoyltransferase I deficiency: acylcarnitine profiles in blood spots are highly specific. R Fingerhut et al. Clin Chem . 2001 Oct;47(10):1763-8.
- Carnitine palmitoyltransferase I deficiency in neonate identified by dried blood spot free carnitine and acylcarnitine profile. Sim KG et al. J Inherit Metab Dis. 2001;24:51–9.
- Reliable Diagnosis of Carnitine Palmitoyltransferase Type IA Deficiency by Analysis of Plasma Acylcarnitine Profiles.M Rebecca Heiner-Fokkema et al. JIMD Rep. 2016 Jun 14;32:33–39.



Cell Death and Disease (2016) 7, e2226; doi:10.1038/cddis.2016.132

CPT1A

Main Artifacts and Challenges in the Acylcarnitine Profile (CPT1A)

✓ Profile Normalization (False Negative):
Unlike other oxidation defects, the acylcarnitine profile may normalize rapidly after the first days of life or during periods when the patient is not in metabolic crisis. An apparently normal profile does not exclude CPT1A deficiency.

- Normal levels of plasma free carnitine and acylcarnitines in follow-up samples from a presymptomatic case of carnitine palmitoyl transferase 1 (CPT1) deficiency detected through newborn screening in Denmark. Borch L, et al JIMD Rep. 2012;3:11-5
- Utility of Genetic Testing for Confirmation of Abnormal Newborn Screening in Disorders of Long-Chain Fatty Acids: A Missed Case of Carnitine Palmitoyltransferase 1A (CPT1A) Deficiency Leah Dowsett et al Int J Neonatal Screen. 2017 Apr 28;3(2):10.
- Wanders R, Vreken P, Ijlst L. Disorders of mitochondrial fatty acyl-CoA β-oxidation. JIMD. 1999;22:442–487.

✓ Carnitine Supplementation:
Patients receiving L-carnitine may show a falsely elevated C0 (free carnitine) level; however, studies show that the C0/(C16+C18) ratio remains specific for CPT1 deficiency.

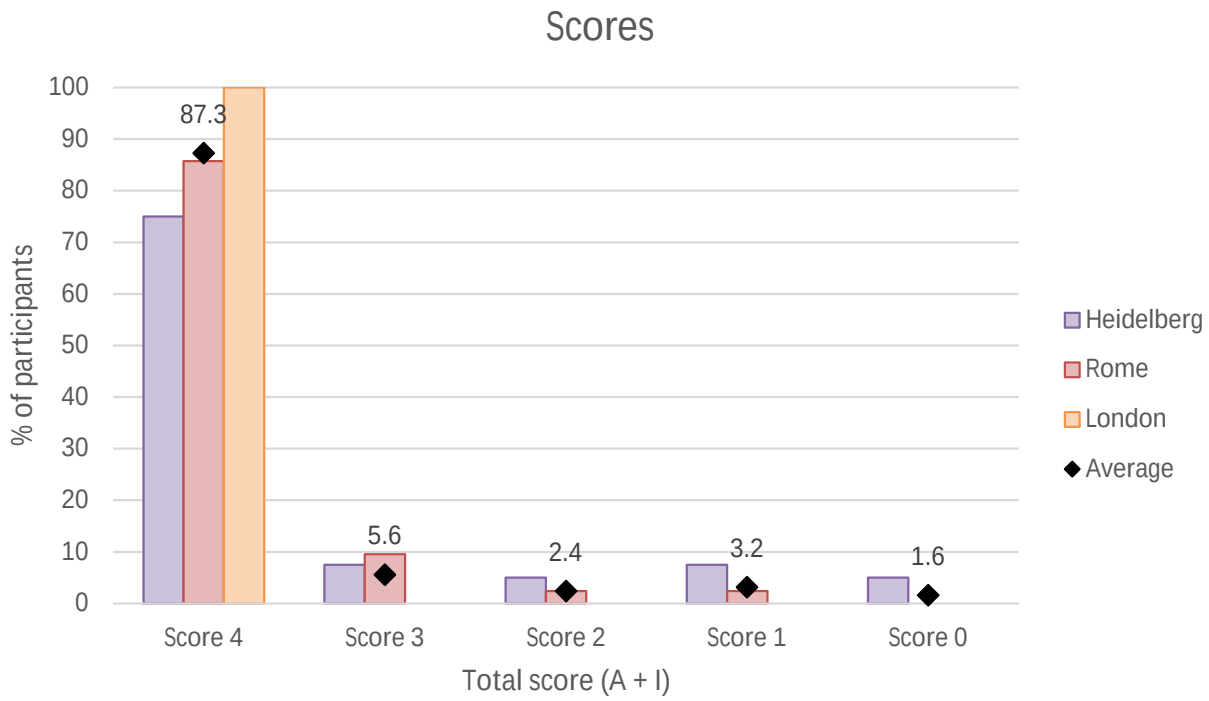
Difference Between Plasma and Dried Blood Spots:
Levels of free carnitine (C0) are generally higher and long-chain acylcarnitines lower in dried blood spots compared with plasma, which affects the cut-off values used for interpretation.

- Differences between acylcarnitine profiles in plasma and bloodspots. de Sain-van der Velden M, Verhoeven-Duif N.Mol Gen Metab. 2010;110:116–121.

Recommendations

- *CPT1A* genetics
- Repeat DBS or plasma acylcarnitines
- Urine organic acids
- Routine Chemistry, e.g. ammonia, glucose, LFTs, CK
- Enzyme analysis in fibroblasts
- Referral to a specialist clinician

Scoring



- Criteria for critical error – normal / incorrect diagnosis with no recommendations that would enable identification of CPT1
- Five critical errors

Summary

	Heidelberg	Rome	London	Total
Analytical prof	87.5	95.2	100	94.2
Interpretative prof	82.5	94.0	100	92.2
Overall prof	85.0	94.6	100	93.2

- High scoring sample with the majority of the 126 participants scoring full marks

Take-home messages

- Multiple causes of increased C0, including supplementation, muscle breakdown, renal dysfunction and peri-/post-mortem, as well as CoPAN and CPT1 deficiency
- Use of the “CPT1 ratio” can help distinguish CPT1 from carnitine supplementation if appropriate reference ranges used

Common sample 2025

Source: London

Participants: 130

Diagnosis: VLCADD

Patient details

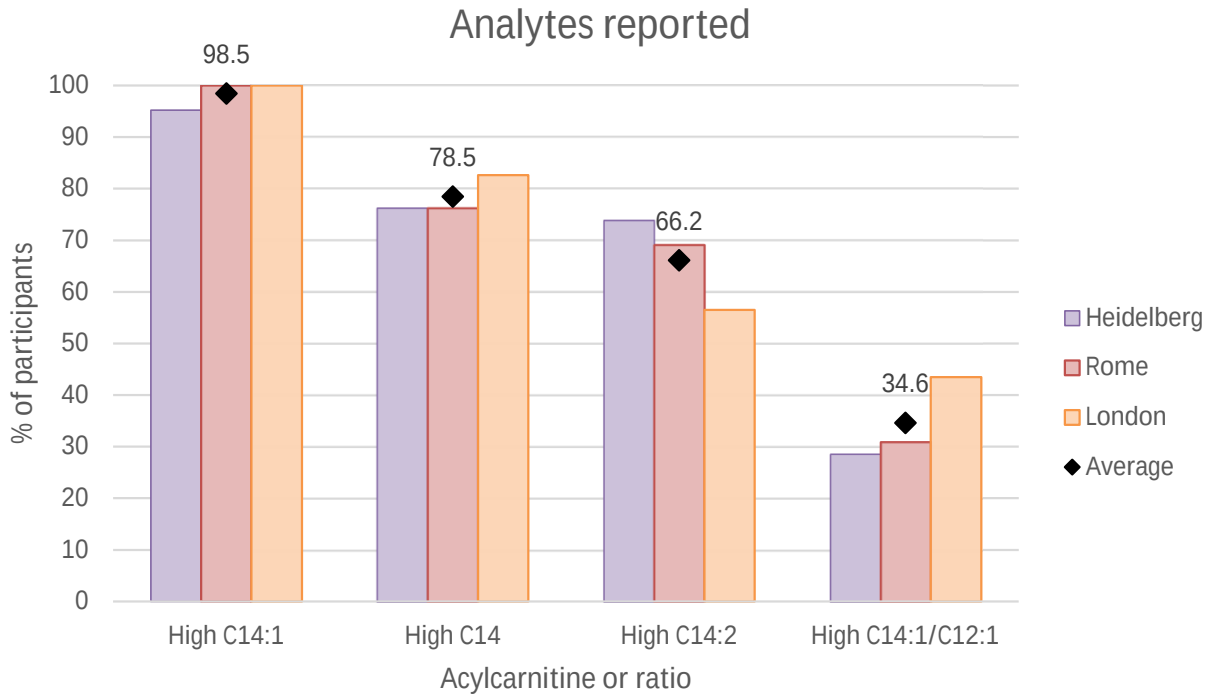
Sample from a 57 year old male

Occasional cramps and dark urine as child. Episodes of rhabdomyolysis as young adult, two episodes requiring dialysis

Confirmed VLCADD age 55

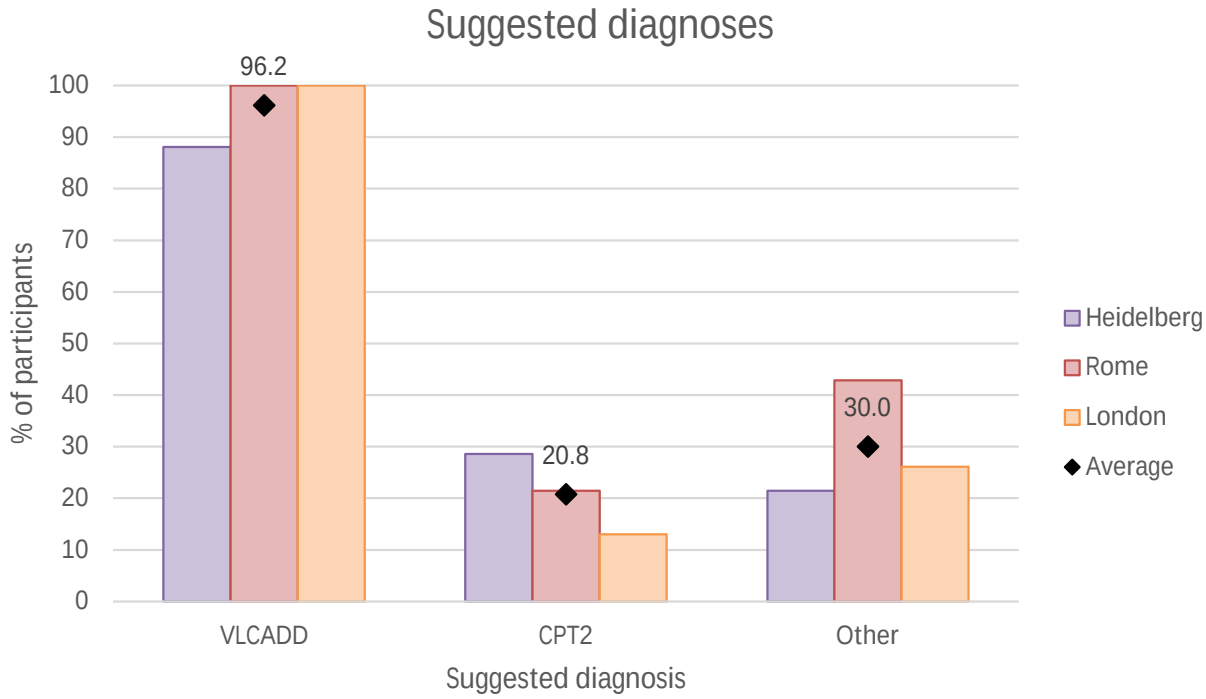
Patient currently well on low fat diet and SOS carbs prior to exercise

Analytical findings



- Other ratios reported: C14:1/C2, C14:1/C4, C14:1/C16
- Agreed scoring across all three centres:
  - 2 points increased C14:1 (or other long chain such as C14, C14:2, C16:1) and/or an appropriate ratio

Interpretation

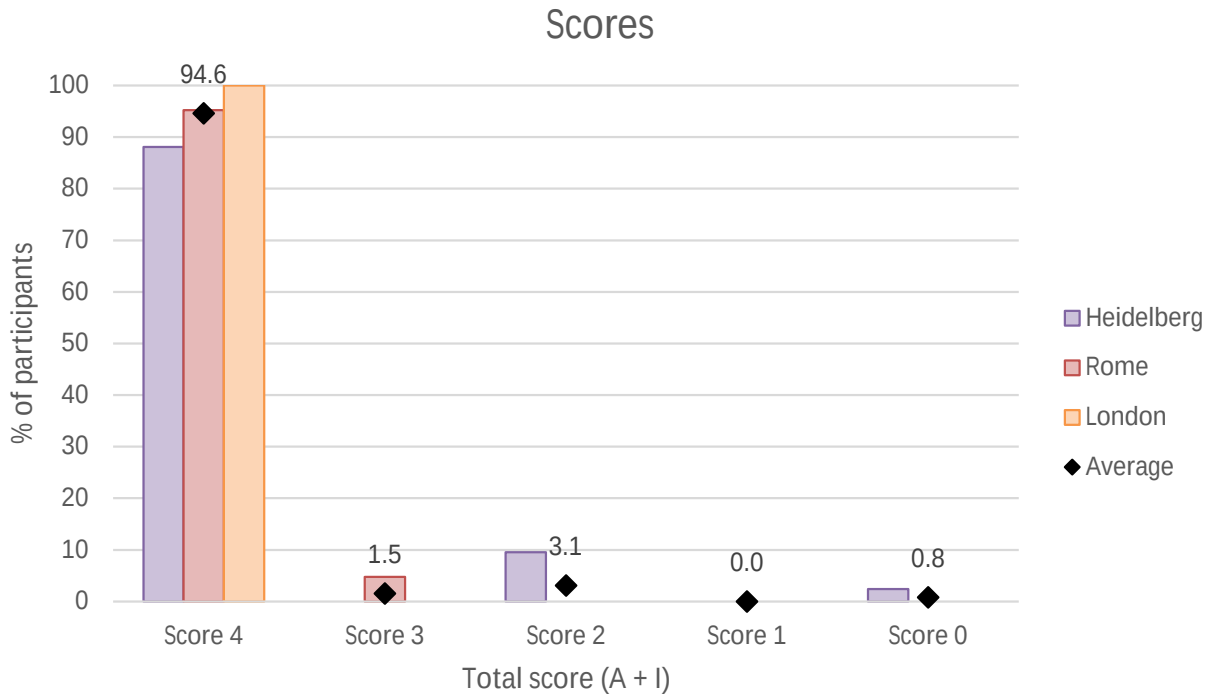


- Other diagnoses included other FAOD (CPT2, CACT, LCHADD, MTP, MCADD, MADD), TANGO2
- Agreed scoring across all three centres:
  - 1 point VLCADD
  - 1 point appropriate recommendations

Recommendations

- *ACADVL* genetics (or FAOD panel)
- Repeat DBS or plasma acylcarnitines
- Urine organic acids
- Plasma CK
- Enzyme analysis
- Referral to a specialist clinician

Scoring



- Criteria for critical error – normal / incorrect diagnosis with no recommendations that would enable identification of VLCADD
- Two critical errors

Summary

	Heidelberg	Rome	London	Total
Analytical prof	97.6	100	100	99.2
Interpretative prof	88.1	97.6	100	95.2
Overall prof	93.8	98.8	100	97.5

- High scoring sample with the majority of the 130 participants scoring full marks

Take-home messages

- This was a relatively clear acylcarnitine pattern, but some cases of VLCADD can be very mild with subtle acylcarnitine abnormalities → use of ratios

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ACDB Rome Scheme

Dr. Cristiano Rizzo

March 27th 2026

Samples included in the 2024 ERNDIM ACDB Rome scheme

2024: 45 lab. were registered with the Rome section of the Scheme

Returned results in the 2024 ERNDIM ACDB Rome scheme.

