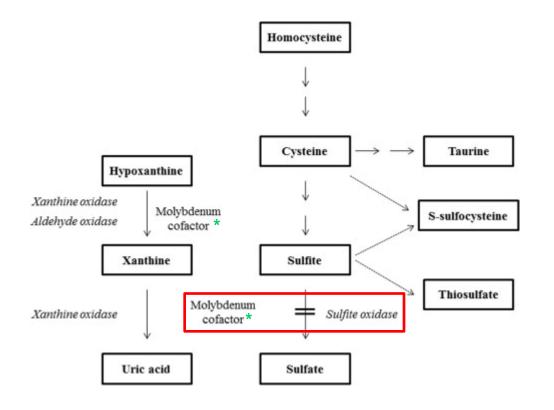
A Short Case Study – Sulphite Oxidase Deficiency picked up on Amino Acid Analysis

MetBio.Net BMS Training Group Annual Conference

14th November 2019

Metabolic Derangement:

• Autosomal recessive disorder caused by mutations in SUOX gene which encodes the sulphite oxidase enzyme.



* Sulphite oxidase requires Molybdenum co-factor which can also be defective in another metabolic disorder (Molybdenum co-factor deficiency (MoCo)).

Clinical Presentation:

- Neonatal:
 - Poor feeding
 - Hypotonia
 - Seizures
 - Followed by spasticity and severe development impairment
- Childhood:
 - Movement disorder
 - Stroke
 - Developmental regression

Diagnostic Tests:

- Plasma amino acids: ↑ Tau, Sulphocysteine, ↓ Cys
- Plasma: ↓ Total Homocysteine, (↓ Urate in MoCo)
- Urine sulphite: Positive on dipstick (fresh urine sample required)
- Urine purine screen (xanthine elevated) in MoCo
- Genetic analysis

Treatment and Prognosis:

- Poor prognosis for neonatal onset cases
- Low cysteine and methionine diet may help patients with mild form of sulphite oxidase deficiency

Ion Exchange Chromatography (IEC):

- Traditionally IEC referred to as the gold standard methodology.
- Routinely used in clinical laboratory.
- Cation exchange chromatography with post column ninhydrin detection
 - Stepwise elution with a series of lithium citrate buffers of increasing pH and ionic strength.
 - More acidic species elute first
- Dual detection:
 - 570nm (purple)
 - 440nm (yellow): better for detection of imino acids (proline, hydroxyproline)



Ion Exchange Chromatography (IEC):

Standard: IS IS 570nm 340 885 DILCAL 340 570nm 20190507_885(V35)885 DILCAL.dat Name Retention Time 320 22 320 Taurine 5.733 Glycine 37.764 38.931 300 49.830 300 1-Methyl Histid 79.595 Histidine 80.661 aminoadipic 35.098 inle.21.630.30 56.863 59.630 53.397 Citrulline 40.397 aminobutyric 41.564 Cystine 47.697 Threonine 22.065 Serine 23.498 Hist 83.328 280 280 Alanine 26.665 nic 28.198 ine 29.731 Phenyalan 60.663 Arginine 91.227 **Notts** NH3 69.962 260 43.997 -Mai Volts 260 64.963 ∢ ne 67.129 Glutamine ne 87.928 agine sobutyric (85.261 B alanine 57,963 240 Gaba ē 240 32.698 0H Proline 20.099 e Carnosir 8 100 7.833 220 36. 뛾 220 Sarcosin 33.998 364 oline B Urea 9 9 200 200 ÷. 180 180 0 10 🖉 15 20 25 30 35 40 45 £30 85 🖌 90 AD 65 70 75 80 Minute 285 DE CAL 20.099 632 440nm 40190507 885/V35/885 Dil CAL dat 120 22.032 49.830 Nam 28. 120 60.663 60.663 Reto 23,498 tion Time OH Proline Histidine 80.661 29.698 33 Isparagine ġ Methionine 83.328 32.698 74.329 110 43.997 rine 110 57.96 8 91.227 85.261 87.928 nVolts AEC. 69.929 033 100 Val 63.263 100 g Arginine NH3 B 8 90 90 80 80 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90

Ion Exchange Chromatography (IEC):

Standard:

