Decreased plasma citrulline

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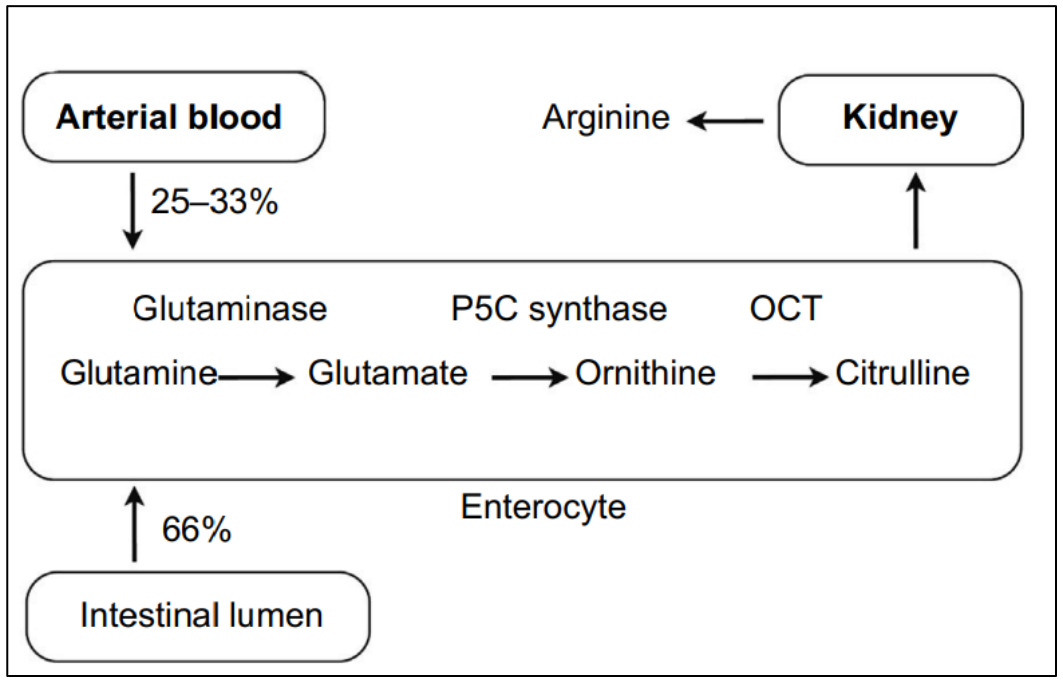
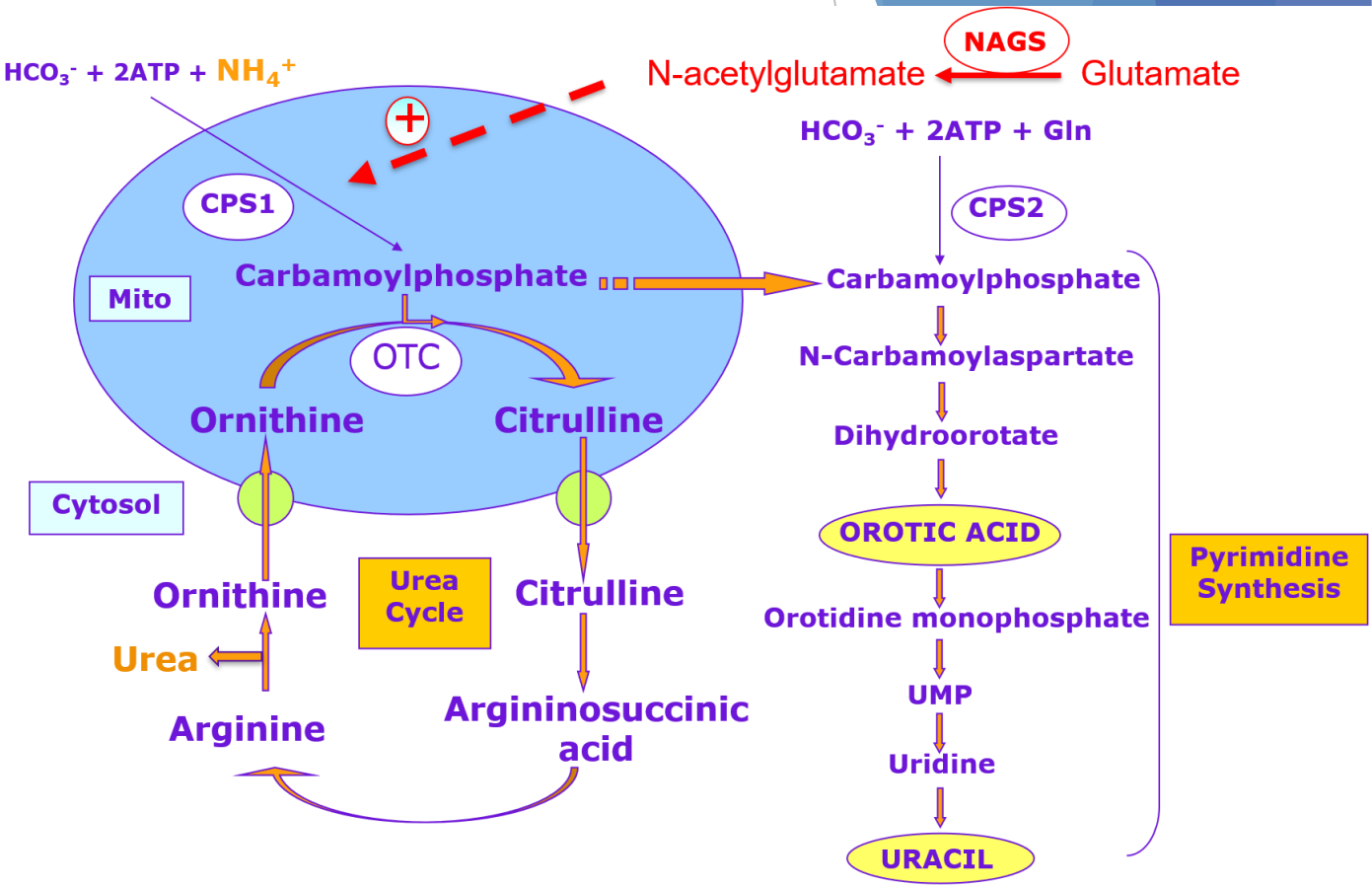
Decreased plasma citrulline – causes:

- Urea cycle defect (OTC / CPS1 / NAGS)
- Compromised small bowel function
- Generalised decrease in amino acids