		Survey 1	Survey 2
Receipt of results		42	44
No answer	3	1	

Score for satisfactory performance: At least 17 points from the maximum of 24 (71%) are required for satisfactory performance

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
ACDB-IR-2024-A	CPT1	95	94	95
ACDB-IR-2024-B	GA1; GCDH	95	96	96
ACDB-IR-2024-C	cblC	64	70	67
ACDB-IR-2024-D	Methylmalonic aciduria cblA type	91	95	93
ACDB-IR-2024-E	SBCADD	85	86	86
ACDB-IR-2024-F	VLCAD	98	98	98

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CoASY

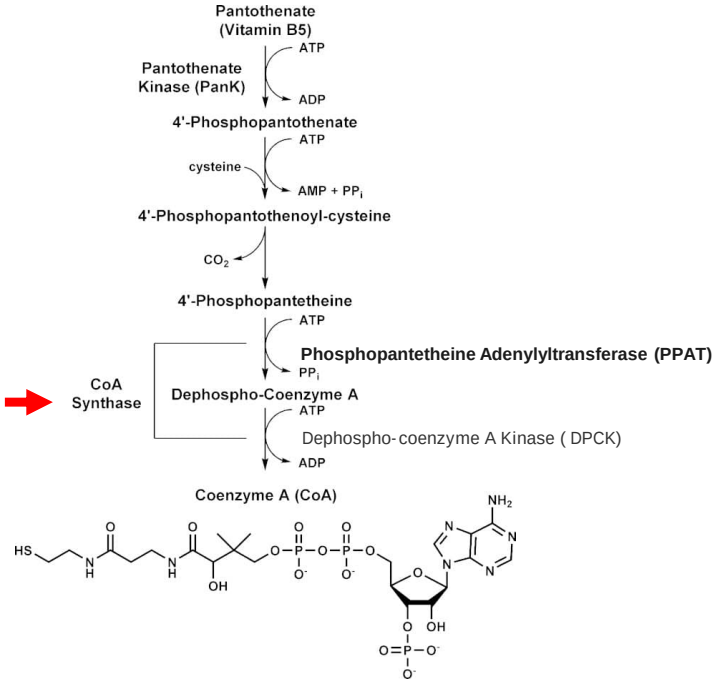
JIMD Reports JIMD Rep. 2026 Feb 26;67(2):e70066

CASE REPORT OPEN ACCESS

Abnormal Newborn Screening Resembling Carnitine Palmitoyltransferase 1a Deficiency in Three Patients With *COASY* Protein Associated Neurodegeneration

Matthew Lynch[1,2,3] | Sophie Manoy[1,3] | Claire Murray[2] | Geoff Wallace[2] | Nolette Pereira[4] | Ricky Price[5] | Anita Inwood[1,6] | Jim McGill[3,7] | David Coman[1,3,8]

COASY gene is located on chromosome 17q21 and functions in the mitochondria where it regulates the final two steps of Coenzyme A synthesis. Biallelic COASY mutations cause two rare autosomal recessive phenotypes – pontocerebellar hypoplasia (PCH) type 12, a foetal onset brain malformation disorder, and COASY protein associated neurodegeneration (CoPAN), a childhood onset neuroferritinopathy.



Elevated C0 and [C0/(C16+C18)] in DBS

C0 and [C0/(C16+C18)] normal in plasma

Progressive brain atrophy and severe neurodevelopmental phenotype in siblings with biallelic COASY variants.
Rosati J, Johnson J, Stander Z, White A, Tortorelli S, Bailey D, Fong CT, Lee BH.Am J Med Genet A. 2023 Mar;191(3):842-845.

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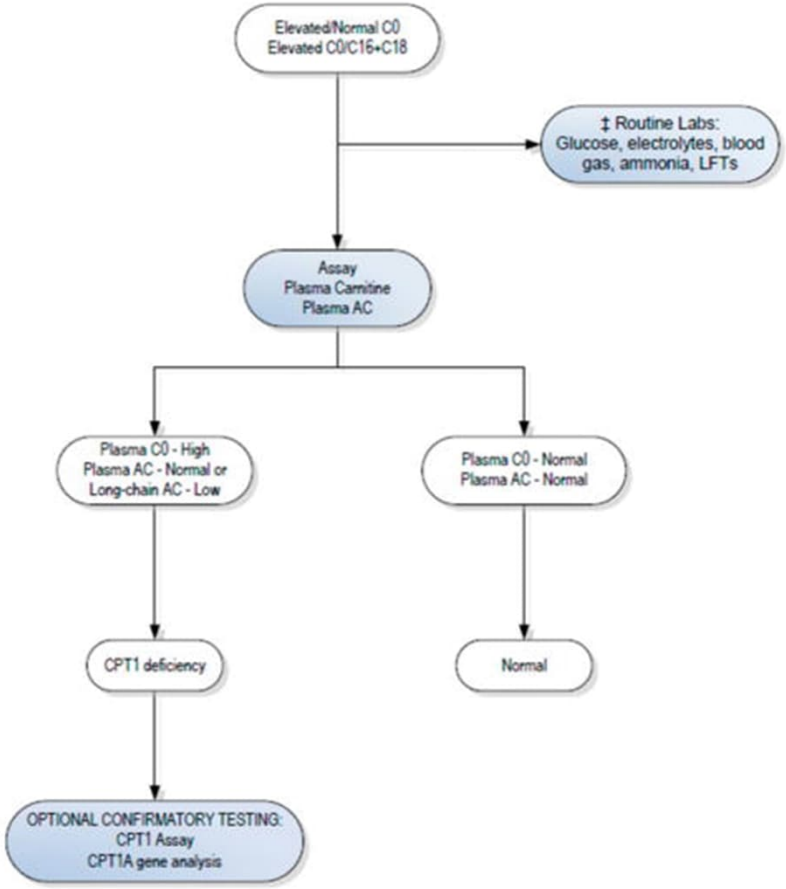
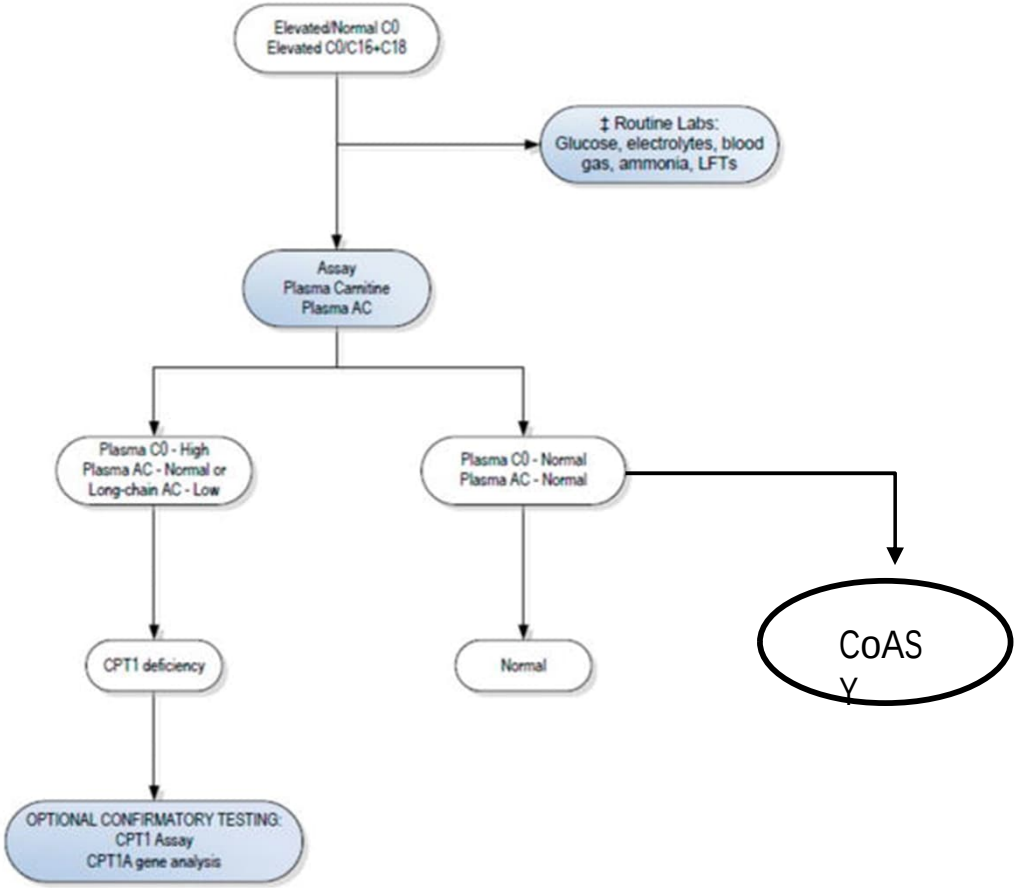
CPT1A VS CoASY





Int J Neonatal Screen. 2017 Apr 28;3(2):10

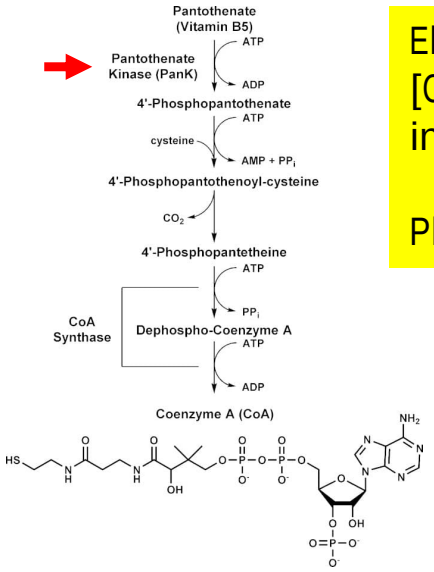
PKAN (Pantothenate Kinase-Associated Neurodegeneration)

Molecular Genetics and Metabolism
Volume 147, Issue 1, January 2026, 109713

Research Paper

The acylcarnitine profile in patients with PKAN may mimic CPT1 deficiency

Emel Yılmaz-Gümüş [a], Emine Genç [a], Sebile Kılavuz [a], Gülten Öztürk [b], Soner Erdin [c], Hatice Ömercikoğlu-Özden [d], Dilşad Türkdoğan [b], Dilek Günal [d], Burcu Öztürk-Hişmi [a]



Elevated C0 and [C0/(C16+C18)] in DBS

Plasma NA

Table 1
Clinical, molecular and biochemical data of the patients.

Case No	Gender	First presentation Age	First presentation Symptom	Age at diagnosis	Molecular analysis (*PANK2* genotype)	Acylcarnitine profile (DBS) Age	C0 Ref:8–90 μmol/L	C0/(C16 + C18) Ref:3–70	Last presentation Age	Last presentation Clinical findings
1	M	6 m 2 y	Poor appetite[a] Unsteady gait[b]	3 y	Homozygous c.628 + 2 T > G	6 m 2 y 3 y 9 m 3 y	121.5 101.8 100.8 125.1	296.5 195.9 162.6 116.9	5 y	Walking with broad-based gait, speaking short sentences
2	M	2 y	Unsteady gait	3.5	Homozygous c.1231G > A (p. Gly411Arg)	3 y 10 m 5 y	59.1 47.1	105.5 58.2	5 y	Walking with a broad-based gait, babbling
3	F	5 y	Unsteady gait	12.5 y	Compound heterozygous c.539G > A (p. Ser180Asn) / c.629 T > C (p. Ile210Thr)	13.5 y	41.3	76.5	19 y	No walking, communication only through blinking, feeding via PEG tube
4	M	12 y	Unsteady gait, speech disorder	16 y	Homozygous c.980C > T (p. Thr327Ile)	18 y	62.2	55.5	20 y	Walking with support, speaking single words
5	M	19 y	Unsteady gait, speech disorder	20 y	Homozygous c.401 A > G (p. Glu184Gly)	33 y	43.2	62.6	35 y	Walking with support, slurred speech, feeding via PEG tube

Abbreviations: C0, free carnitine; C0/(C16 + C18), ratio of free carnitine to the sum of palmitoylcarnitine and stearylcarnitine; DBS, dried blood spot; F, female; M, male; PEG, percutaneous endoscopic gastrostomy; m, month; y, years.
[a] Symptom observed at presymptomatic admission.
[b] Initial disease-related clinical symptom.

CPT1-like acylcarnitine profiles were detected in patients with classic PKAN. Two patients exhibited elevations in both C0 levels and C0/(C16 + C18) ratio; however, these elevated parameters normalized during the clinical follow-up for Patient 2. In the third patient with classic PKAN, only the C0/(C16 + C18) ratio was elevated, while C0 remained within the normal range. Conversely, the acylcarnitine profiles for patients with atypical PKAN were found to be normal

PPCS (Phosphopantothenoylcysteine Synthetase)

ARTICLE

Mutations in *PPCS*, Encoding Phosphopantothenoylcysteine Synthetase, Cause Autosomal-Recessive Dilated Cardiomyopathy

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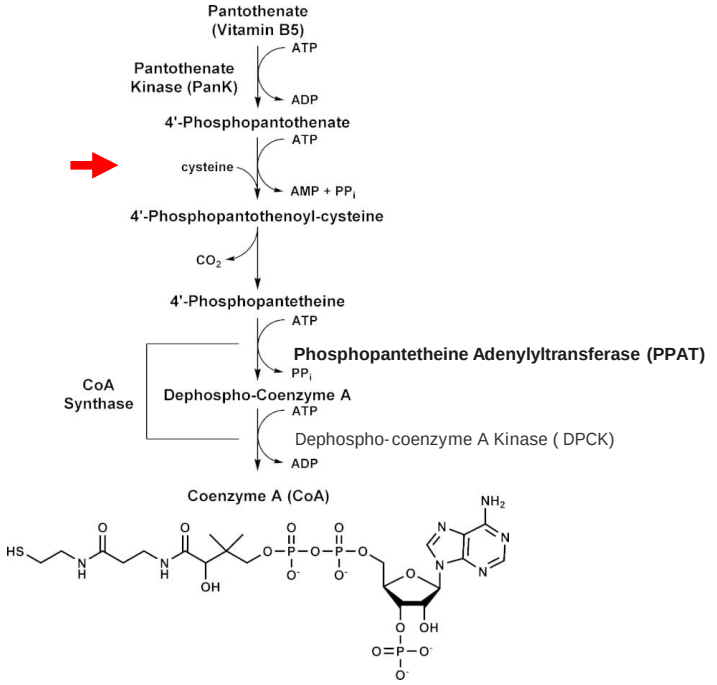
- DBS acylcarnitines C0 normal and Elevated [C0/(C16+C18)]
- Plasma NA



ERNDiM

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ACDB-IR-2024-B Glutaric acidemia type 1

Patient details provided to participants: Age 3 Y Female. Patient presented dystonic spastic hemiparesis at the age of 2 years

Analytical performance. Results were returned by 42 labs (92%).

Investigations

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c5dc	39	40	0,62	0,39
c5dc/c8	9	9	11,60	9,12
c5dc/c16	9	9	1,35	6,83
c0	13	14	32,90	4,56
c5dc/c5oh	3	3	3,06	2,11

Diagnosis / Interpretative proficiency: 41/42 (97%) respondents considered glutaryl-CoA dehydrogenase deficiency (glutaric acidemia type I) as the most likely diagnosis. One respondent considered the sample normal.

Suggested follow up test to confirm the diagnosis were: mutation analysis of glutaryl-CoA dehydrogenase (GCDH) gene (n=35), urinary organic acids (n=35), enzyme assay in cultured fibroblast (n=13), plasma carnitine/acylcanitines analysis (n=6), urinary glutarylcarnitine (n=4)

ACDB-IR-2024-C Combined methylmalonic aciduria and homocystinuria type cblC

Patient details provided to participants: Age 12 Y male.Patient presented ipotonia, neutropenia and microcephaly at birth. Maculopathy was found at 2 months of age. He currently has intellectual disability and visual impairment.
Analytical performance: Results were returned by 42 labs (92%).

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c3	26	26	2,40	0,45
c3/c2	17	17	0,46	0,74
c3/c16	5	5	2,94	1,16
methylmalonyl carnitine (c4-dc	8	8	0,39	0,34
2nd-tier: methylmalonic	3	3	3,98	1,37
2nd-tier: homocysteine	1	1	44,90	0,00

Diagnosis / Interpretative proficiency: 33/42 (78%) respondents considered a disorders of intracellular cobalamin metabolism, 10 respondents suggested a normal profile, 1 respondent suggested mitochondrial acetoacetyl-CoA-thiolase deficiency, 1 respondent suggested long chain Acyl-CoA dehydrogenase deficiency, 1 respondent suggested Carnitine uptake disorder, as the most likely diagnosis.

