Uncertainty in laboratory reports – does it affect patients?

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Newborn Screening: PKU

- Day 5 bloodspot phenylalanine 1321µmol/L
- Day 10 phenylalanine
  - Bloodspot (TMS) 1911µmol/L
  - Plasma (Biochrom) 2276µmol/L
  - Difference of 365µmol/L
  - Different samples
  - Different methodology

Newborn Screening: MCADD

- Day 5 blood spot acylcarnitine
  - C8 0.56µmol/L (cut off 0.5)
  - C8/C10 ratio 0.93 (cut off 1.0)
- Diagnostic samples day 12
  - Plasma C8 0.67µmol/L (<0.22)
  - Blood spot C8 0.25µmol/L (<0.3)

Urine organic acids

- Organic acids day 12: Mild dicarboxylic acid with equal levels of suberate and adipate. No suberylglycine was detected and importantly no clearly increased peak of hexanoylglycine was evident. This organic acid profile is not clearly indicative of MCAD.
- Quantitative hexanoylglycine: 3.4 micromol/mmol creat (MCAD range >2.1)

Intensity
20,803,332
TIC
20000000
15000000
10000000
5000000
0
7.0
10.0
20.0
30.0
min
LACTATE
OXALATE
SUCCINATE
CITRATE
INTERNAL STANDARD (HEPTADECANOATE)

Molecular Genetics

- Sequencing of the *ACADM* gene
  - Homozygous for the 199T>C mutation
  - Genotype not previously described
  - Compound heterozygosity for 985G>A/199T>C
    - Probable ‘mild’ phenotype with residual enzyme activity
    - Temperature sensitive
    - Only rare reports of clinical presentations

Fat oxidation

- Fat oxidation flux in fibroblasts was normal at 37°
  - Myristate 86%
  - Palmitate 97%
  - Oleate 105%
  - Octanoate 153%
- At 41 ℃:
  - Myristate 52%
  - Palmitate 44%
  - oleate 67%
  - Octanoate 19%
- ‘Temperature sensitive MCADD’

Qualitative Organic acids

- Previously healthy 2 year old
- 2 days of gastroenteritis
- Unresponsive episode associated with a blood sugar of 0.6 mmol/L
- Metabolic acidosis with slowly resolving ketosis
- On recovery, he remained quite sleepy and was not as mobile as he had been previously and was initially quite wobbly and ataxic.

Qualitative Organic acids

‘Gross ketonuria with appropriate dicarboxylic aciduria but also with substantial peaks of 2-methyl-3-hydroxybutyrate, 2-methylacetoacetate and tiglylglycine, all 3 of which are associated with β-ketothiolase deficiency. However the excretion, given the degree of ketosis, is not consistent with β-ketothiolase deficiency being the primary defect. Potentially it is due to a high degree of catabolism causing a secondary build up of metabolites.’

Qualitative organic acids

‘There is a significant peak of hexanoylglycine (but not suberylglycine or phenylpropionylglycine) ruling out MCAD deficiency and a mildly increased excretion of ethylmalonic acid, two metabolites associated with MADD (multiple acylCoA dehydrogenase deficiency). The degree of ketosis and the presence of some but not all metabolites associated with MADDD could suggest a diagnosis of riboflavin responsive MADD….There are substantial peaks of two unknown compounds – structurally they appear to be acylglycines…