Peters et al 2017 Clinical and Experimental Gastroenterology 2017:10 75–81

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661–668 (2023)

the Clinical Chemist

Genetic Metabolic Series

Low Plasma Citrulline Guiding the Diagnosis of a Mitochondrial Disorder

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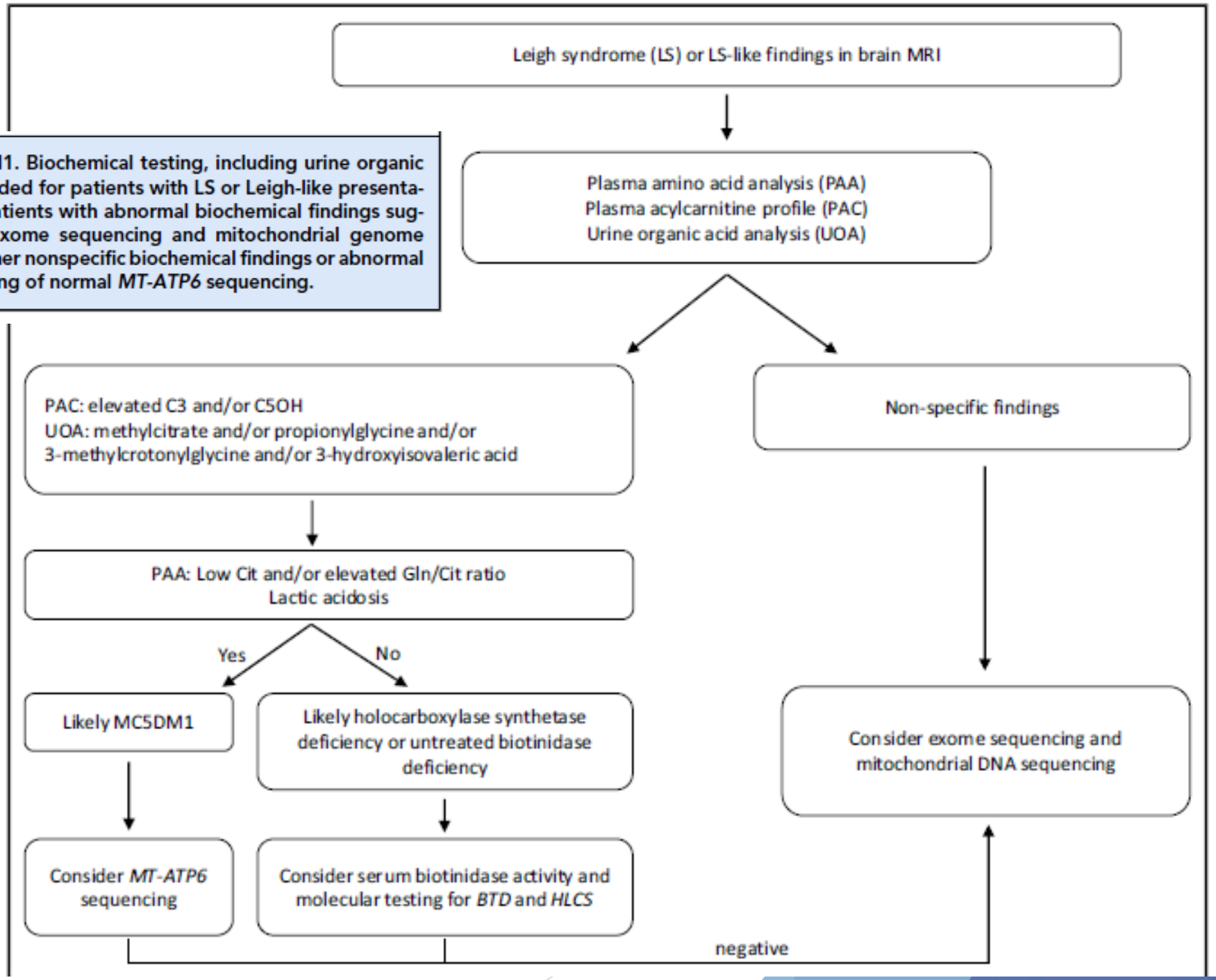


Fig. 2. Recommended diagnostic algorithm for MC5DM1. Biochemical testing, including urine organic acid, plasma amino acid and PAC analyses, is recommended for patients with LS or Leigh-like presentation. Sequencing of *MT-ATP6* is recommended in the patients with abnormal biochemical findings suggestive of MC5DM1, while broader tests, including exome sequencing and mitochondrial genome sequencing, are recommended for patients who have either nonspecific biochemical findings or abnormal biochemical findings suggestive of MC5DM1 in the setting of normal *MT-ATP6* sequencing.

Plasma amino acids

Amino acids [Plasma]

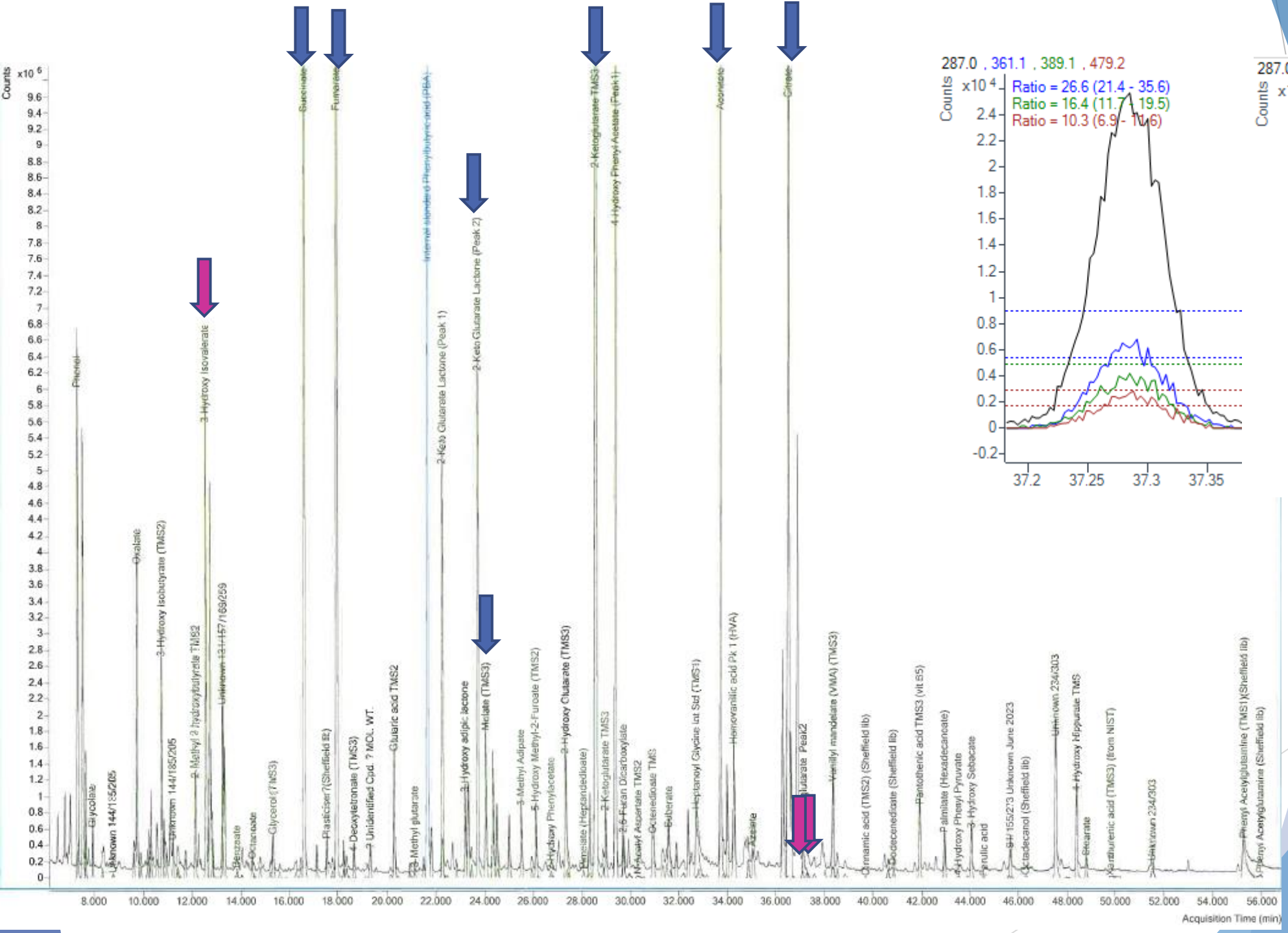
Taurine	43	umol/L	(28-212)	Valine	193	umol/L	(77-335)
Aspartic acid	8	umol/L	(4-52)	Cystine	27	umol/L	(10-80)
Hydroxyproline	20	umol/L	(6-75)	Methionine	18	umol/L	(11-43)
Threonine	181	umol/L	(47-228)	Isoleucine	**110 H**	umol/L	(27-101)
Serine	139	umol/L	(66-231)	Leucine	151	umol/L	(52-206)
Asparagine	44	umol/L	(23-80)	Tyrosine	47	umol/L	(32-125)
Glutamic acid	74	umol/L	(21-194)	Phenylalanine	42	umol/L	(35-103)
Glutamine	574	umol/L	(227-735)	Ornithine	41	umol/L	(24-144)
Proline	163	umol/L	(98-429)	Lysine	134	umol/L	(70-259)
Glycine	171	umol/L	(133-455)	Histidine	52	umol/L	(42-108)
Alanine	**646 H**	umol/L	(138-565)	Tryptophan	73	umol/L	(19-107)
Citrulline	**6 L**	umol/L	(7-48)	Arginine	28	umol/L	(12-145)