Suggested follow up test to confirm the diagnosis were: genetic analyses of genes related to elevation of C3-carnitine (panel including MMACHC gene and other genes involved in the absorption, transport and intracellular metabolism of cobalamins and propionic pathway disorders) (n=22), urinary organic acids (n=28), plasma total Hcys (n=20), serum Vit. B12 (n=18), plasma aminoacids (n=11), plasma methylmalonate (n=21), cblC complementation studies in fibroblasts (n=2), plasma carnitine/acylcanitines analysis (n=3), plasma, SAM and SAH (n=2)

Diagnosis of CblC by remains challenging

2022

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
ACDB-IR-2022-A	HMGCLD	98	99	98
ACDB-IR-2022-B	(EE); ETHE1	88	94	91
ACDB-IR-2022-C	VLCADD	97	98	97
ACDB-IR-2022-D	cblC	99	99	99
ACDB-IR-2022-E	Normal	100	75	88
ACDB-IR-2022-F	MCAD	99	100	99

Investigations

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c3	39	40	3,69	0,68
c3/c2	25	25	0,83	0,15
c3/c16	11	11	4,00	0,86
c4dc	15	16	0,41	0,36
c3/met	7	7	1,01	0,67
methylmalonic acid (2nd tier)	3	3	11,94	1,00

2024

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
ACDB-IR-2024-A	CPT1	95	94	95
ACDB-IR-2024-B	GA1; GCDH	95	96	96
ACDB-IR-2024-C	cblC	64	70	67
ACDB-IR-2024-D	Methylmalonic aciduria cblA type	91	95	93
ACDB-IR-2024-E	SBCADD	85	86	86
ACDB-IR-2024-F	VLCAD	98	98	98

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c3	26	26	2,40	0,45
c3/c2	17	17	0,46	0,74
c3/c16	5	5	2,94	1,16
methylmalonyl carnitine (c4-dc	8	8	0,39	0,34
2nd-tier: methylmalonic	3	3	3,98	1,37
2nd-tier: homocysteine	1	1	44,90	0,00

ACDB-IR-2024-D Methylmalonic aciduria, vitamin B12-responsive, cblA type

Patient details provided to participants: Age 11 Y male. Patient presented with recurrent vomiting, dysphagia nystagmus and hypotonus of the lower limbs at two months of life. He currently has psychomotor retardation and language delay. In treatment with carnitine, vitamin B12 and folate.
Analytical performance 45 participants. 2 participants did not provide a response.

Investigations

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c3	36	37	3,53	0,83
c3/c2	28	28	0,54	0,13
c3/c16	13	13	4,12	0,78
methylmalonylcarnitine	5	5	0,22	0,27
methylmalonil-succinil-3-oh-	7	7	0,35	0,11
mma	2	2	11,03	2,10

Diagnosis / Interpretative proficiency: 40/43 (93%) respondents considered a disorders of intracellular cobalamin metabolism, methylmalonic acidemia, or propionic acidemia, as the most likely diagnosis. 1 respondent suggested carnitine uptake deficiency, 1 respondent suggested b-ketothiolase deficency, 1 respondent mt-ATPase6 deficency as the most likely diagnosis.

Suggested follow up test to confirm the diagnosis were: genetic analyses of genes related to elevation of C3-carnitine, (transport and intracellular metabolism of cobalamins and propionic pathway disorders) (n=34), urinary organic acids (n=40), plasma total Hcys (n=28), serum Vit. B12 (n=21), plasma aminoacids (n=17), plasma methylmalonate(n=16), DBS methylmalonate (n=4)complementation studies in fibroblasts (n=3), plasma carnitine/acylcanitines analysis (n=6), plasmaSAM and SAH (n=3)

ACDB-IR-2024-E Short/Branched-Chain Acyl-CoA Dehydrogenase Deficiency

Patient details provided to participants Age 9 Y male. Patient presented hyporeactivity, sleepiness and hypertonic crises at 20 days of life. He currently has no symptoms and is on a free diet

Analytical performance

Results were returned by 43 labs (95%).

6 laboratories reported normal C5-carnitine levels

Investigations

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c5	39	39	0,54	0,11
c5/c2	16	17	0,23	0,08
c5/c3	12	12	1,09	0,23

Diagnosis / Interpretative proficiency: 37/43 (86%) respondents considered Short/Branched-Chain Acyl-CoA Dehydrogenase or Isovaleryl-CoA Dehydrogenase Deficiency (Isovaleric acidemia;IVA) as the most likely diagnosis. 2 respondents considered normal the acylcarnitine profile. 3 respondents considered Carnitine palmitoyltransferase II deficiency, 1 Very long Chain Acyl-CoA Dehydrogenase deficiency and 1 a 3-Methylcrotoyl-CoA Carboxylase deficency as the most likely diagnosis.

2 respondent suggested in addition, the possibility of false positives due to contamination by pivalate containing drugs or cosmetic products containing neopentanoate

Suggested follow up test to confirm the diagnosis were: mutation analysis of Short/Branched-Chain Acyl-CoA dehydrogenase (ACADSB) or/and Isovaleryl-CoA Dehydrogenase (IVD) genes (n=31), urinary organic acids (n=37), enzyme assay in cultured fibroblast (n=3), plasma carnitine/acylcanitinesanalysis (n=5).

5 respondents performed a II tier test which provided the chromatographyc separation of the C5 isomers(isovalerylcarnitine, 2-methylbutyrilcarnitine, pivaloylcarnitine, valerylcarnitine) on the blood spot.

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ACDB-IR-2024-F VLCADD

Patient details provided to participants: Age 1 Y male. Diagnosis at birth by NBS. Sporadic episodes of myalgia in the lower limbs at the age of 6 years.
Analytical performance: Results were returned by 44 labs(98%).

Investigations

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c14:1	43	43	2,37	0,77
c14	36	37	0,83	0,19
c14:2	34	34	0,37	0,14
c16:1	21	21	0,58	0,19
c14:1/c2	14	14	0,89	0,21
c14:1/c16	12	13	1,46	0,33

Diagnosis / Interpretative proficiency 44/44 (100%) respondents considered VLCADD as the most likely diagnosis

The alternative differential diagnosis suggested by respondents included:
a) Carnitine-acylcarnitine translocase (CACT) deficiency (n=3)
b) Carnitine Palmitoyl transferase type 2 (CPT2) deficiency (n=11)
c) Mitochondrial trifunctional protein (MTP) deficiency/long-chain 3-Hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency/long-chain 3-Ketoacyl-CoA Thiolase (LCKAT) deficiency (n=2)
d) Multiple Dehydrogenase Deficency (glutaric aciduria type 2) (n=2)
e) TANGO2 (n=1)

Suggested follow up test to confirm the diagnosis were: mutation analysis of ACADVL gene (n=41), urinary organic acids (n=24), VLCAD activity in fibroblasts and lymphocytes (n=22), plasma carnitine/acylcanitines analysis (n=19)

Samples included in the 2025 ERNDIM ACDB Rome scheme

46 lab. were registered with the Rome section of the Scheme

Returned results in the 2025 ERNDIM ACDB Rome scheme.

		Survey 1	Survey 2
Receipt of results		43	41
No answer	3	5	

Score for satisfactory performance At least 17 points from the maximum of 24 (71%) are required for satisfactory performance

Overall Proficiency

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
ACDB-IR-2025-A	VLCADD	91	89	90
ACDB-IR-2025-B	MCADD	89	85	87
ACDB-IR-2025-C	SCADD	85	83	84
ACDB-IR-2025-D	VLCADD	89	88	89
ACDB-IR-2025-E	GAII	89	88	89
ACDB-IR-2025-F	GAI	87	87	87

ERNDiM

ACDB-IR-2025-B MCADD

Patient details provided to participants: Age 14 Y male. Neonatal onset, with hypoglycemic and hyperammonemic decompensation on the second day of life. To date, stable clinical picture and follows a normolipidic diet.

Analytical performance: 46 lab. were registered in Rome section of the s (89%). All respondents considered C6, C8 concentration increased.

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c6	40	41	1,20	0,25
c8	40	41	2,90	0,68
c10:1	34	35	0,68	0,33
c10	17	18	0,19	0,04
c8/c10	24	24	15,71	2,46
c8/c2	17	17	1,22	2,17

Interpretative proficiency: 40/41 (97%) respondents considered MCADD as the most likely diagnosis and 1 respondent considered SCADD/IBDD as the most likely diagnosis.

Suggested follow up test to confirm the diagnosis were: mutation analysis of ACADM gene (n=39), urinary organic acids (n=34), urinary acylglycines (n=14) enzyme assay in cultured fibroblast (n=7), plasma carnitine/acylcarnitines analysis (n=20).

ERNDiM

ERNDiM

ACDB-IR-2025-C SCADD

Patient details provided to participants: Age 15 Y male. At the age of 4, a diagnosis of epilepsy was made. Therapy started first with Valproate then with ethosuximide with disappearance of the crises

Analytical performance Results were turned by 41 labs (89%). 39 respondents found increased C4-carnitine. 1 laboratory reported no alterations in the acylcarnitine profile.

Interpretative proficiency 39/41 (95%) respondents considered SCADD, 1 respondent considered Ethylmalonic encephalopathy (ETHE1), 1 respondent considered normal acylcarnitines profile as the most likely diagnosis.

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c4	38	40	1,35	0,23
c4/c3	10	10	1,04	0,13
c4/c2	11	11	0,17	0,04
c4/c8	7	7	20,56	1,80
c5	13	13	0,20	0,05

ERNDiM

ERNDiM

ACDB-IR-2025-C SCADD

The alternative differential diagnosis suggested by respondents included:

a) Isobutyryl-CoA Dehydrogenase Deficiency (n=23)
b) Ethylmalonic encephalopathy (ETHE1) (n=10)
c) Glutaric aciduria type II (n=1)
d) Secondary effect of valproate therapy (n=2)

Suggested follow up test to confirm the diagnosis were: mutation analysis of ACADS, ACAD8, ETHE1 genes (n=36), urinary organic acids (n=36), enzyme assay in cultured fibroblast (n=6), plasma acylcarnitines analysis (n=18), urine acylcarnitines analysis (n=6).

Urinary organic acid analysis and resolution between butyrylcarnitine and isobutyrylcarnitine using the second-tier LC-MS/MS method in DBS are required to further establish the diagnosis. The final confirmation is by molecular genetic testing

ERNDiM

ERNDiM

ACDB-IR-2025-D VLCADD

Patient details provided to participants: Age 30 Y female. She underwent multiple hospitalizations for rhabdomyolysis and metabolic decompensation. To date, she follows the low-lipid home diet integrated with MCT and evening cornstarch with good compliance.

Analytical performance: Analytical results were returned by 41 labs (89%). 100% respondents founded C14:1-carnitine elevated.

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c14:1	41	41	1,49	0,49
c14:2	35	35	0,24	0,06
c14	34	34	0,63	0,11
c16:1	22	22	0,66	0,14
c14:1/c2	14	14	0,22	0,04
c14:1/c16	15	16	0,90	0,20

Interpretative proficiency: Interpretative proficiency results were returned by 41 labs (89%). 41/41 (100%) respondents considered Very VLCADD.

Suggested follow up test to confirm the diagnosis were: mutation analysis of ACADVL gene (n=37), urinary organic acids (n=19), VLCAD activity in fibroblasts and lymphocytes (n=16), plasma carnitine/acylcanitines analysis (n=23).

ERNDiM

ACDB-IR-2025-E MADD

Patient details provided to participants: Age 10 Y female. At one year of age she was hospitalized for hyperthermia with asthenia and hypotonia, To date, she follows therapy with riboflavin and carnitine; psychomotor development and language are appropriate to age.

Analytical performance: Results were turned by 41 labs (89%). 100% respondents founded C4, C5, C6, C8, C10, C12, C14, C14: 1, C14:2, C18:1 and C18:2 carnitines elevated.

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c8	41	41	0,79	0,14
c10	40	40	1,35	0,42
c5	41	41	0,98	0,34
c14:1	38	38	0,78	0,27
c6	37	37	0,44	0,10
c12	29	29	0,71	0,17

Interpretative proficiency: Interpretative proficiency results were returned by 41 labs (89%). 40/41 (97%) respondents considered Multiple Acyl-CoA dehydrogenase deficiency (MADD) and one respondent considered Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD) as the most likely diagnosis.

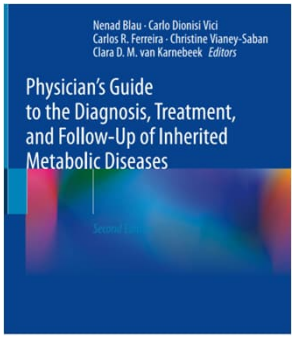
ACDB-IR-2025-E MADD

The suggested alternative diagnoses were:

- Defects in FAD synthase, riboflavin transporters or the mitochondrial flavin adenine dinucleotide transporter (MTF) (14 respondents);
- Medium chain Acyl-CoA deficiency (3 respondents);
- Carnitine palmitoyltransferase II (5 respondents);
- Very Long Chain Acyl-CoA dehydrogenase deficiency (3 respondent);
- Carnitine-acylcarnitine translocase deficiency (3 respondent);
- Riboflavin deficiency (4 respondents);
- Short Chain Acyl-CoA dehydrogrnase deficiency (1 respondent);
- TANGO 2 (1 respondent);
- Unspecified mitochondrial disorder (1 respondent);
- autosomal recessive polycystic kidney disease (1 respondent),
- Zellweger syndrome (1 respondent),
- Alterations in sterol biosynthesis (1 respondent),
- MADD like disease associated with Sertraline use (1 respondent) ,
- Nitric oxide abuse (1 respondent).

Suggested follow up test to confirm the diagnosis were mutation analysis of ETFA, ETFB, ETFDH, FLAD1, SLC52A1, SLC52A2 SLC52A3, SLC25A32 genes (n=39), urinary organic acids (n=35), enzyme assay in cultured fibroblast (n=4), plasma acylcarnitines analysis (n=17), plasma riboflavin (n=5)

MADD-like pattern of acylcarnitines associated with



Disorders of Riboflavin Metabolism 32

Christine Vianey-Saban, Cécile Acquaviva,
and Annet M. Bosch

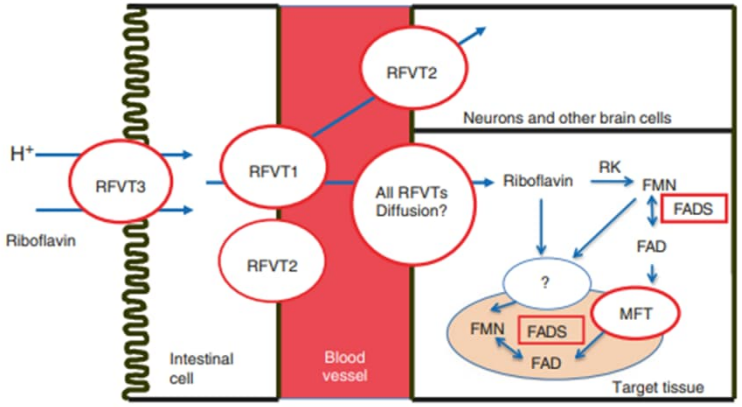


Fig. 32.3 Simplified scheme of riboflavin metabolism. *FAD* Flavin adenine dinucleotide, *FADS* FAD synthase, *FMN* Flavin mononucleotide, *MFT* Mitochondrial FAD transporter, *RFVT* Riboflavin transporter, *RK* Riboflavin kinase

No.	Disorder	Alternative name	Abbreviation	Inheritance	Gene symbol	Chromosomal localisation	Affected protein	OMIM N°
32.1	Riboflavin transporter 2 deficiency	Brown-Vialetto-van Laere syndrome type 1	RFVT2 deficiency	AR	*SLC52A3*	20p13	Riboflavin transporter 2	613350
32.2	Riboflavin transporter 3 deficiency	Brown-Vialetto-van Laere syndrome type 2	RFVT3 deficiency	AR	*SLC52A2*	8q24.3	Riboflavin transporter 3	607882
32.3	Flavin adenine dinucleotide synthase deficiency (early-onset form)	FAD synthase deficiency (early-onset form)	FADS deficiency	AR	*FLAD1*	1q21.3	FAD synthase	610595
32.4	Flavin adenine dinucleotide synthase deficiency (late-onset form)	FAD synthase deficiency (late-onset form)	FADS deficiency	AR	*FLAD1*	1q21.3	FAD synthase	610595
32.5	Mitochondrial flavin adenine dinucleotide transporter deficiency	Riboflavin-responsive exercise intolerance	MFT deficiency	AR	*SLC25A32*	8q22.3	Mitochondrial FAD transporter	610815
32.6	Electron transfer flavoprotein deficiency	Multiple acyl-CoA dehydrogenase deficiency (ETF)	MADD (ETF)	AR	*ETFA*, *ETFB*	*ETFA* 15q24.2-q24.3 *ETFB* 19q13.41	Electron transfer flavoprotein (ETF)	ETFA 608053 ETFB 130410
32.7	Electron transfer flavoprotein dehydrogenase deficiency	Multiple acyl-CoA dehydrogenase deficiency (ETFDH)	MADD (ETFDH)	AR	*ETFDH*	4q32-qter	ETF-ubiquinone oxidoreductase (ETF-QO)	231675
32.8	Riboflavin-responsive electron transfer flavoprotein dehydrogenase deficiency	Riboflavin-responsive multiple acyl-CoA dehydrogenase deficiency (ETFDH)	RR-MADD (ETFDH)	AR	*ETFDH*	4q32-qter	ETF-ubiquinone oxidoreductase (ETF-QO)	231675
32.9	Riboflavin transporter 1 deficiency	Transient riboflavin deficiency	RFVT1 deficiency		*SLC52A1*	17p13.2	Riboflavin transporter 1	607883

MADD-like pattern of acylcarnitines associated with:

FAD synthase

- Riboflavin-Responsive and -Non-responsive Mutations in FAD Synthase Cause Multiple Acyl-CoA Dehydrogenase and Combined Respiratory-Chain Deficiency
  Rikke K J Olsen, et al. Am J Hum Genet. 2016 Jun 2;98(6):1130-1145.