10	w aminoadibic	200 CAL 35.1	1862160 4.97
16	Proline	200 CAL 36.7	52748 0.09
17	Glycine	200 CAL 37.8	
18	Alanine	200 CAL 38.9	
19	Citrulline	200 CAL 40.4	1966673 6.57
20	A aminobutyric	200 CAL 41.6	2130421 4.94
21	Valine		2023232 6.04
22	- GALLIC	200 CAL 44.0	1861393 6.18
23	Cystine	0 46.9	29809
24	CASCIUS	100 CAL 47.7	1224033 1.91
25	N	0 48.4	10009
26	Methionine	200 CAL 49.8	2075141 5.43
	Cystathionine-1	100 CAL 51.3	809876 3.43
27	Cystathionine-2	100 CAL 51.5	1279892 4.06
28	Isoleucine	200 CAL 53.4	
29	Leucine	200 CAL 54.3	
30	Norleucine	200 CAL 55.5	2114650 5.35
31	Tyrosine	200 CAL 56.9	2058942 5.48
32	Balanine		2113141 5.23
33	Phenyalanine	200 CAL 58.0	437570 4.00
34	B	200 CAL 59.6	2051648 5.56
54	-	200 CAL 60.7	493377 5.96
35	aminoisobutyric		
- +	Homocystine	200 CAL 63.3	3909184 3.39
36	Gaba 🥻	200 CAL 65.0	1335058 4.05
Page 3 of 7			1333038 4.03

Use channel ratio (570nm:440nm) for all standard compounds to compare against patient values to confirm where peaks are coeluting with interfering substances or presence of unusual (atypical) amino acids which can coelute (e.g. homocitrulline and methionine).

Ion Exchange Chromatography (IEC):

- Advantages:
 - Dedicated instrument
 - Minimal sample preparation required no derivitisation step
 - Stable and precise
 - Identifies all analytes of interest
 - Identifies unusual (atypical) amino acids
 - Commercial kit
 - In routine use for a number of years

Ion Exchange Chromatography (IEC):

- Disadvantages:
 - Long analysis time ~130mins for standard profile
 - Structural analogue internal standard
 - Single point calibration, sporadic frequency
 - Expensive capital cost
 - Expertise in technology required
 - Significant maintenance
 - Often running to capacity
 - Method lacks specificity
 - Identification based on retention time alone (channel ratio used as a second marker for reassurance)
 - Co-eluting substances
 - Interferences from drugs
 - Poor resolution of some analytes e.g. sulphocysteine





Clinical Presentation:

- Baby girl, first child of non-consanguineous parents of Polish origin.
- Born at term following normal pregnancy and delivery
- Presented at 90 minutes of life with seizures that were unresponsive to Phenobarbitone, Valproate, Keppra and Midazolam
- On examination she had increased tone in limbs
- Brain MRI showed appearances suggestive of hypoxic-ischaemic change

Case 1

Biochemistry:

- Plasma Ammonia: Normal
- Plasma Urate: Normal
- Acylcarnitines: Not suggestive of a Fatty Acid Oxidation Disorder.
- Urine Organic Acids: No specific abnormality detected.
- Amino Acid results....



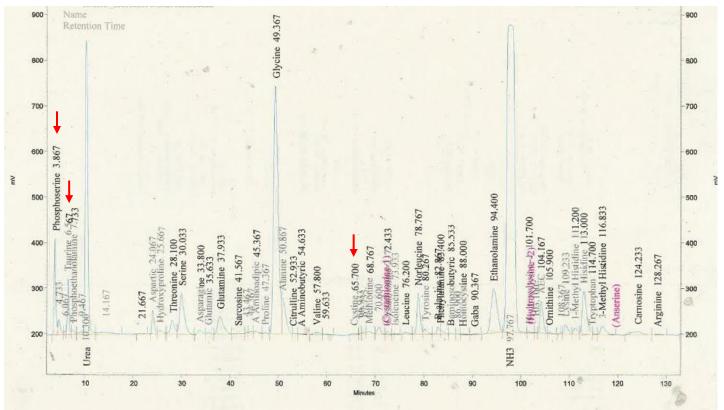
Plasma Amino Acids:

60 - 00 - 00 - 00 - 00 - 00 - 00 - 00 -	00	Aspartic 22.433	Threonine 27.333 35.000		e 57.200	 0 75.833	33	*		AEC 104.133 Ornithine 105.900 I vsine 109.267	7 113.033		T
40 - Hosphoserine	5.967 Phosphoethanolamine 7.700	21.567 Aspa (Hydroxyproline)	Asparagine 33.167 Glutamic 35	a 37.300 Sageosite A Aminoadipic 44.867 Proline 47.067	50.500 A Aminobutyric 53.933	Gystinge 66.500 ◆ Methionine 68.600 70.300 70.300 Systatritionines2172-133.500 Systatritionines2172-133.500 Loucine 75	B-atanine 81/105the 80.067 Phenylatanine 83.633 (Bamin osobutyric)	Homocystine) (Gaba) Ethanolamine 94.233	99.800 NH3 97.700	-	1-Methyl Histidine 111.267 Histidine Histidine 115.133 -3-Methyl Histidine 116.733 (Anserine)	(Carnosine)	122 067