Urine organic acids

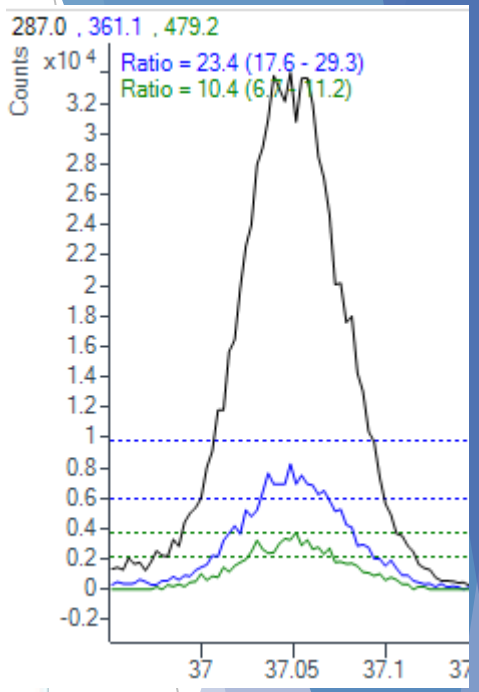
Organic acids [Urine]

Organic acids [Urine] Comment

Marked increases in TCA cycle intermediates (succinate, fumarate, malate and 2-ketoglutarate). Suggest further investigation to exclude a mitochondrial disorder in this patient.


Counts
x10 6
Succinate
Fumarate
3-Hydroxy Isovalerate
2-Keto Glutarate Lactone (Peak 1)
2-Keto Glutarate Lactone (Peak 2)
2-Ketoglutarate TMS3
4-Hydroxy Phenyl Acetate (Peak 1)
Apomorphine
Citrate
Phenol
Glycolate
Oxalate
3-Hydroxy Isobutyrate (TMS2)
Unknown 144/185/205
2-Methyl 3-hydroxybutyrate TMS2
Unknown 131+157/169/259
Benzoate
Octanoate
Glycerol (TMS3)
Plasticizer? (Sheffield lib)
4-Deoxytetronate (TMS3)
? Unidentified Cpd. ? MOL. WT.
Glutaric acid TMS2
2-Methyl glutarate
Internal standard Phenylbutyric acid (PBA)
3-Hydroxy adipic lactone
Malate (TMS3)
3-Methyl Adipate
5-Hydroxy Methyl-2-Furoate (TMS2)
2-Hydroxy Phenylacetate
2-Hydroxy Glutarate (TMS3)
Benzoate (Heptanedioate)
2-Ketoglutarate TMS3
2,5-Furan Dicarboxylate
N-Acetyl Aspartate TMS2
Octenedioate TMS
Suberate
Heptanoyl Glycine Int Std (TMS1)
Homovanillic acid Pk 1 (HVA)
Azelaate
Glutarate Peak2
Vanillyl mandelate (VMA) (TMS3)
Cinnamic acid (TMS2) (Sheffield lib)
Dodecenedioate (Sheffield lib)
Pantothenic acid TMS3 (vit B5)
Palmitate (Hexadecanoate)
4-Hydroxy Phenyl Pyruvate
3-Hydroxy Sebacate
Ferulic acid
1-Hexadecanol (Sheffield lib)
Unknown 234/303
4-Hydroxy Hippurate TMS
Stearate
Xanthurenic acid (TMS3) (from NIST)
Unknown 234/303
Phenyl Acetylglutamine (TMS1) (Sheffield lib)
Phenyl Acetylglutamine (Sheffield lib)
8.000
10.000
12.000
14.000
16.000
18.000
20.000
22.000
24.000
26.000
28.000
30.000
32.000
34.000
36.000
38.000
40.000
42.000
44.000
46.000
48.000
50.000
52.000
54.000
56.000
Acquisition Time (min)

287.0 , 361.1 , 389.1 , 479.2
Counts
x10 4
Ratio = 26.6 (21.4 - 35.6)
Ratio = 16.4 (11.7 - 19.5)
Ratio = 10.3 (6.9 - 11.6)
37.2
37.25
37.3
37.35


287.0 , 361.1 , 479.2
Counts
x10 4
Ratio = 23.4 (17.6 - 29.3)
Ratio = 10.4 (6.7 - 11.2)
37
37.05
37.1

Plasma acylcarnitines

Plasma acylcarnitine profile:
Tandem Mass Spectrometric Analysis of Acylcarnitines

Free Carnitine	38	umol/L	15-53
C3	4.29	umol/L	<1.30
C5-OH	0.14	umol/L	<0.06

Biotinidase

Biotinidase

Biotinidase	10.9	U/L	(5.0-14.9)

Biotinidase activity analysed Spectrophotometrically.
Normal biotinidase activity detected.