- Flavin adenine dinucleotide synthase deficiency due to FLAD1 mutation presenting as multiple acyl-CoA dehydrogenation deficiency-like disease: A case report
  Kenji Yamada 1, et al, Brain Dev. 2019 Aug;41(7):638-642.

Acilcarnitines
Organic acids
like MADD

Mitochondrial FAD transporter

- Novel SLC25A32 mutation in a patient with a severe neuromuscular phenotype Debby M E I Hellebrekers et al. Eur J Hum Genet. 2017 Jun;25(7):886-888.

- SLC25A32 Mutations and Riboflavin-Responsive Exercise Intolerance. Manuel Schiff et al. N Engl J Med. 2016 Feb 25;374(8):795-7.

MADD-like pattern of acylcarnitines associated with:

TANGO 2

TANGO2 (transport and Golgi organization 2 def.) :metabolic encephalopathy; recurrent metabolic crises with rhabdomyolysis, cardiac arrhythmias, and neurodegeneration

- Transport and Golgi organization 2 deficiency with a prominent elevation of C14:1 during a metabolic crisis: A case report. Katsuyuki Yokoi et al. JIMD Rep. 2022 Oct 27;64(1):3–9.

- Mitochondrial dysfunction associated with TANGO2 deficiency. Paige Heiman, et al. Scientific Reports volume 12, Article number: 3045 (2022)

Acilcarnitines and OA like MADD

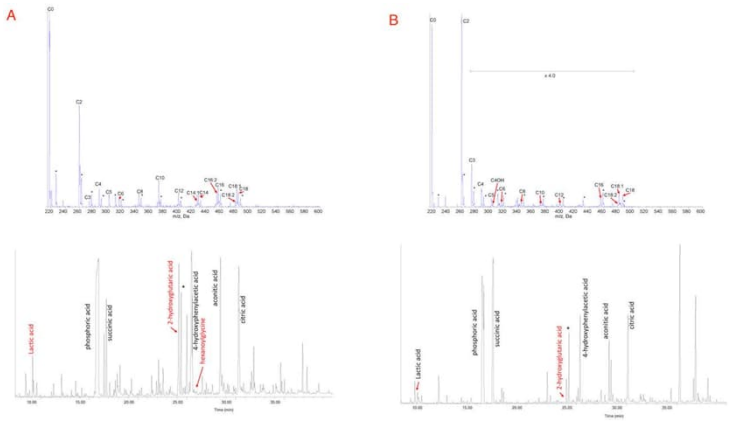
MT-CO2

- Mitochondrial complex IV deficiency caused by a novel frameshift variant in MT-CO2 associated with myopathy and perturbed acylcarnitine profile. Sara Roos et al. Eur J Hum Genet. 2019 Feb;27(2):331-335.

MADD-like pattern of acylcarnitines associated with Sertraline

- MADD-like pattern of acylcarnitines associated with sertraline use. Ingoglia F, et al.Mol Genet Metab Rep. 2024 Sep 16;41:101142.
- The Complex Etiology of Sertraline-Induced Lipid Storage Myopathy and Acquired Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)-Like Syndromes: Hidden Genetic Variation, Nutritional Deficiency, and Mitochondrial Vulnerability. McCarron EP.Muscle Nerve. 2026 Feb 10. doi: 10.1002/mus.70178.
- Sertraline-Associated Riboflavin-Responsive Lipid Storage Myopathy: Report of Two Case. Shaibani A, Taylor A.Muscle Nerve. 2026 Feb;73(2):309-312.
- Late-onset multiple-acyl-CoA-dehydrogenase deficiency-like condition: a case series from the West of Scotland. Watson-Fargie T et al.Neuromuscul Disord. 2025 Apr;49:105343.
- Acquired multiple acyl-CoA dehydrogenase deficiency (MADD) provoked by sertraline: an emerging and treatable disorder. Alungulese AL et .Pract Neurol. 2026 Feb 9:pn-2025-005016.

Acilcarnitines
Organic acids
like MADD



ERNDiM

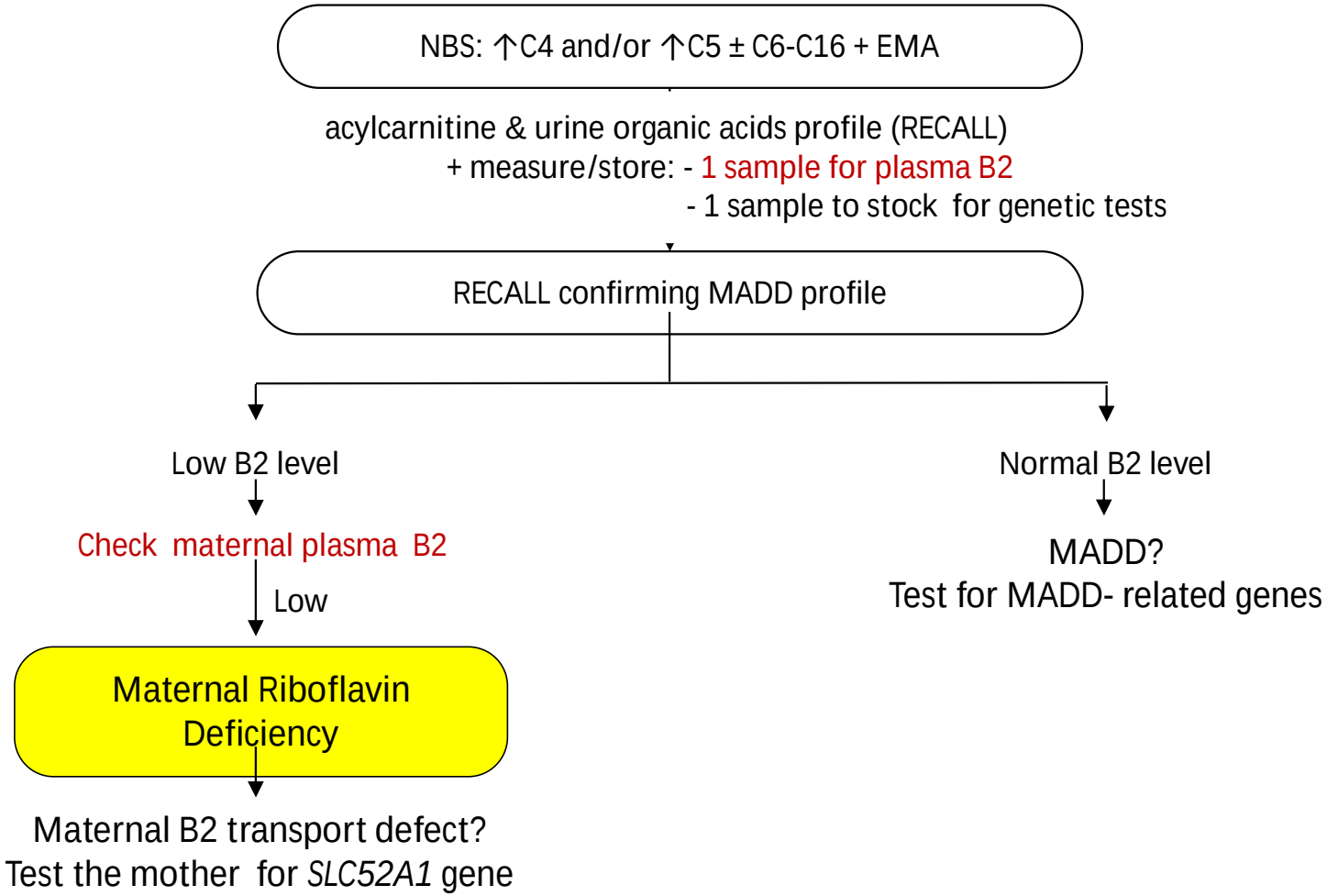
ERNDiM

MADD-like pattern of acylcarnitines associated with Renal insufficiency

- Mass Spectrometric Analysis of L-carnitine and its Esters: Potential Biomarkers of Disturbances in Carnitine Homeostasis Judit Bene et al. Mol Med. 2020;20(5):336-354

- Associations of Plasma Amino Acid and Acylcarnitine Profiles with Incident Reduced Glomerular Filtration Rate Feijie Wang et al Clin J Am Soc Nephrol. 2018 Apr 6;13(4):560-568.

Proposal for implementation of the diagnostic algorithm



ERNDiM

ACDB-IR-2025-F GA I

Patient details provided to participants: Age 9 Y male. Diagnosed at 17 days of age. At birth he presented with large pseudocysts in the bilateral frontal paraventricular area. Follows pharmacological therapy (carnitine) and dietary therapy (reduced intake of lysine). To date, the child's clinical picture is very good: never metabolic decompensations, absence of neurological signs and macrocrania.

Analytical performance: Analytical results were returned by 40 labs (86%). 100% respondents founded glutarylcarnitine elevated.

Interpretative proficiency: 40/40 (100%) respondents considered glutaryl-CoA dehydrogenase deficiency (glutaric acidemia type I) as the most likely diagnosis.

Acylcarnitines	n (quant)	n (qual)	Median value	SD
c5dc	40	40	2,92	1,45
c5dc/c16	12	12	3,71	1,40
c5dc/c8	12	12	86,02	757,65
c0	13	13	33,00	8,33
c5dc/c5oh	7	7	6,39	2,24

Suggested follow up test to confirm the diagnosis were mutation analysis of glutaryl-CoA dehydrogenase (GCDH) gene (n=37), urinary organic acids (n=38), enzyme assay in cultured fibroblast (n=14), plasma carnitine/acylcarnitines analysis (n=14), urinary glutarylcarnitine (n=3)

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Overview 2024 / 25

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
ACDB-DH-2024-A *	CPT1	88	83	85
ACDB-DH-2024-B	IVA	100	100	100
ACDB-DH-2024-C	PA	98	100	99
ACDB-DH-2024-D	HMG CLD	83	90	87
ACDB-DH-2024-E	Normal control	95	95	95
ACDB-DH-2024-F	3-MGA	95	66	80
ACDB-DH-2025-A *	VLCADD	98	88	93
ACDB-DH-2025-B	VLCADD	64	71	68
ACDB-DH-2025-C	MADD	99	68	83
ACDB-DH-2025-D	Normal control	63	63	63
ACDB-DH-2025-E	MMA mut(0)	100	91	96
ACDB-DH-2025-F	HMG CLD	83	74	78

* common samples

ACDB Heidelberg

Focus I: Mild VLCAD – (ACBD-DH-2025-B)

Patient details provided to participants

Girl aged 11, diagnosed by NBS. Now recurrent nausea without vomiting.

Further details:

- elevated C14:1 level in newborn screening, which normalised in the second card
- VLCAD enzyme activity in leukocytes was 18% of controls
- molecular analysis of ACADVL → compound heterozygosity for two deletions confirming VLCAD deficiency
- never had any metabolic decompensations

ACDB Heidelberg

Focus I: Mild VLCAD – (ACBD-DH-2025-B)

Comparison of analytical results in HD from sample validation and participation

Sample	2023-E	2025-A	2025-B	range	
C0	14.5	30.4	23.5	4.3 - 56.8	µmol/L
C14:0	0.42	0.64	0.11	0 - 0.41	µmol/L
C14:1	0.94	2.3	0.34	0 - 0.28	µmol/L
C14:1/C4	10.4	15.3	3.09	0 - 1.57	
C14:1/C6	13.4	76.7	11.3	0 - 6.47	
C14:1/C8	13.4	57.5	5.67	0 - 4.49	
Analytical prof.	97	98	64		%
Interpretation prof.	95	88	71		%

further frequently used diagnostic ratios for VLCAD:
C14:1 / C12:1, C14:1 / C2, C14:1 / C16

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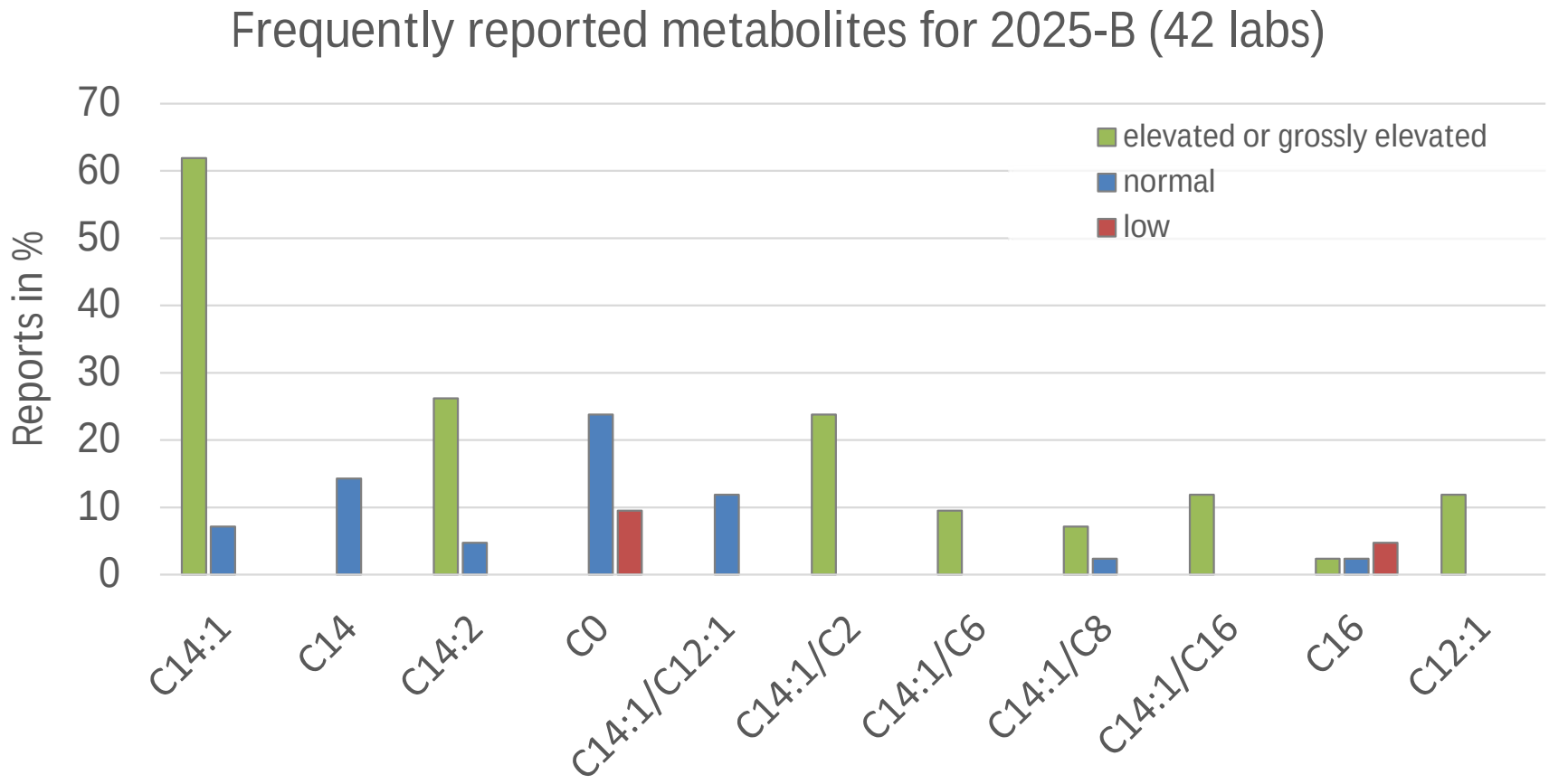
Focus I: Mild VLCAD – (ACBD-DH-2025-B)



Evaluation criteria were harmonized with (common) sample A:
2 pts for C14:1 and/or analytical ratios at least as elevated