- Plasma Tau = 144umol/L (26-169)
- Plasma Cys = 2umol/L (21-53)
- Plasma Phosphoserine channel ratio: 1.37 (5.08) ? sulphocysteine



Urine Amino Acids:



- Urine Tau = 121umol/mmol creatinine (8-266)
- Urine Cys = 4umol/mmol creatinine (24-78)
- Urine Phosphoserine channel ratio: 1.23 (5.37) ? sulphocysteine



Biochemistry:

- Urine Sulphocysteine: 90.6 umol/mmol (0-10)
- Genetic Analysis: SUOX homozygous gene mutation c.90C>A (p.(Cys30Ter))



Clinical Presentation:

- Baby girl, second child of consanguineous parents
- FH:
 - 10yr old brother with severe global developmental delay, ? undiagnosed metabolic disorder
- Presented at 2 months of age with:
 - 1 day history of irritability
 - Attended to local hospital with seizures and abnormal posturing that eventually progressed to apnoeas
 - Previously fit and well
 - Abnormal brain MRI

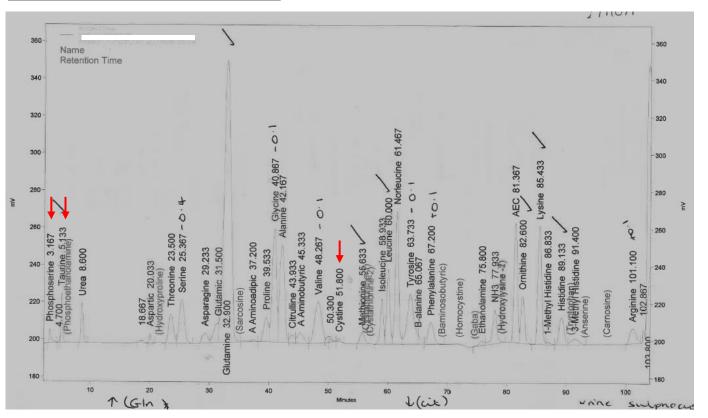


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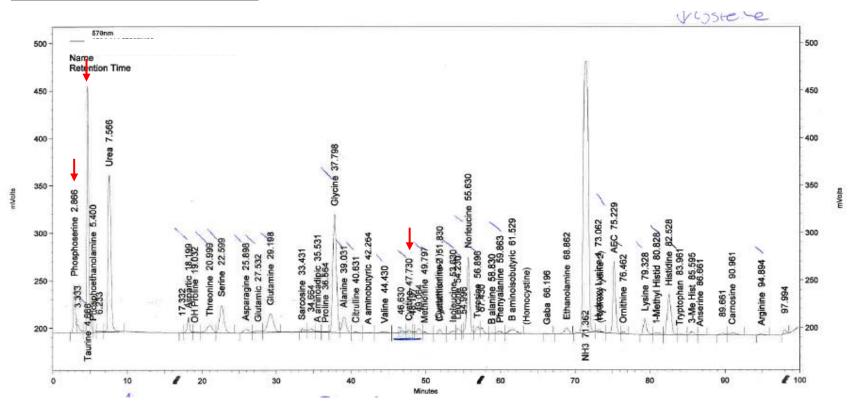
Plasma Amino Acids:



- Plasma Tau = 26umol/L (26-169)
- Plasma Cys = 3umol/L (21-53)
- Plasma Phosphoserine channel ratio: 1.44 (5.71) ? sulphocysteine



Urine Amino Acids:



- Urine Tau = 1400umol/mmol creatinine (6-89)
- Urine Cys = 25umol/mmol creatinine (13-48)
- Urine Phosphoserine channel ratio: 2.03 (5.92) ? sulphocysteine



Biochemistry:

- Urine Sulphocysteine: 112.8 umol/mmol (0-10)
- Genetic Analysis: SUOX homozygous gene mutation c.1097G>A (p.(Arg366His))

Summary

- Sulphide oxidase deficiency can be picked up on amino acid analysis using IEC (Biochrom).
 - Plasma/ Urine: \uparrow Tau, Sulphocysteine, \downarrow Cys
- However....
 - Sulphocysteine:
 - Difficult to detect as coelution with phosphoserine
 - Separation difficult to adjust as at the beginning of the run immediately start with buffer 1 and there is no buffer or temperature change over the period in which it elutes.
 - Channel ratio can be an indicator that there is a coeluting substance present but not specific to sulphocysteine.
 - Taurine is not always raised.
 - Most reliable marker is absence of plasma cystine (although there is a buffer change in the region so often get a small peak).
 - Interpret results in conjugation with clinical details and other biochemical markers.