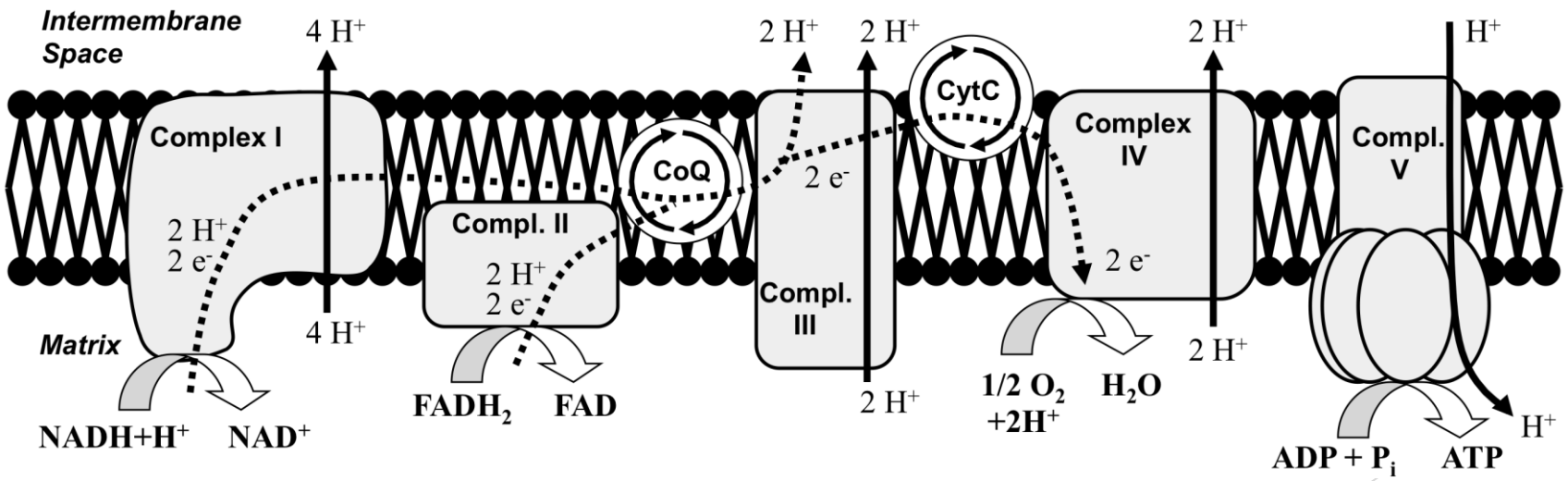
- NB: Doesn’t exclude holocarboxylase synthetase deficiency

Summary of biochemistry MT-ATP6

	Plasma lactate (mmol/L)	Plasma citrulline (umol/L)	Plasma alanine (umol/L)	Organic acid increases	Acylcarnitine increases	Biotinidase
CASE 1	6.8	6	646	TCA cycle (3OH-IVA)	C3 & C5OH	Normal
CASE 2	4.8 - 5.3	4 & 0	793 & 739	3OH-IVA	C5OH	Normal
CASE 3	5.9	4 & 4	517 & 618	TCA cycle & 3OH-IVA	C3 & C5OH	Normal

MT-ATP6 & MC5DM1

- MT-ATP6 encodes subunit of Complex V of respiratory chain = ATP synthase
- MC5DM1: Mitochondrial complex V mitochondrial type 1 (MT-ATP6)
- MC5DM2: Mitochondrial complex V mitochondrial type 2 (MT-ATP8)
- Ganetsky et al 2019: 218 published cases of MT-ATP6 + 14 new cases
- m.8993T>G variant most prevalent (>100 cases)
- 18 other pathogenic variants described



MC5DM1 – Clinical presentation

- Developmental delay
- Epilepsy
- Cardiomyopathy
- Neuropathy
- Ataxia
- Retinitis pigmentosa
- Brain MRI abnormalities consistent with Leigh syndrome

- Variation in severity:

  Infantile-onset Leigh syndrome → adult-onset progressive ataxia
- Dependent on genotype & heteroplasmy