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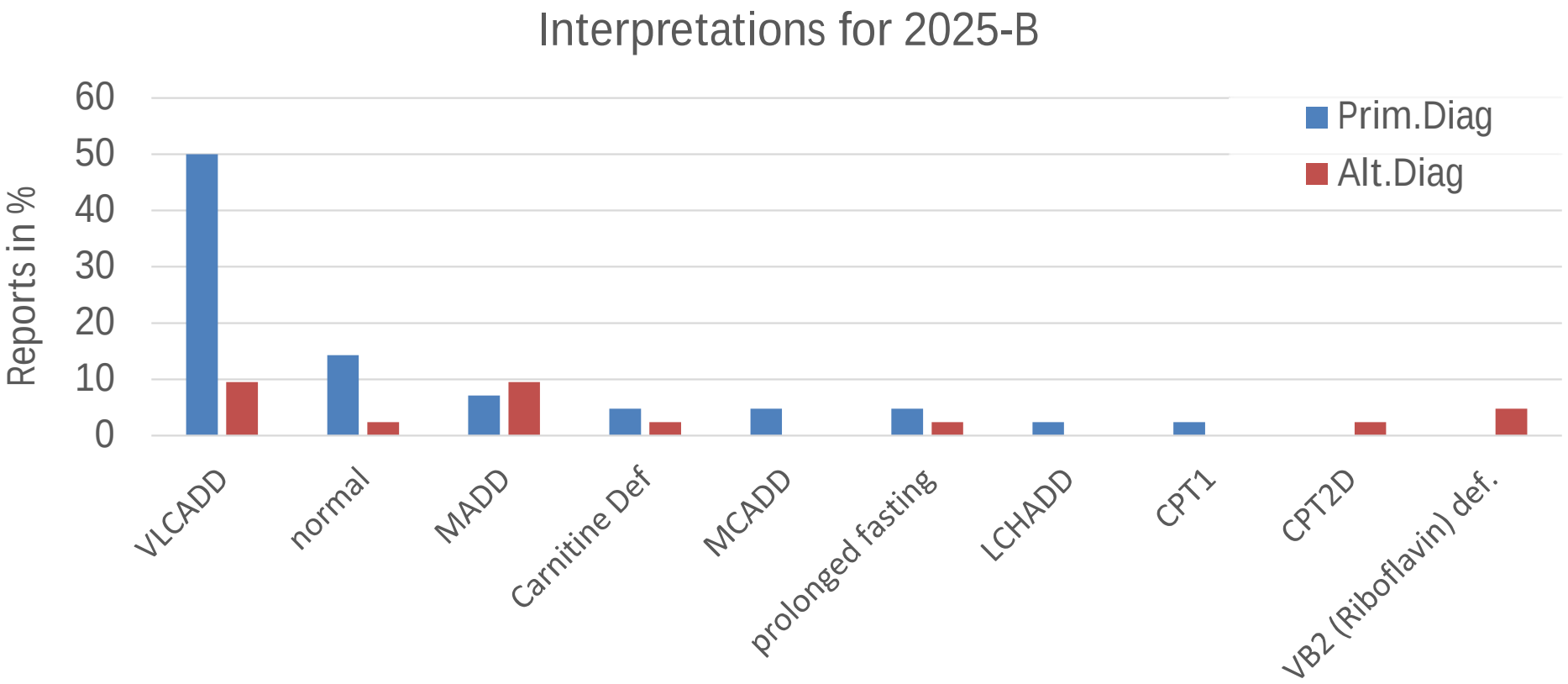
Focus I: Mild VLCAD – (ACBD-DH-2025-B)



Evaluation criteria:

- 1 pt for reporting VLCADD (principal or alternative diag.) and
- 1 pt for recommending an appropriate test for confirmation

ACDB Heidelberg

Focus I: Mild VLCAD – (ACBD-DH-2025-B)

- Consider including AC ratios in addition to absolute concentrations in your diagnostic decision making.
- Ratios can vary in diagnostic sensitivity

- Upadia, J., Noh, G., Lefante, J. J. & Andersson, H. C. Biochemical and molecular characteristics among infants with abnormal newborn screen for very-long-chain acyl-CoA dehydrogenase deficiency: A single center experience. Mol. Genet. Metab. Rep. 37, 101002 (2023).
- Wang, B. et al. New Ratios for Performance Improvement for Identifying Acyl-CoA Dehydrogenase Deficiencies in Expanded Newborn Screening: A Retrospective Study. Front. Genet. 10, 811 (2019).
- Hesse, J. et al. The diagnostic challenge in very-long chain acyl-CoA dehydrogenase deficiency (VLCADD). J. Inherit. Metab. Dis. 41, 1169–1178 (2018).

ACDB Heidelberg

Focus II: MGA1 / HMG CLD

Patient details provided to participants (same sample)

- 2023-B: 17-year-old teenager with global developmental delay in infancy, now moderate intellectual disability.
- 2024-F: Teenager with recurrent headaches.

Patient details

- Detection of elevated 3-OH isovaleric acid and 3-methylglutaconate in urine at the age of 6 y induced further investigations
- 3-Methylglutaconyl-CoA hydratase activity in lymphocytes was clearly reduced.
- Subsequently a homozygous deletion of exons 5-6 in the AUH gene confirmed the diagnosis.

ACDB Heidelberg

Focus II: MGA1 / HMG CLD

Comparison of ACs occurring in disorders of BCAA metabolism

Group	Acylcarnitine	3MCC	HMG CLD	BIO / HOLO	MGA1	BKT	MHBD
C5OH (+C4CD)*	3OH-isovaleryl	X	X	X	X		
	3OH-2Me-butyryl					X	X
C5:1	Tiglyl						
	3Me-crotonyl	X				X	X
C3	Propionyl			X			
C6DC	Adipoyl						
	3Me-glutaryl		X				

Excerpt from Miller et al. (2021), Laboratory analysis of acylcarnitines, 2020 update. Genet. Med. 23, 249–258.

Consider that further conditions can mimick biochemical features of multiple carboxylase def.:

- Leigh syndrome
- Variants of *MT-ATP6*

Balasubramaniam et al. (2017), JIMD Rep 33:99–107
Larson et al. (2023), Mitochondrion 44:58–64

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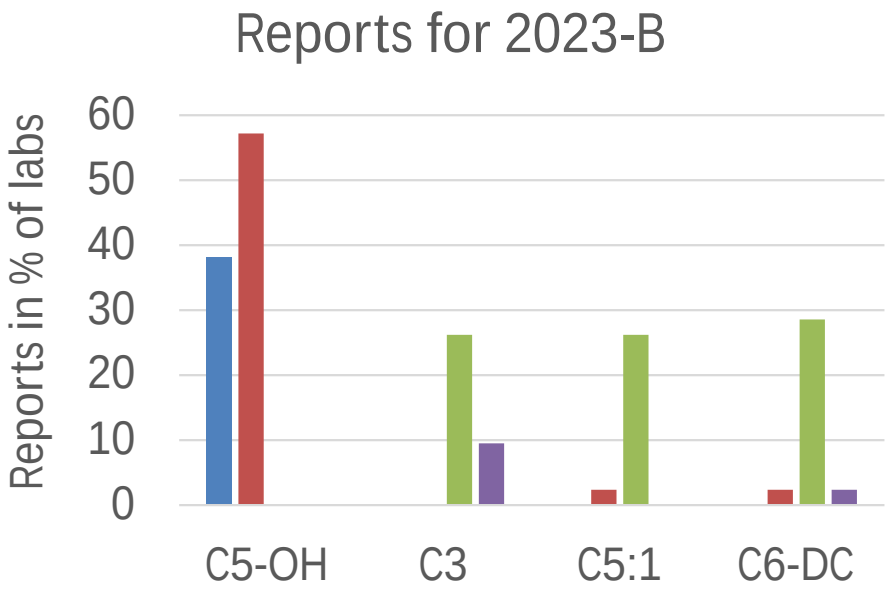
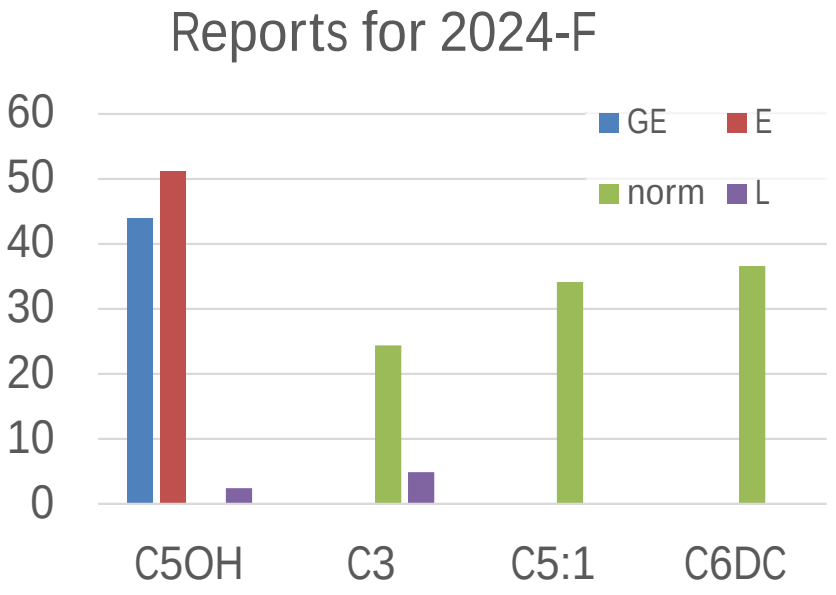
Focus II: MGA1 / HMG CLD
Analytical results





HD results

analyte	value	ref.rng	
C5OH	1.8	0 – 0.68	µM
C5OH/C2	0.17	0 – 0.02	

GE / E: (grossly) elevated
norm: normal
L: Low

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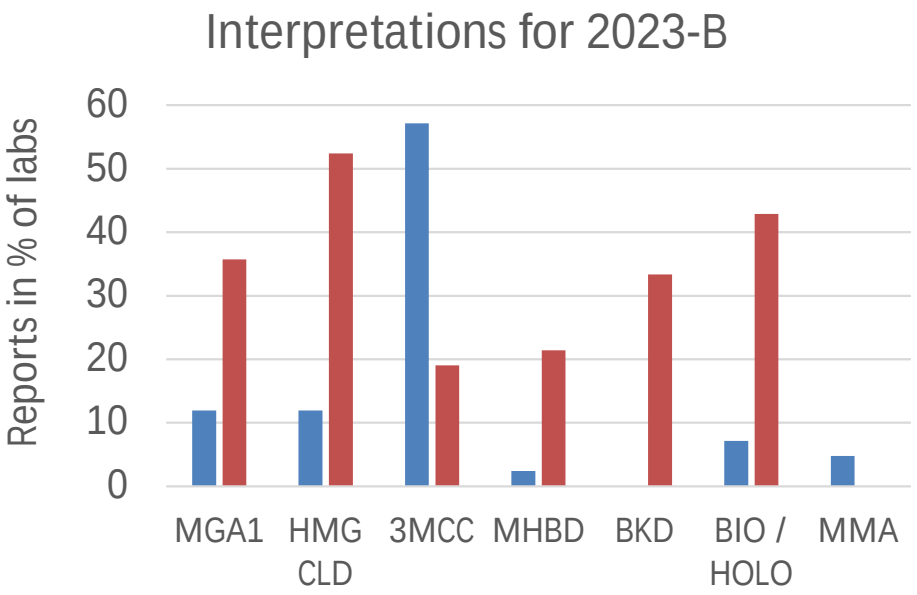
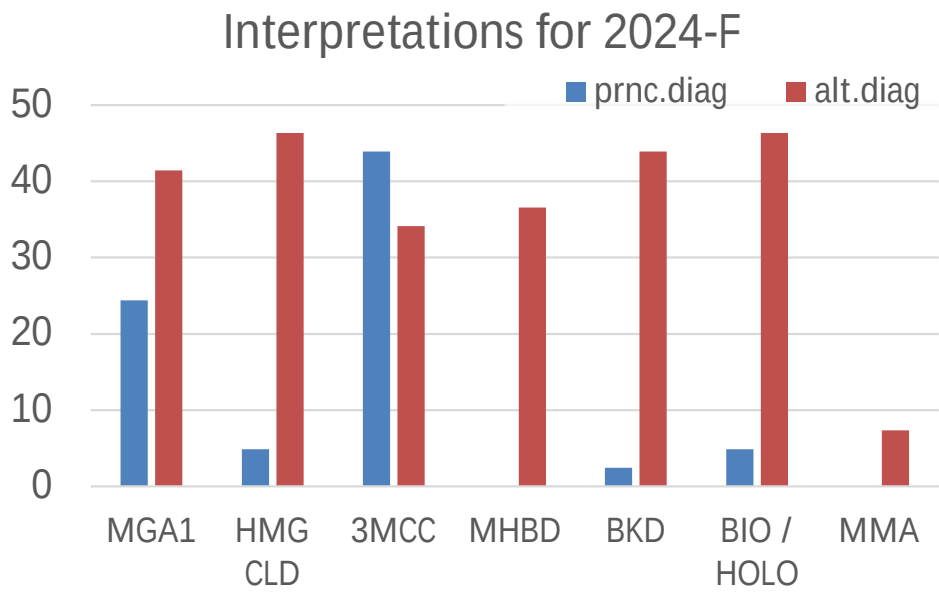
Focus II: MGA1 / HMG CLD
Interpretation results





Proficiency	analytical	interpretive
2023-B	95	48
2024-F	95	66

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Focus II: MGA1 / HMG CLD

Patient details provided to participants (same sample)

- 2024-D: Boy of eight years who presented first 3 years ago with hepatomegaly and seizures.
- 2025-F: Seven-year-old girl with a hypoglycemic seizure as a toddler. Today well.

Patient details

- 2024-D sample was donation
- 2025-F sample was from a patient with suspected HMG CLD in NBS, which was confirmed biochemically.

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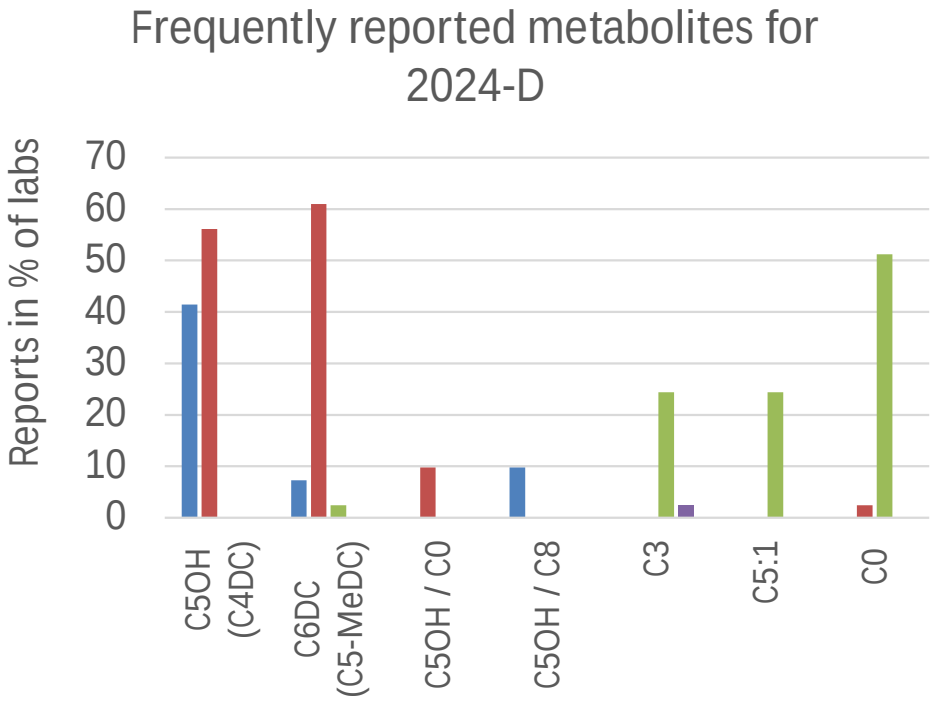
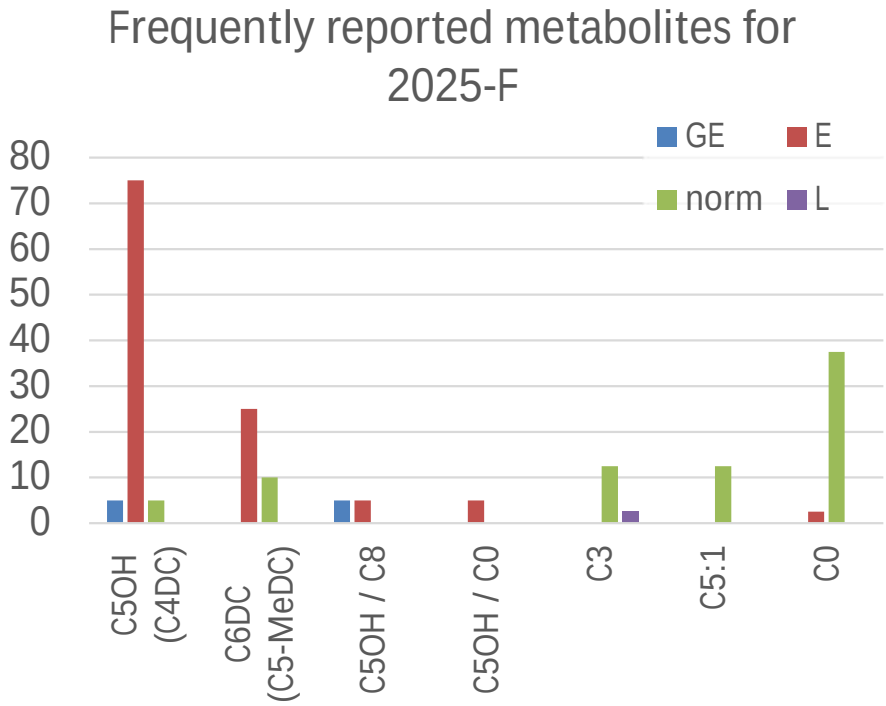
Focus II: MGA1 / HMG CLD
Analytical results