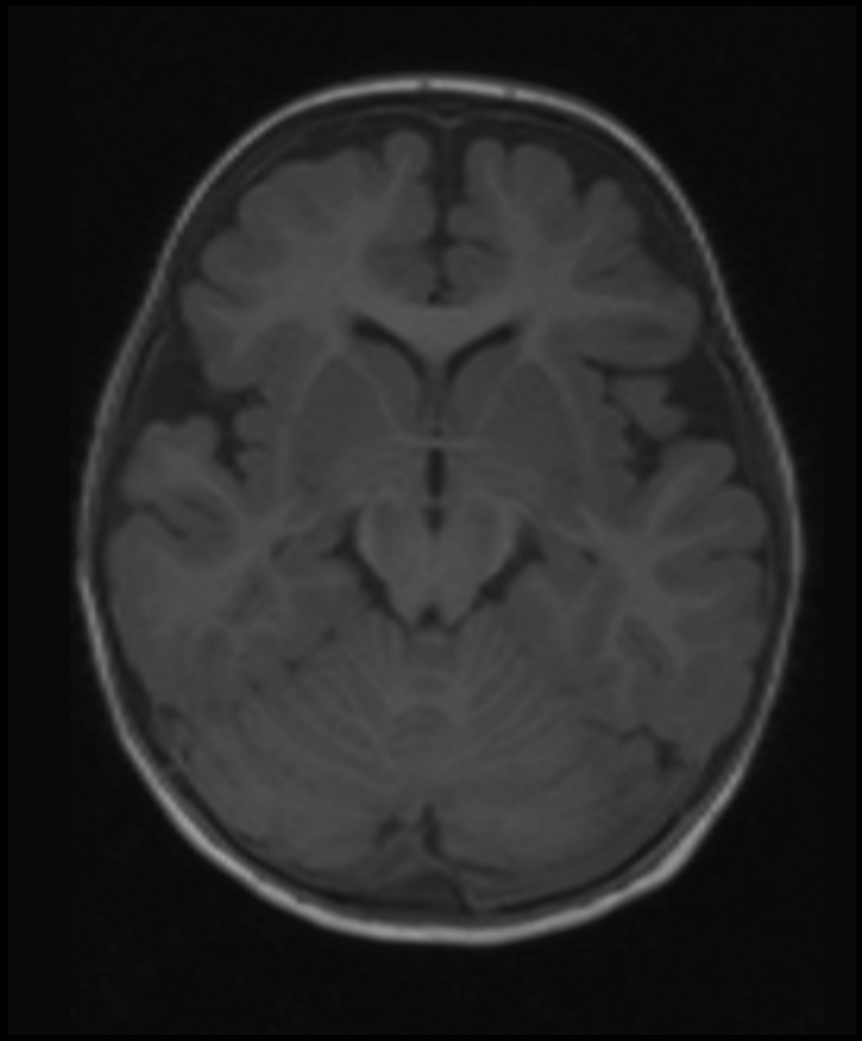
To summarise – this is a highly unusual and atypical organic acid profile but it is unclear what the underlying defect is, or indeed if there is one. Suggest repeat samples before and after giving riboflavin…’

Progress

- Given riboflavin:
  - Clinically improved
  - Organic acids normalised
- Fat oxidation in fibroblasts normal
- Does he have a riboflavin responsive disorder?

Glutaric aciduria type 1

- 18 month old boy
- Previously healthy
- Consanguineous parents
- Episode of gastroenteritis
- Encephalopathy
- Dystonia and loss of motor skills on recovery



Metabolic investigation

- Plasma acylcarnitines:
  - A significant peak of glutarylcarnitine (0.46micromol/L ref<0.06) was detected in this sample This isolated increase in glutaryl carnitine can be indicative of a defect in glutaryl-CoA dehydrogenase (GA1)
- Organic acids:
  - No significant abnormality

Genetic analysis: Sequencing of *GCDH*

P[(Thr261Ile)];[(Thr261Ile)]

‘This variant has not been reported in the literature; it is a substitution affecting a highly conserved residue of the GCDH protein and *in silico* analysis predicts it to be pathogenic. However, without any other functional or clinical data, at present this variant must be regarded as a variant of uncertain clinical significance. Enzyme analysis of fibroblasts from the individual would be helpful to clarify the pathogenicity of this variant.’

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Enzyme analysis from fibroblasts:

15.94% of simultaneous control

Monitoring in GA1

- Aim to keep plasma lysine in lower 1/3 of reference range (historic advice from S.Kolker)
- Lab A reference range (age 6 years)
  - 112 - 238µmol/L
- Lab B reference range (age 6 years)
  - 50 - 233µmol/L

Plasma amino acids

	Lab A	Lab B		Lab A	Lab B
Alanine	213-628	150-650	Lysine	112-238	50-233
Arginine	42-179	0-160	Methionine	16-69	0-54
Aspartate	7-46	0-80	Ornithine	51-186	40-160
Citrulline	3-56	0-54	Phenylalanine	38-129	20-130
Glutamine	226-732	550-830	Serine	80-231	60-240
Glycine	162-516	120-480	Threonine	67-201	40-180
Histidine	72-143	50-130	Tyrosine	40-120	30-130
Isoleucine	37-88	0-135	Valine	110-302	50-375
Leucine	60-178	60-260			