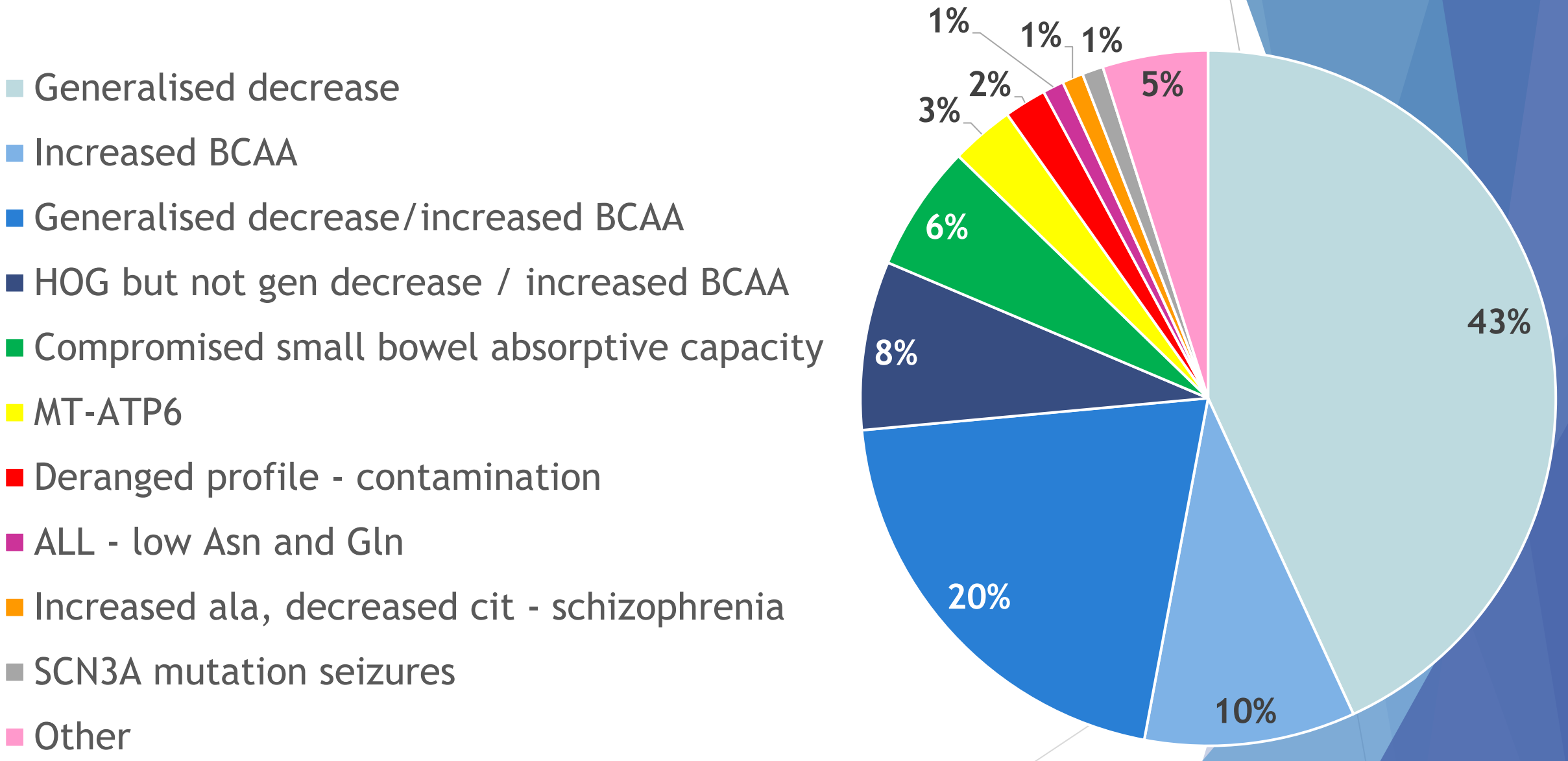
Mechanism for biochemical abnormalities

- Larson et al 2019: ? Secondary deficiencies in ATP-dependent enzymes:
  - CPS1
  - Δ_1-pyrroline-5-carboxylate synthetase
  - Biotin-protein ligase
  - Propionyl-CoA carboxylase
  - 3-Methylcrotonyl carboxylase

Review of decreased citrulline results

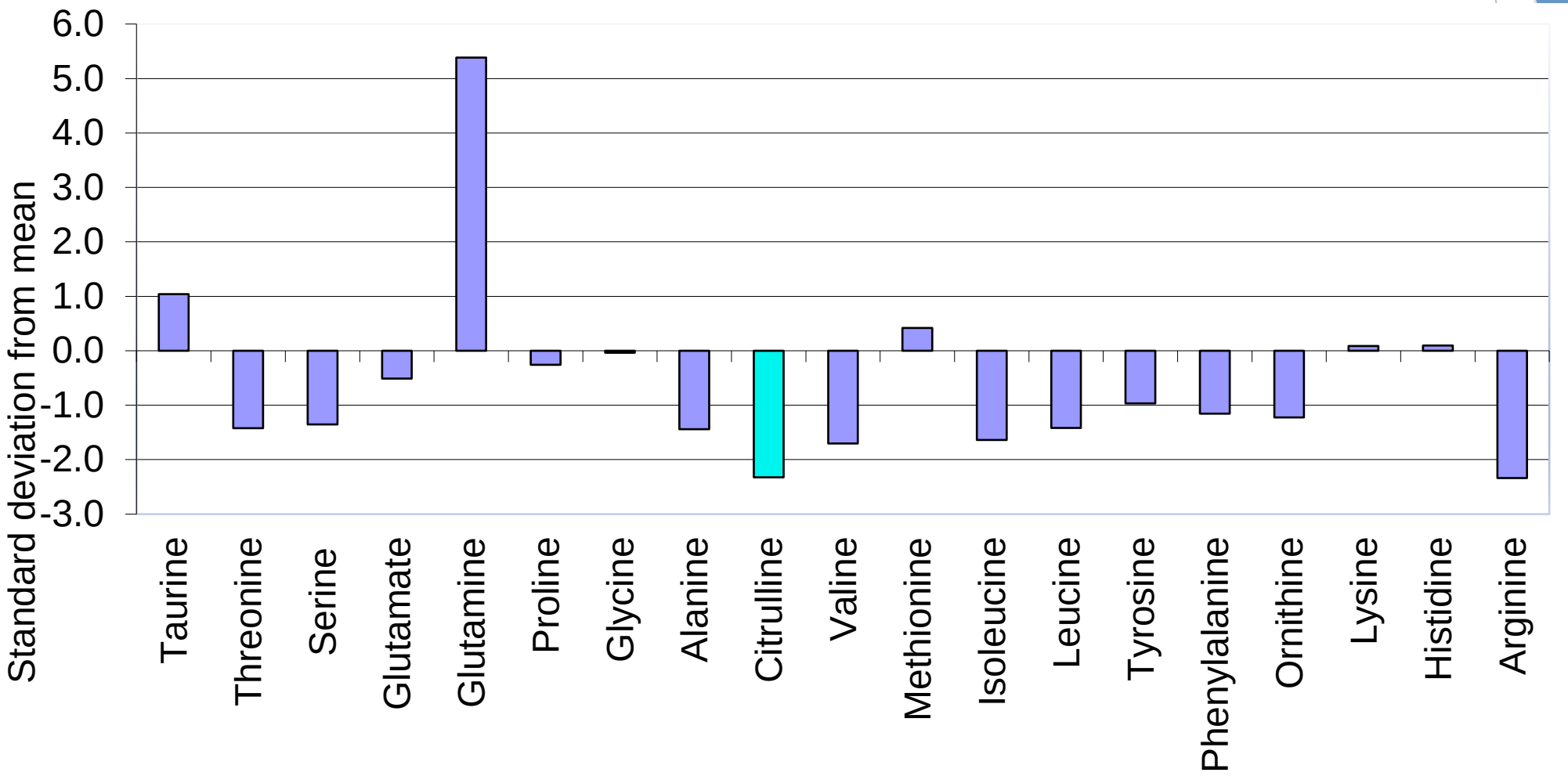
- 3459 plasma amino acid profiles
- 102 citrulline (3%) <LLN of reference range (<7 umol/L)
- 63 (62%) from HYPOGLYCAEMIA SCREENS