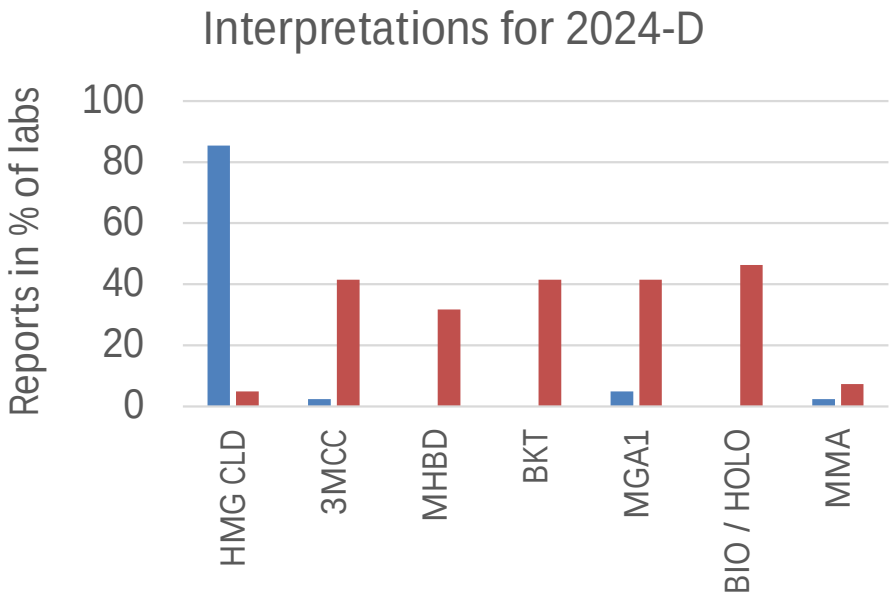


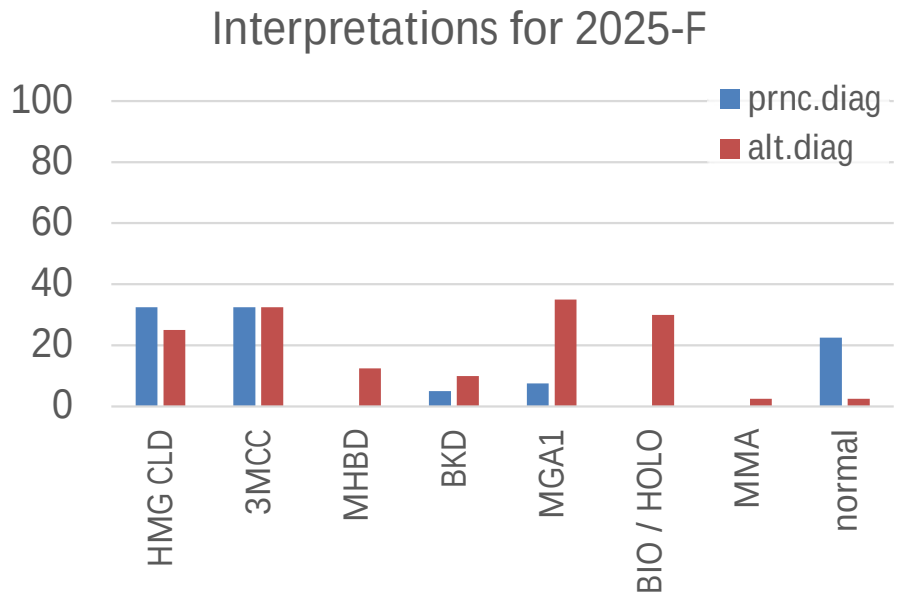


GE / E: (grossly) elevated
norm: normal
L: Low

ACDB Heidelberg

Focus II: MGA1 / HMG CLD
Interpretation results





Proficiency	analytical	interpretive
2024-D	83	90
2025-F	83	74

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Focus II: MGA1 / HMG CLD

Consider a (more) complex differential diagnosis when obtaining C5OH-based AC profiles.

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Focus III: MCAD vs MADD

Patient details provided to participants

Toddler with normal development and recurrent episodes of trembling.

Further patient details

- now 3-year-old patient identified in newborn screening with elevations of medium chain acylcarnitines (C8 < C10) and elevated C12, C14 and C14:1.
- Clinically asymptomatic.
- Confirmatory biochemical analyses:
  - elevated medium chain acylcarnitines in DBS
  - normal urinary organic acids
  - Molecular genetic analysis indicated MADD (two variants in *ETFDH*)
  - VLCAD and MCAD enzyme activity was normal.
- On riboflavine treatment the patient is still asymptomatic with normal development and no decompensations.

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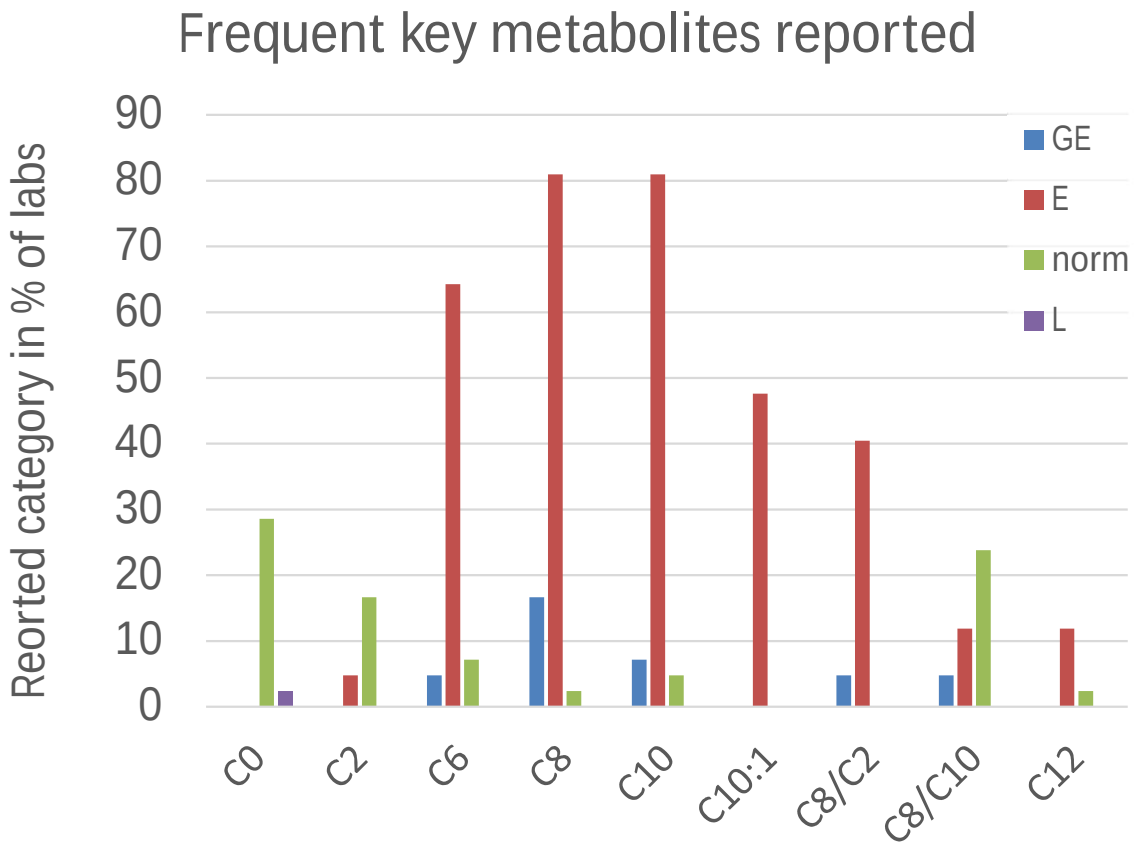
Focus III: MADD vs MCAD
Analytical results

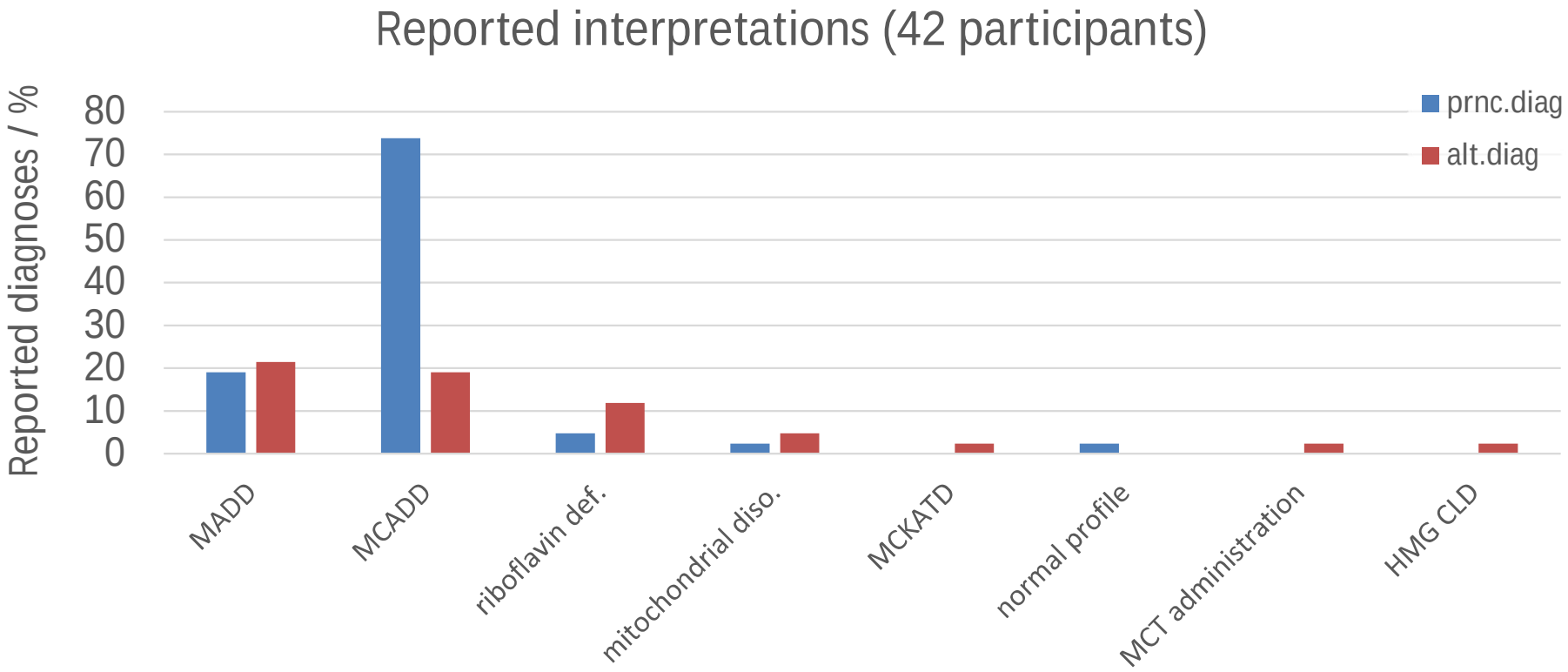
HD results

analyte	value	ref.rng
C0	34.5	4.3 – 56.8
C6	0.28	0 – 0.12
C8	0.83	0 – 0.15
C10	0.76	0 – 0.38
C10:1	0.16	0 – 0.09
C8/C2	0.064	0 – 0.01
C8/C6	2.96	0 – 0.23
C8/C10	1.09	0 – 1.39
C8/C12	5.93	0 – 1.9



ACDB Heidelberg

Focus III: MADD vs MCAD Interpretation



Proficiency	analytical	interpretive
2025-C	99	68

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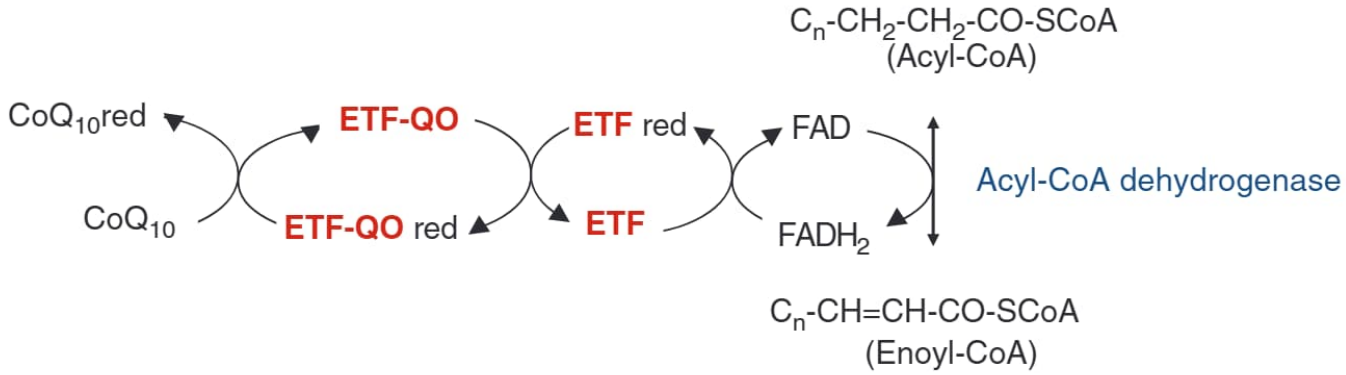
Focus III: MADD vs. MCADD

Fig. 32.2 Simplified scheme of electron transfer from acyl-CoA dehydrogenases to coenzyme Q_{10}. *CoQ_{10}* Coenzyme Q_{10}, *ETF* Electron transfer flavoprotein, *ETF-QO* ETF-ubiQuinone Oxidoreductase, *red*, Reduced *



- ETF is involved in electron transfer for Acyl-CoA Dehydrogenases (SCAD, MCAD, VLCAD)
- In case of MADD, AC profiles are highly variable (C4 – C18 ACs)
- Consider MADD in differential diagnosis of FAOs!

* Vianey-Saban et al. in: Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases. (2022).

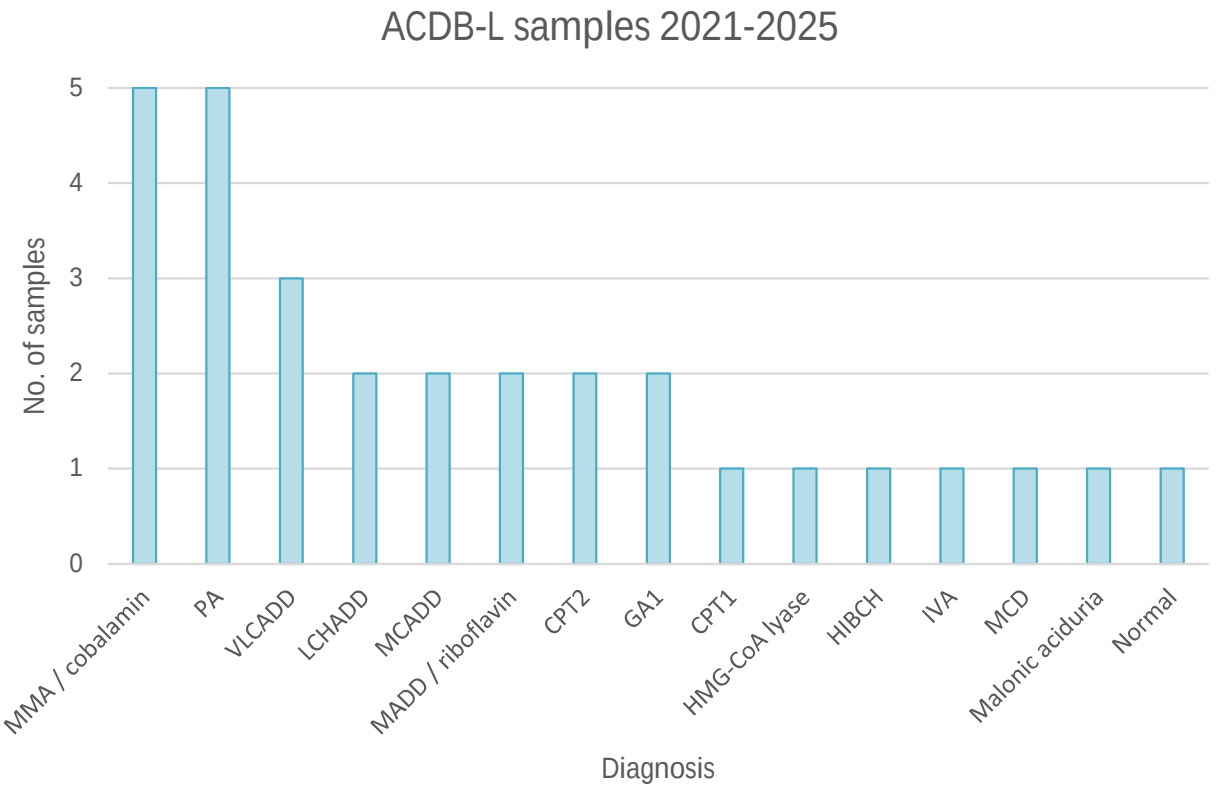
ERNDiM

Quality Assurance in Laboratory Testing for IEM

ACDB Participant's meeting 2026
London scheme

Erin Emmett

Diagnosis summary



- Primarily from treated and otherwise well patients with genetically confirmed diagnoses
- Thank you to other labs for donations over the years