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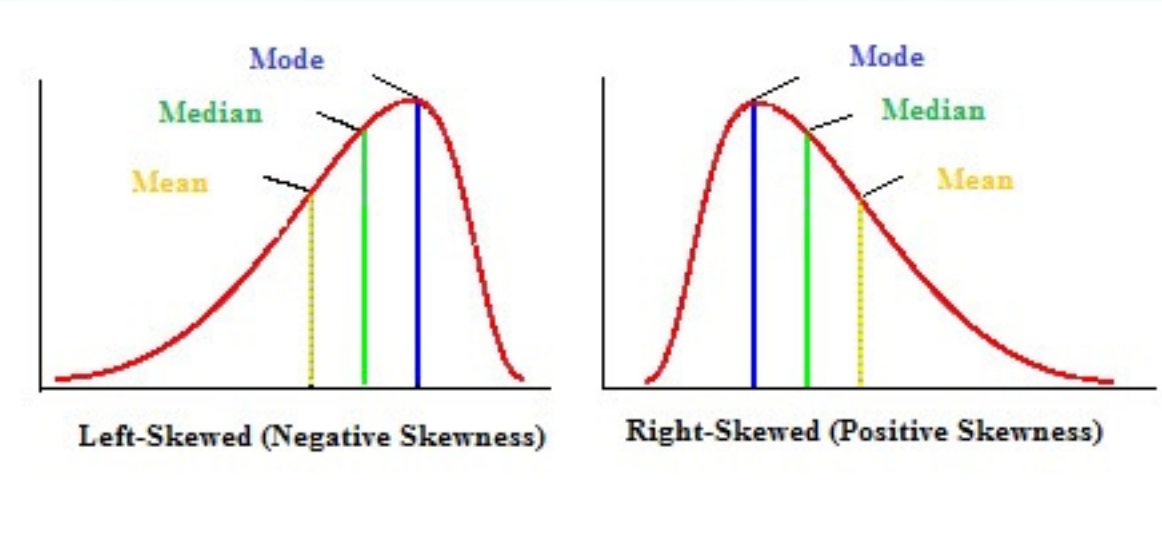
Reference Ranges

- May be constructed many years ago
- Different technology to current
- Characteristics of population may be unknown
- Appropriate statistical analysis



Glucosaminoglycans

- 2 year old child
  - Coarse facial features
  - Mild developmental delay
  - Possible heart lesion
  - Hepatomegaly
- Urine GAG
  - GAG/creatinine 16.4mg/mmol creat (9.7-19.5)
  - GAG electrophoresis – increase in chondroitin sulphate
  - Chondroitin sulphate
    - normal
    - MPS VII
    - MPS IX
  - Normal leukocyte β-glucuronidase 426 (100-800)
  - MPS IX (Natowicz) – hyaluronidase deficiency (uncertain phenotype)

Biotinidase

- 3 month old developed West syndrome
- Increased plasma lactate
- Plasma biotinidase requested – sample sent by post to lab
- Activity 1.8 U/L (2.5-10.5)
- Started on biotin
- Repeat biotinidase 5.7 U/L
- 2nd repeat biotinidase 11 U/L
- Conclusion by referring neurologist ‘biotin responsive biotinidase deficiency’

Biotinidase

Laboratory information sheet:

‘Cases of biotinidase deficiency have activities close to zero.
A slightly low result may reflect a deteriorated sample, for example the activity will be lower if the plasma is left at room temperature for more than two days. A repeat fresh sample should confirm this (the plasma can usually be transported at room temperature but ideally sent by courier on dry ice)’.

Conclusions

- Differences in sample type – different results
- Ratios often effective, problem of small denominators
- Reference ranges – population, technology, analysis
- Molecular analysis – changes of uncertain significance, only one change found
- Enzyme analysis – stability of sample, conditions of analysis
- Qualitative abnormality in quantitatively normal sample