Plasma samples with decreased citrulline concentration



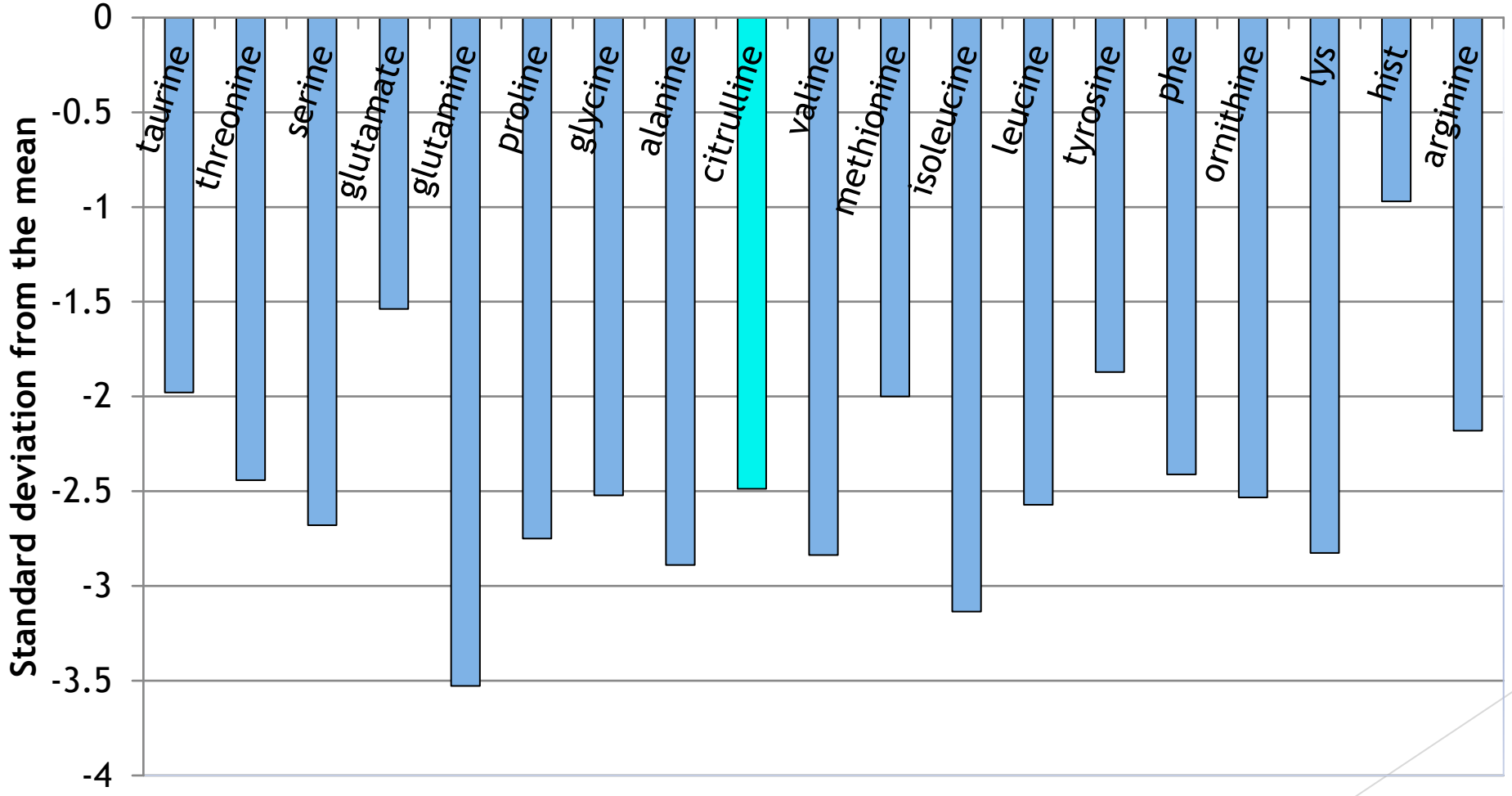
Use of SD plots to interpret low citrulline