Performance 2024 and 2025

2024						
	A	B	C	D	E	F
Diagnosis	CPT1	Riboflavin	GA1	VLCADD	PA	MCADD
Analytical prof	100	96.6	95.5	83.0	100	98.9
Interpretative prof	100	86.4	94.3	90.9	94.3	97.7
Overall prof	100	91.5	94.9	86.9	97.2	98.3
			(2 CE)		(1 CE)	(1 CE)

2025						
	A	B	C	D	E	F
Diagnosis	VLCADD	HIBCH *	IVA	MCADD	Cbl C	Malonic
Analytical prof	100	60.9	100	100	54.3	82.6
Interpretative prof	100	48.9	100	95.7	67.4	88.0
Overall prof	100	54.9	100	97.8	60.9	85.3
						(4 CE)

* Educational

Performance 2024 and 2025

2024						
	A	B	C	D	E	F
Diagnosis	CPT1	Riboflavin	GA1	VLCADD	PA	MCADD
Analytical prof	100	96.6	95.5	83.0	100	98.9
Interpretative prof	100	86.4	94.3	90.9	94.3	97.7
Overall prof	100	91.5	94.9	86.9	97.2	98.3
			(2 CE)		(1 CE)	(1 CE)

2025						
	A	B	C	D	E	F
Diagnosis	VLCADD	HIBCH *	IVA	MCADD	Cbl C	Malonic
Analytical prof	100	60.9	100	100	54.3	82.6
Interpretative prof	100	48.9	100	95.7	67.4	88.0
Overall prof	100	54.9	100	97.8	60.9	85.3
						(4 CE)

* Educational

2024.B – Riboflavin

Participants: 44

Diagnosis: Not known

Overall prof.: 91.5%

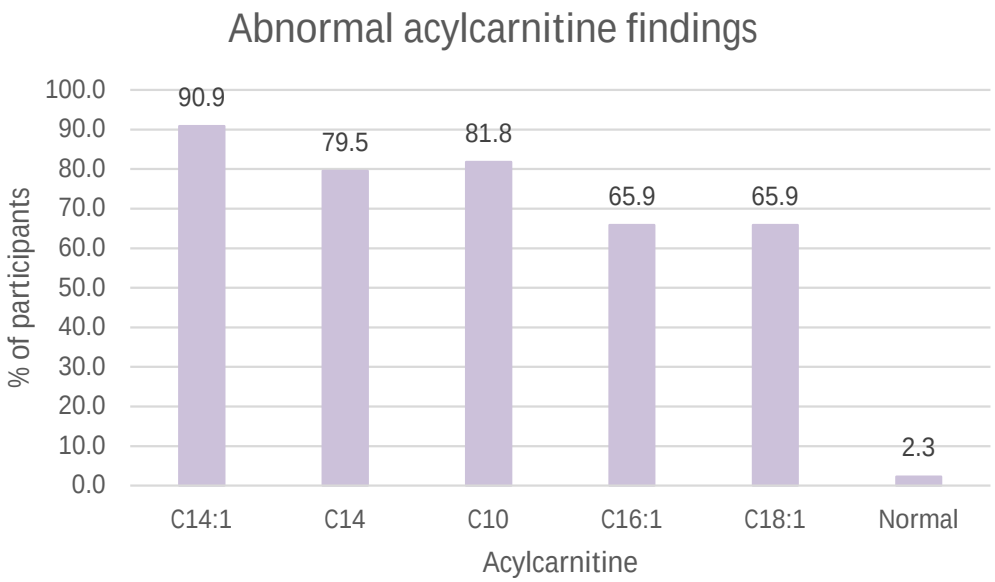
Patient details

Acute sample from a 33 year old female

Weakness with subacute degeneration of spinal cord

2024.B – Riboflavin

- Wide range of abnormalities, primarily in medium- and long-chain acylcarnitines
- Analytical scoring: 2 points for any abnormality in medium/long chains
- 42/44 participants scored 2 points



Analytical proficiency
96.6%

2024.B – Riboflavin

- Majority of participants suggested MADD/riboflavin disorder (23/44) or VLCADD (15/44) as the primary diagnosis
- Interpretative scoring:
  - 2 points MADD/riboflavin with recommendations
  - 1 point if mention another IMD but suggest appropriate follow-up tests
- Agreed no critical errors as there was/is no diagnosis in this patient

Alternative diagnoses	
Other FAOD (SCAD, MCAD, CPT2, CACT, ACAD9, TANGO2)	10
General B vitamin deficiencies / B12 / cobalamin	9
NO abuse	3
EMA encephalopathy / Jamaican vomiting sickness	2
Sertraline	2
CoQ10 deficiency	2
Secondary mitochondrial dysfunction	1
B12 deficiency causing low Hct and inaccurate calculation	1
MCT diet	1
Medication/dietary causes	1

Interpretative proficiency 86.4%

Recommendations	
Urine organic acids	35
Repeat DBS or plasma/urine acylcarnitine	26
B12 / MMA / tHcy / folate	21
MADD genetics	15
Referral including management and family testing	15
VLCAD genetics	14
General genetics (WGS, FAOD panel etc)	13
Riboflavin	11
Other enzyme analysis	6
Riboflavin transporter genetics	5
VLCAD enzyme in fibroblasts	3
Riboflavin challenge/responsiveness	3
Other routine Chemistry (e.g. CK, ammonia)	21

2024.B – Riboflavin

- Long complex clinical history, including chronic fatigue, deteriorating vision, sensory disturbance and an episode of metabolic acidosis
- The hospital admission was preceded by a significant worsening of symptoms over a few months including falls, weakness and inability to lift arms above her head. As well as subacute degeneration of the spinal cord, bilateral retinal ganglion pathology, peripheral neuropathy and proximal myopathy were also confirmed
- Started on riboflavin and B12
- Biochemical response: One week after supplementation, the bloodspot acylcarnitine and urine organic acid profiles had normalised
- Clinical response: Initial significant improvement in proximal muscle function and improved swallowing/eating. But ongoing rehabilitation required due to initial neurological injury
- Genetics: No pathogenic mutations on R98 gene panel

Most likely cause: Dietary riboflavin deficiency, ? Complicated by sertraline

2024.B – Riboflavin

Acta Neuropathologica (2024) 148:73
https://doi.org/10.1007/s00401-024-02830-x

ORIGINAL PAPER

Lipid storage myopathy associated with sertraline treatment is an acquired mitochondrial disorder with respiratory chain deficiency

Carola Hedberg-Oldfors[1] · Ulrika Lindgren[1,2] · Kittichate Visuttijai[1] · Yan Shen[1] · Andreea Ilinca[3,4] · Sara Nordström[2] · Christopher Lindberg[2] · Anders Oldfors[1]

▸ Mol Genet Metab Rep. 2024 Sep 16;41:101142. doi: 10.1016/j.ymgmr.2024.101142

MADD-like pattern of acylcarnitines associated with sertraline use

Filippo Ingoglia [a,b,*], Mohsen Tanfous [c], Benjamin Ellezam [d], Katherine J Anderson [e], Marzia Pasquali [a,c,f], Lorenzo D Botto [f]

Annals of NEUROLOGY
An Official Journal of the American Neurological Association & the Child Neurology Society
ANA AMERICAN NEUROLOGICAL ASSOCIATION THE HOME FOR ACADEMIC NEUROLOGY

Research Article | Open Access

Multiple Acyl-Coenzyme A Dehydrogenase Deficiency Is Associated with Sertraline Use – Is There an Acquired Form?

Sofie Sunebo MD, Hanna Appelqvist PhD, Bo Häggqvist PhD, Olof Danielsson MD, PhD

2024.F – MCADD

Participants: 44

Diagnosis: MCADD

Overall prof.: 98.3%

Patient details

Acute sample from a 4 day old male

Encephalopathy and respiratory distress. Metabolic acidosis, hypoglycaemia, high ketones

2024.F – MCADD

- 43/44 participants identified the medium-chain abnormalities and gave a diagnosis of MCADD (either primary or alternative)
- One critical error
- Despite the unusual clinical presentation, ketotic hypoglycaemia and the increased C4OH in the sample did not deter most participants from suggesting a potential fatty acid oxidation defect, with 43/44 participants scoring full marks

2025.B – HIBCH deficiency

Participants: 46

Diagnosis: HIBCH

Overall prof.: 54.9%

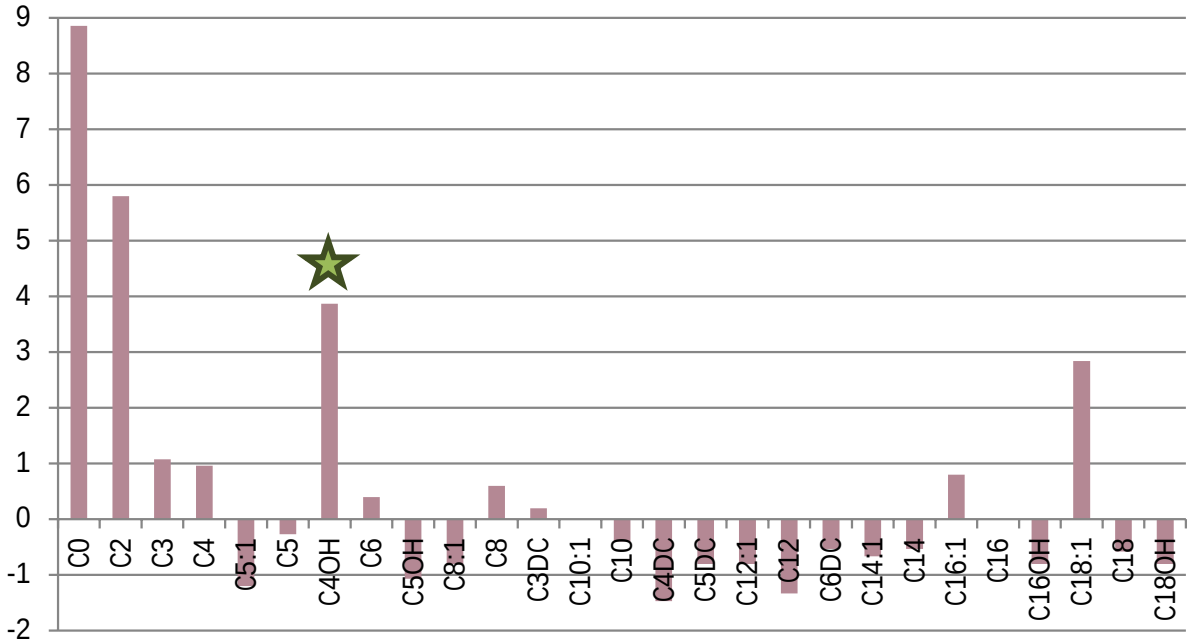
Patient details

Treated sample from a 4 year old male

Viral illness age 3 triggering metabolic decompensation and encephalopathy. Seizures, abnormal brain MRI. Now on carnitine

2025.B – HIBCH deficiency

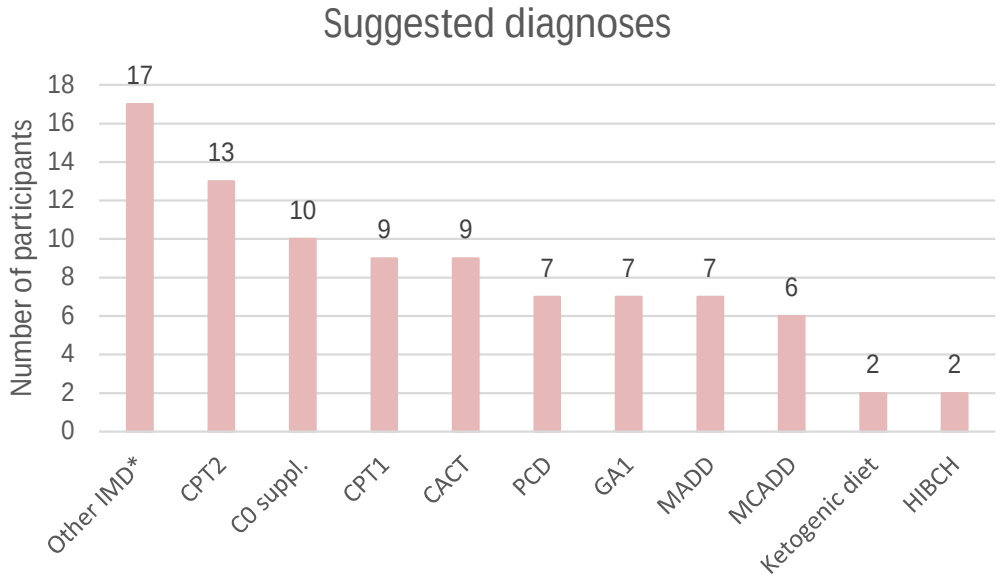
- 45/46 participants identified gross increase in free carnitine
  - As stated in the clinical information provided, patient is receiving carnitine
- 11/46 noted subtle increase in C4OH
- Analytical scoring:
  - 2 points for increased C0 and C4OH
  - 1 point for increased C0 (and any other abnormal acylcarnitines)



Analytical proficiency
60.9%

2025.B – HIBCH deficiency

- Interpretative scoring:
  - 2 points for HIBCH with appropriate recommendations
  - 1 point for any recommendations that could lead to the diagnosis (primarily urine organic acid analysis)
  - 1 point if only carnitine supplementation or CPT1 suggested (as clinical details clearly state 'now on carnitine') and no appropriate recommendations (i.e. UORG or referral)
- Two participants scored full marks

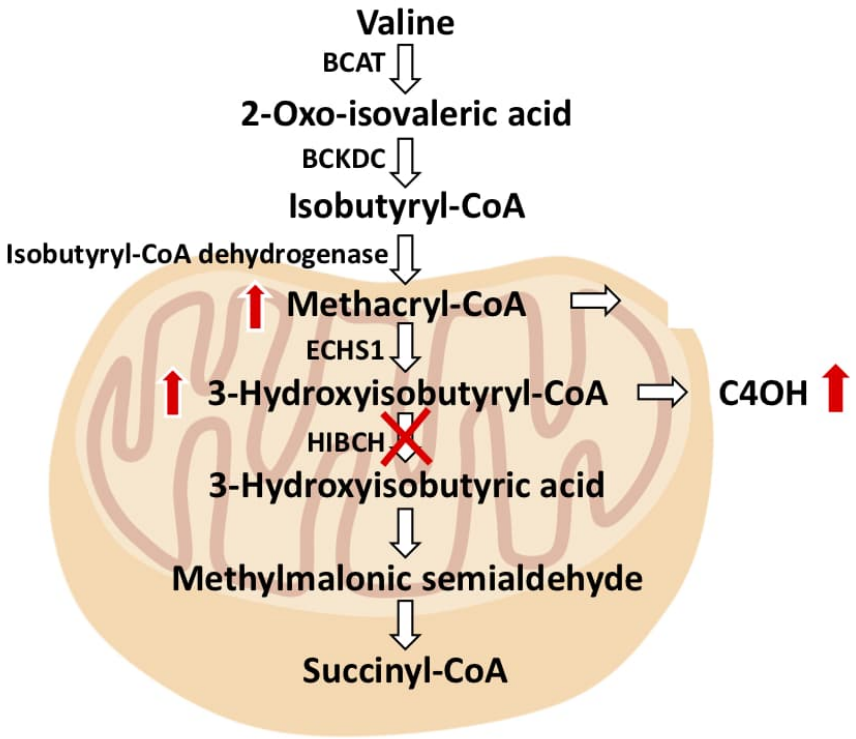


Interpretative proficiency 48.9%

*Other IMD – includes FAOD, ketogenesis defects, CoPAN and PA/MMA

2025.B – HIBCH deficiency

- HIBCH – 3-hydroxyisobutyryl-CoA hydrolase
- Deficiency results in Leigh-like disease and hypotonia
- Increased C4OH is often observed in HIBCH deficiency, but not in ECHS1 deficiency
- Compounds increased in both HIBCH and ECHS1 deficiencies can be seen on:
  - Urine organic acid analysis (2-methyl-2,3-dihydroxybutyrate)
  - Urine amino acid analysis (cysteine and cysteamine metabolites of methacrylyl-CoA and acryloyl-CoA)
  - Urine acylcarnitines (N-acetyl-S-(2-carboxypropyl)cysteine carnitine ester)



G K Brown et al, 1982. Pediatrics, 70(4):532-8
F J Loupatty et al, 2007. AJHG, 80:195-9
H Peters et al, 2015. Mol Genet Metab, 115(4):168-73

2025.E – Cbl C deficiency

Participants: 46

Diagnosis: Cbl C deficiency

Overall prof.: 60.9%

Patient details

Treated sample from a 11 year old female

Mild dev delay, diagnosed after younger sister investigated for seizures

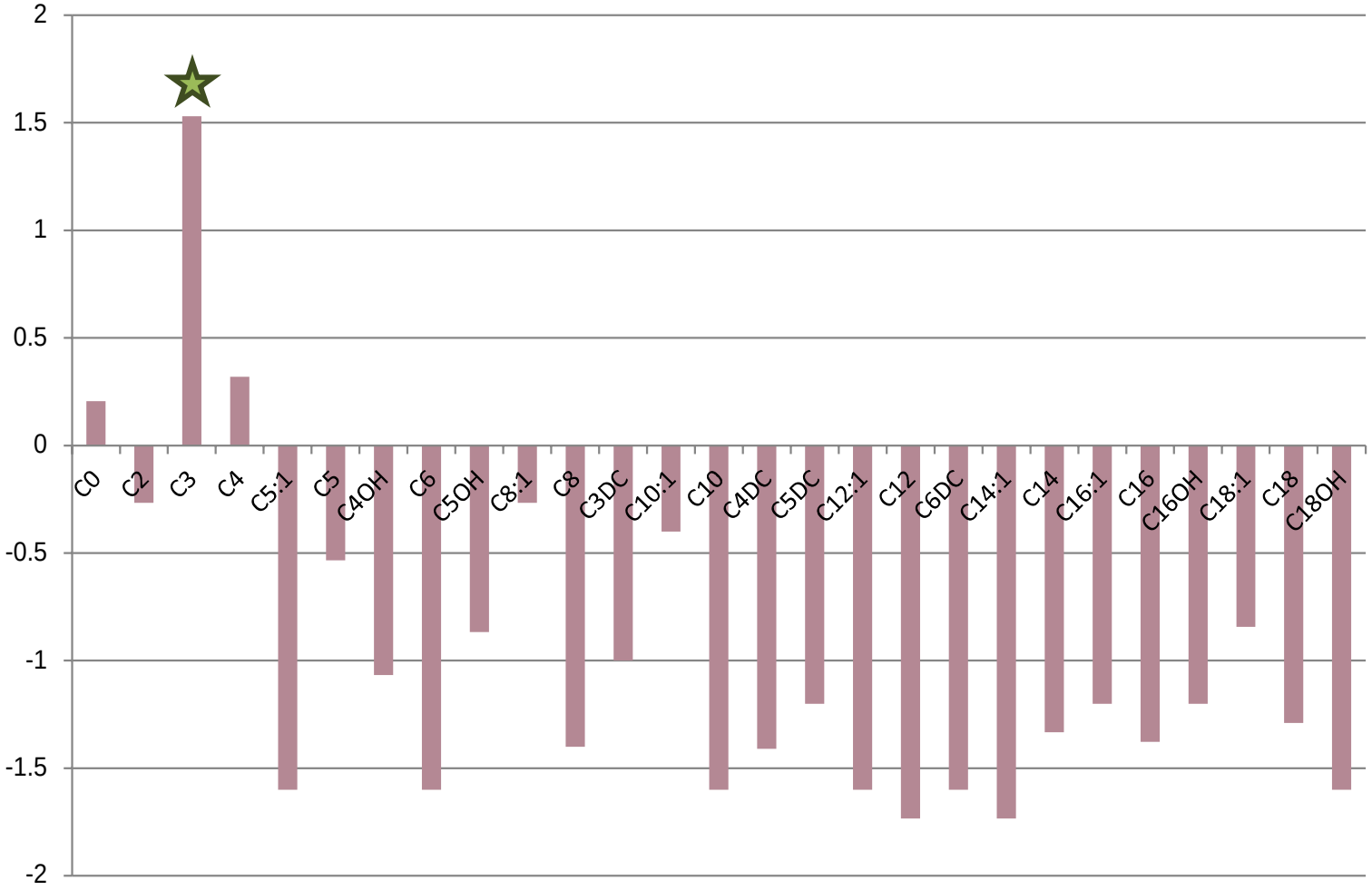
2025.E – Cbl C deficiency

- 26/46 participants identified the subtle abnormality in C3 and/or ratios
- 19/46 reported a normal profile
- 1/46 reported profile consistent with GA1
- Analytical scoring:
  - 2 points for increased C3 and/or appropriate ratios (e.g. C3/C2, C3/C16)

Acylcarnitines	n	Median value	Range
c3	23	2.36	1.36 - 3.02
c3/c2	18	0.275	0.16 - 0.35
c3/c16	9	4.18	3.09 - 6.21

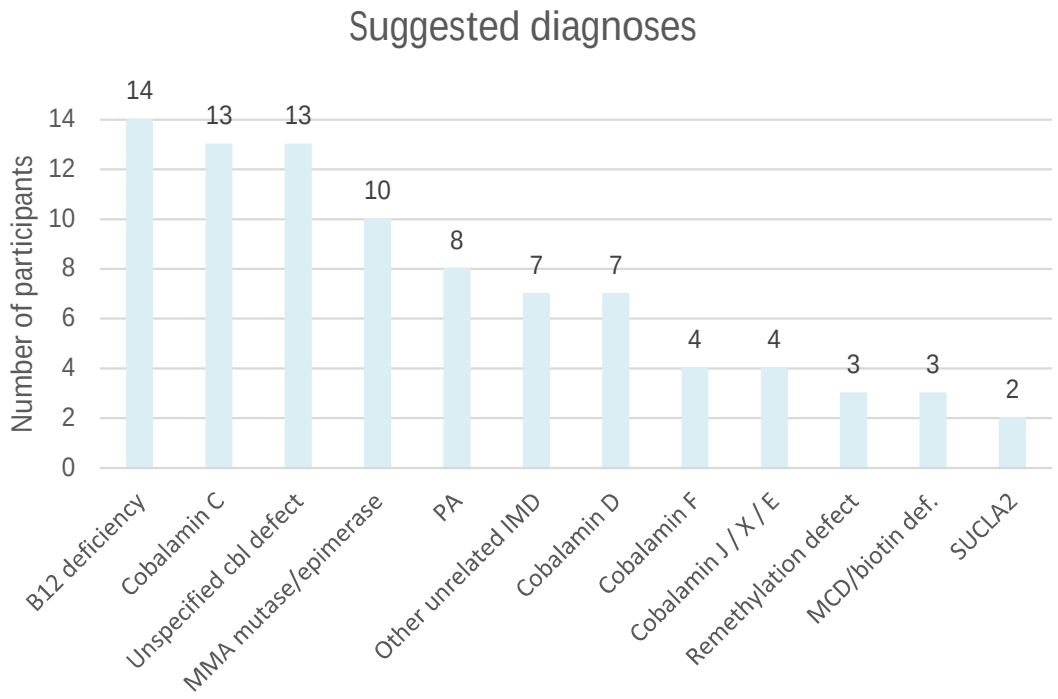
Analytical proficiency
54.3%

2025.E – Cbl C deficiency



2025.E – Cbl C deficiency

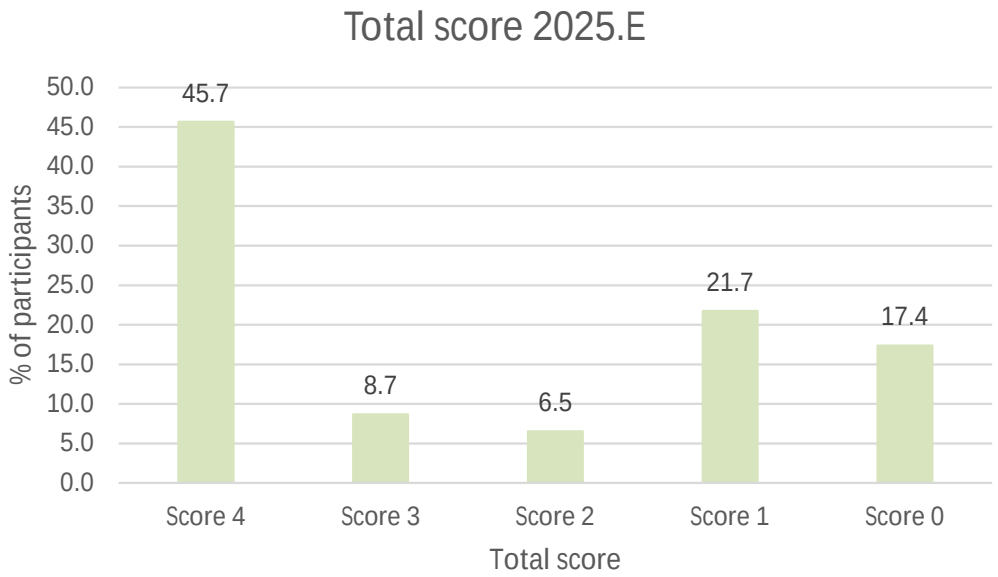
- Interpretative scoring:
  - 1 point MMA/PA/cobalamin or appropriate other diagnosis (as primary or alternative)
  - 1 point for appropriate recommendations (UORG, plasma MMA and tHcy, vitamin B12, relevant genetics, enzyme analysis)



Interpretative proficiency 67.4%

2025.E – Cbl C deficiency

- Agreed at the SAB not to give any critical errors due to the subtle abnormality and relatively low overall proficiency
- Correct diagnoses resulting from second-line analyses, including in this sample total homocysteine, score only one point



2025.F – Malonic aciduria

Participants: 46

Diagnosis: Malonic aciduria

Overall prof.: 85.3%

Patient details

Treated sample from a 14 year old female

Mild motor and speech delay and mild left ventricular hypertrophy aged 3

2025.F – Malonic aciduria

- 39/46 participants identified the increased C3DC (or C3DC+C4OH for methods not using derivatisation)
- All 39 suggested malonic aciduria as the diagnosis and scored the full 4 points
- 12/46 also suggested combined malonic and methylmalonic aciduria
- 7/46 did not identify the abnormality:
  - Based on the clinical details, 3 participants scored 1 as they suggesting urine organic acid analysis (which would enable identification of the correct diagnosis)
  - The remaining 4 suggested a normal profile and no recommendations, therefore were given critical errors

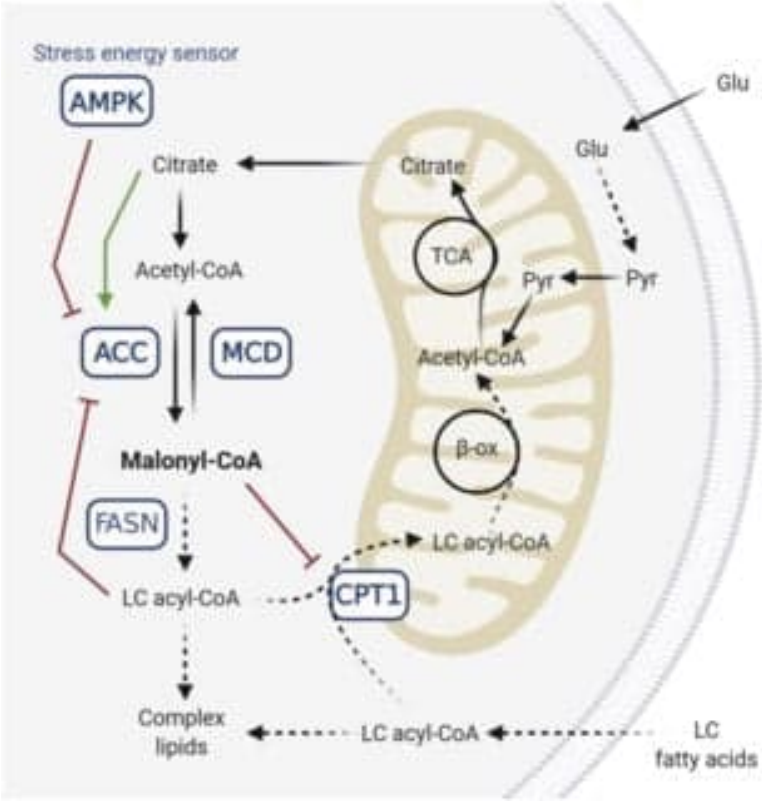
Acylcarnitines	n	Median value	Range
c3dc	39	0.575	0.18 - 4.49

Analytical proficiency 82.6%

Interpretative proficiency 88.0%

2025.F – Malonic aciduria

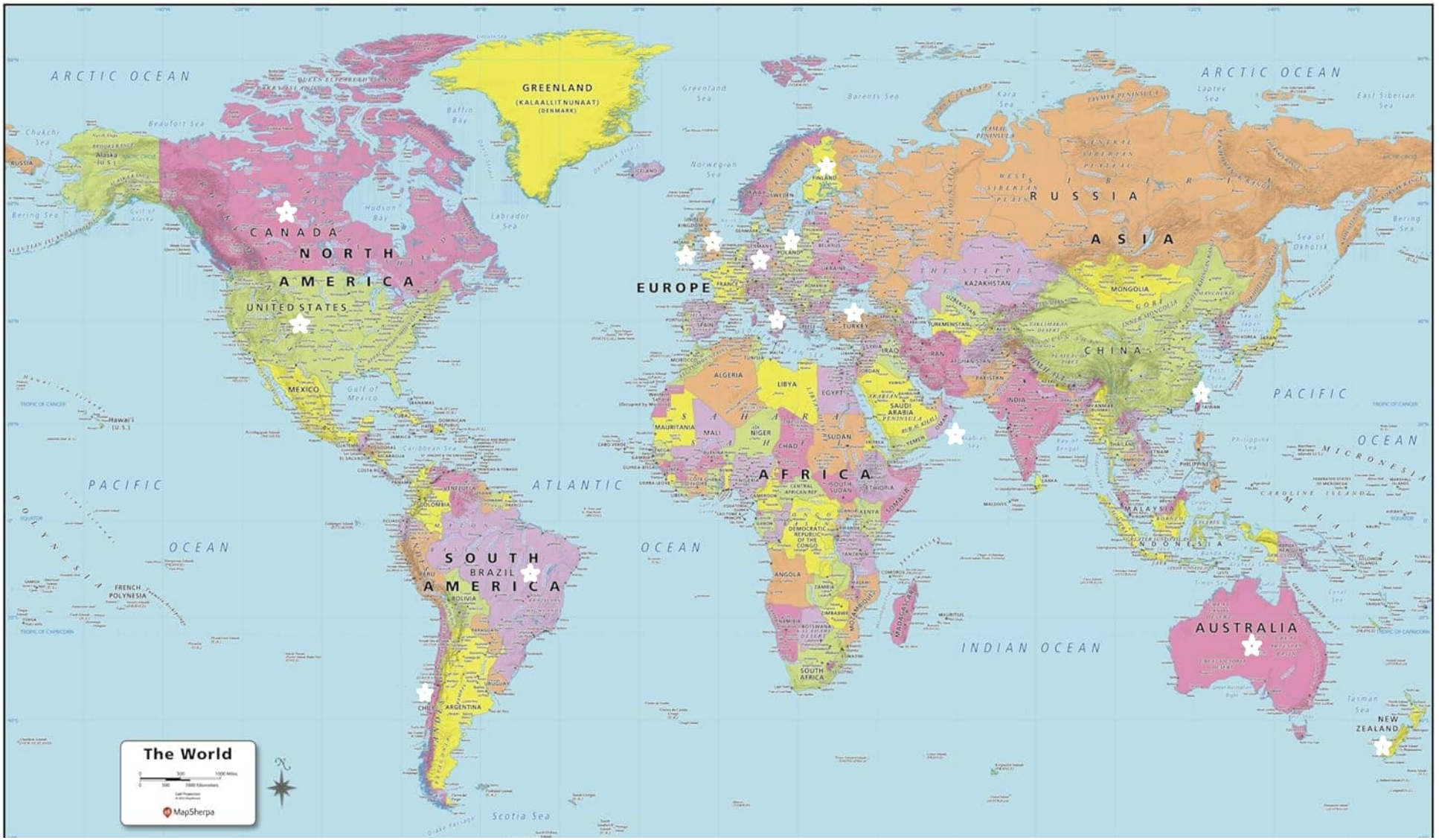
- Malonyl-CoA decarboxylase deficiency (*MLYCD*)
- Developmental delay
- Hypotonia, seizures, hypoglycaemia
- Cardiomyopathy
- Other causes of increased C3DC:
  - Renal dysfunction (dicarboxylics, short- and medium-chains)
  - Exogenous contamination
  - Combined malonic and methylmalonic aciduria (*ACSF3*)



> Pediatrics. 2012 Nov;130(5):e1363-8. doi: 10.1542/peds.2012-0569. Epub 2012 Oct 15.

Sani-cloth wipe mimics rare enzyme deficiency malonic aciduria on newborn screen

Bailey A Reindl [1], Douglas W Lynch, Michael Ramirez, Marcia Valbracht, Laura Davis-Keppen, Kimberlee C Tams, Sarah Groeneveld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Thank you for your participation in
the ACDB schemes and
for attending the workshop