Citrulline
2 µmol/L



OTC
Deficiency

Use of SD plots to interpret low citrulline

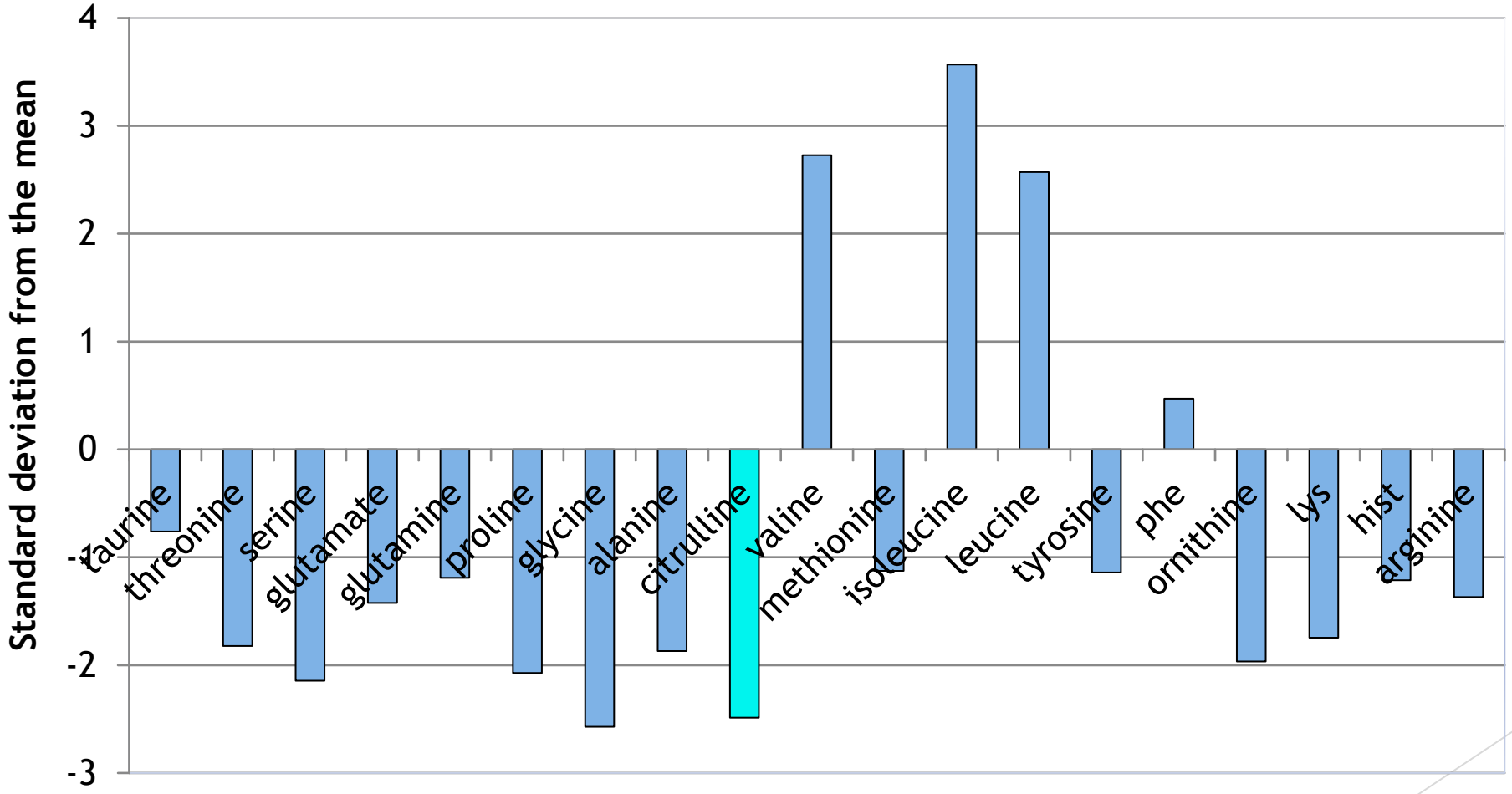


Citrulline
2 µmol/L

Generalised decrease

Use of SD plots to interpret low citrulline

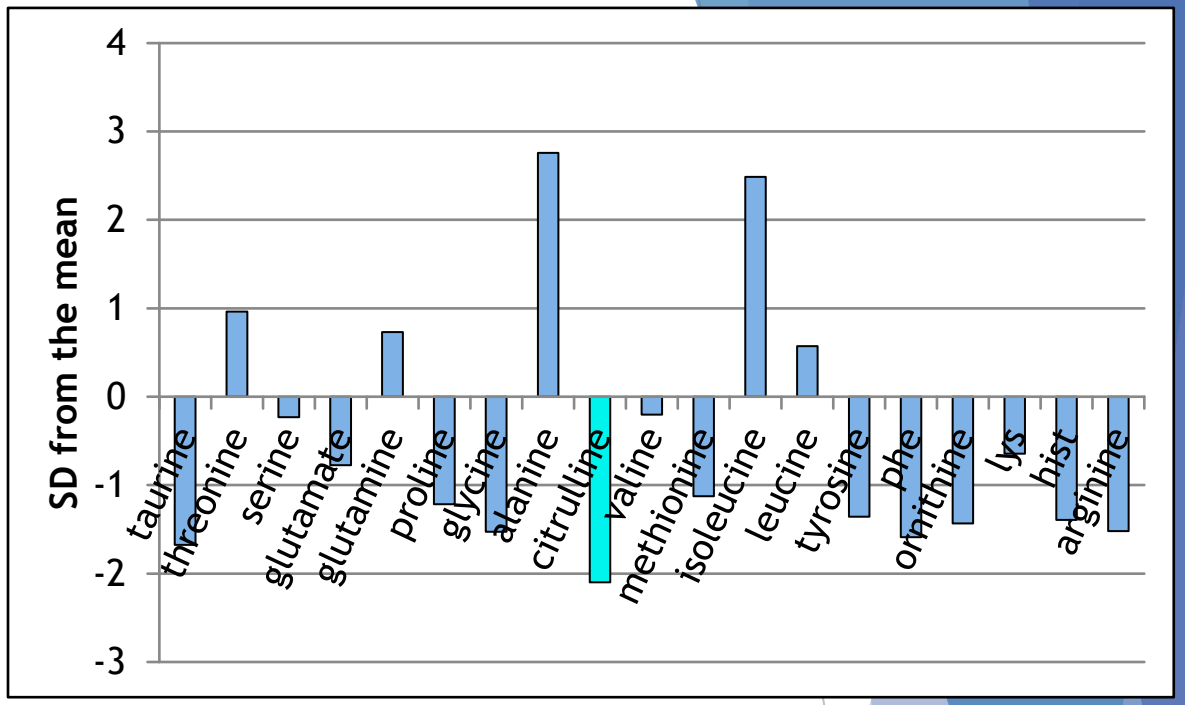
Citrulline
2 µmol/L

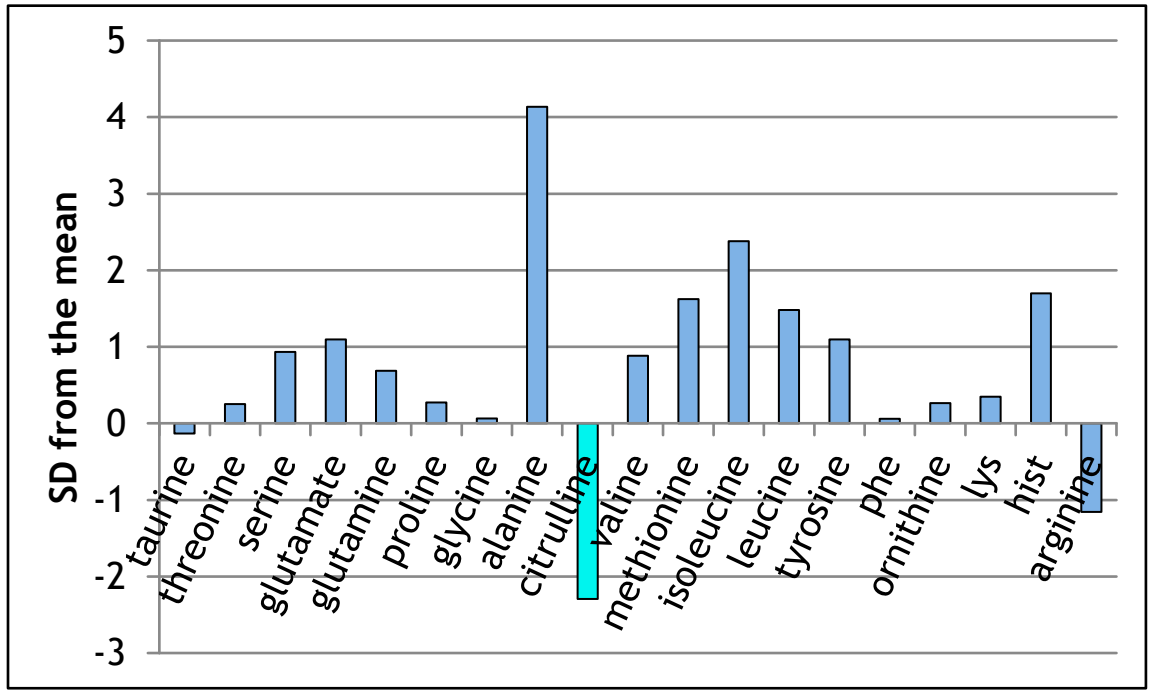


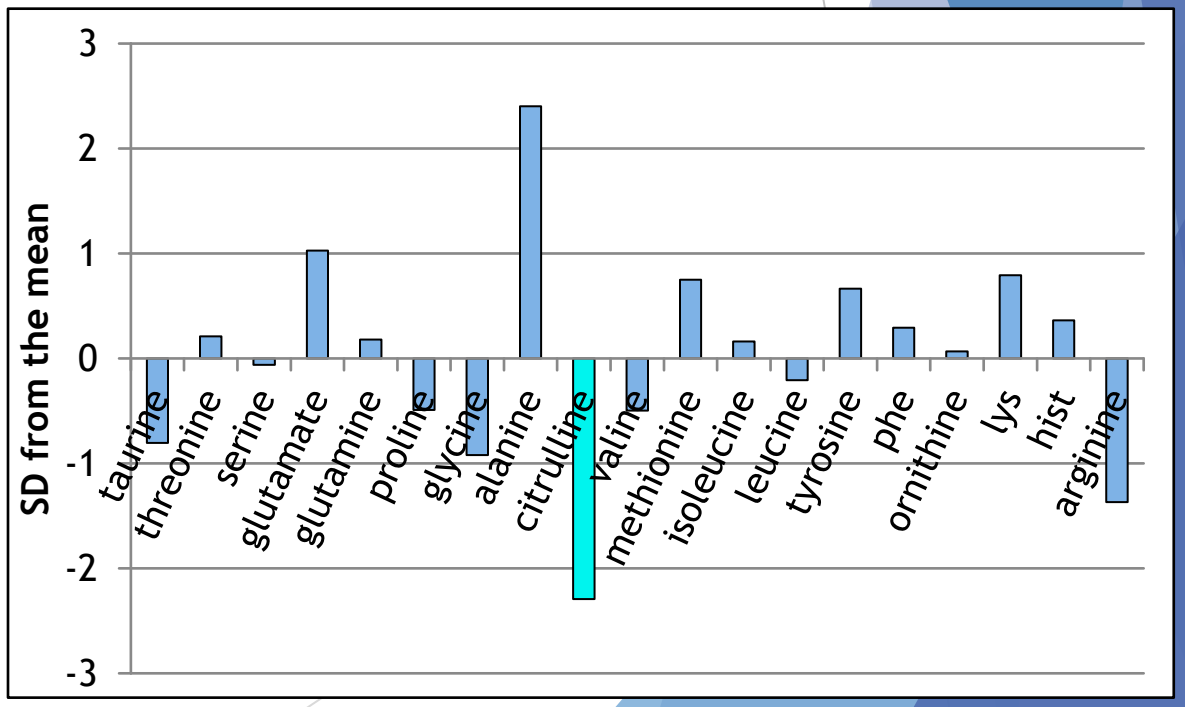
Generalised decrease with increased BCAA (Ketotic)

MT-ATP6 cases

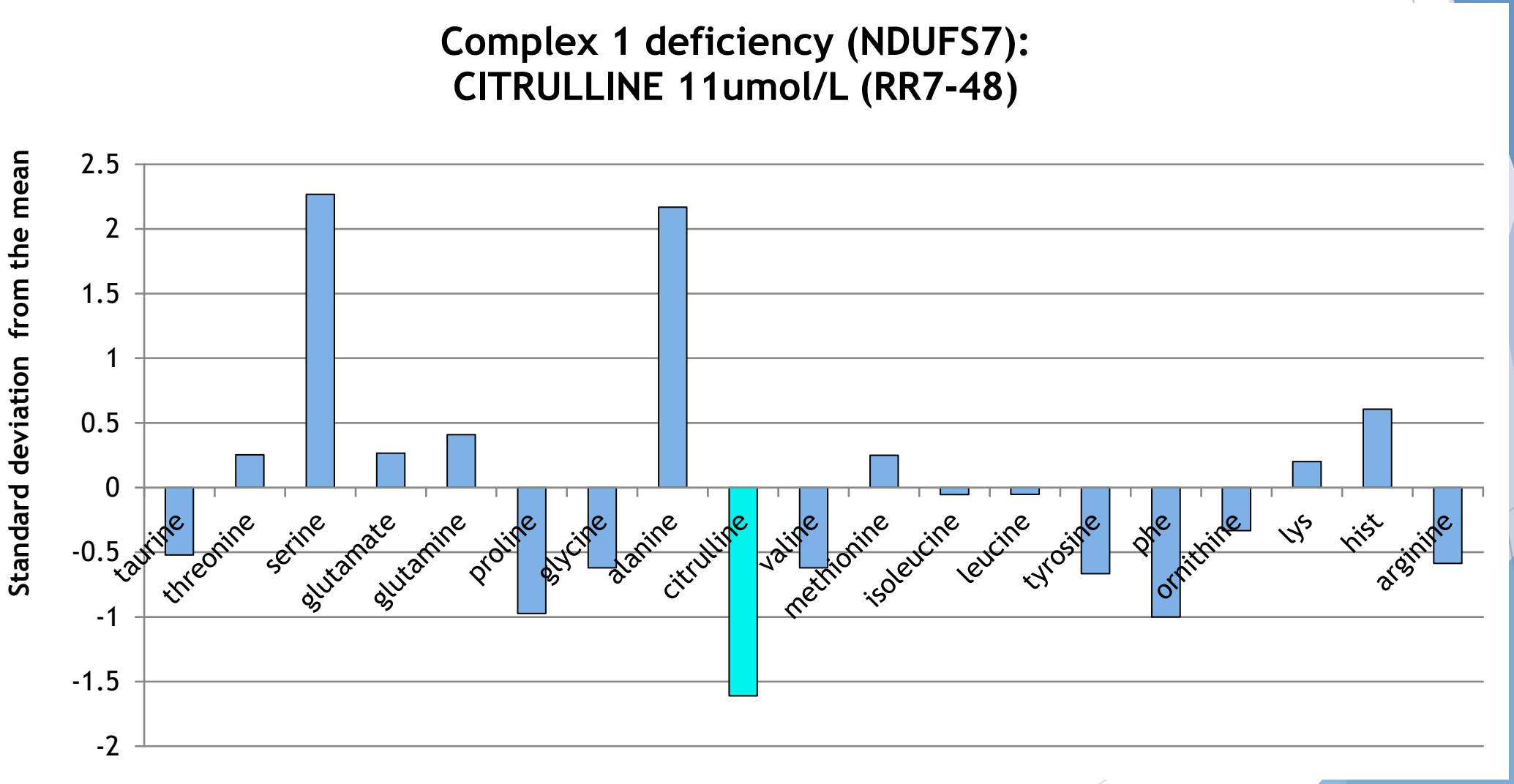
- No generalised decrease
- Citrulline decreased
- Alanine increased 2° to lactic acidosis







Is decreased citrulline present in all mitochondrial / respiratory chain disorders?



Summary

- Think MC5DM1 due to MT-ATP6 if you see:

Lactate	Plasma citrulline	Plasma alanine	Organic acid increases	Acylcarnitine increases	Biotinidase
↑	↓	↑	TCA +/- 3OH-IVA +/- Me.cit	C3 +/- C5OH	Normal

- SD Plots can be useful to highlight relative decreases (or increases)
- ? Other respiratory chain disorders / secondary mitochondrial dysfunction

References

- Peters et al Intravenous citrulline generation test to assess intestinal function in intensive care unit patients. Clinical and Experimental Gastroenterology 2017:10 75–81
- Ganetsky et al *MT-ATP6* Mitochondrial Disease Variants: Phenotypic and biochemical features analysis in 218 published cases and cohort of 14 new cases. Hum Mutat 2019; 40(5): 499–515.
- Capiau et al. Clinical Heterogeneity in MT-ATP6 Pathogenic Variants: Same Genotype—Different Onset. Cells 2022; 11: 489.
- Wongkittichote et al. Low Plasma Citrulline Guiding the Diagnosis of a Mitochondrial Disorder. Clin Chem 2023; 69(6): 661-668
- Larson et al. Biochemical signatures mimicking multiple carboxylase deficiency in children with mutations in MT-ATP6. Mitochondrion 2019; 44: 58-64

Thank you!