Organic Acids – some things to think about…

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Content

- Discussion of 3-methylglutaconic aciduria
- Discussion of TCA cycle intermediates
- A series of unusual / rare cases or cases with a point to make

The 3-Methylglutaconic Acidurias – Previous categorization

- Type 1 = 3-Methylglutaconyl-CoA hydratase deficiency
- Type 2 = Barth Syndrome (Tafazzin deficiency)
- Type 3 = Costeff Syndrome (OPA3 gene)
- Type 4 = Catch all category of disorders where 3MGA may be seen as a secondary finding incl. Smith Lemli Opitz, UCD (esp CPS1), various mitochondrial disorders (primary and secondary), peroxisomal disorders
- Type 5 = DCMA Syndrome (DNAJC19)
- Type 6= ???
- Type 7A = Heterozygous Dominant negative mutation in CLPB gene
- Type 7B = Biallelic recessive mutations in CLPB gene

But better to categorise as PRIMARY and SECONDARY CAUSES

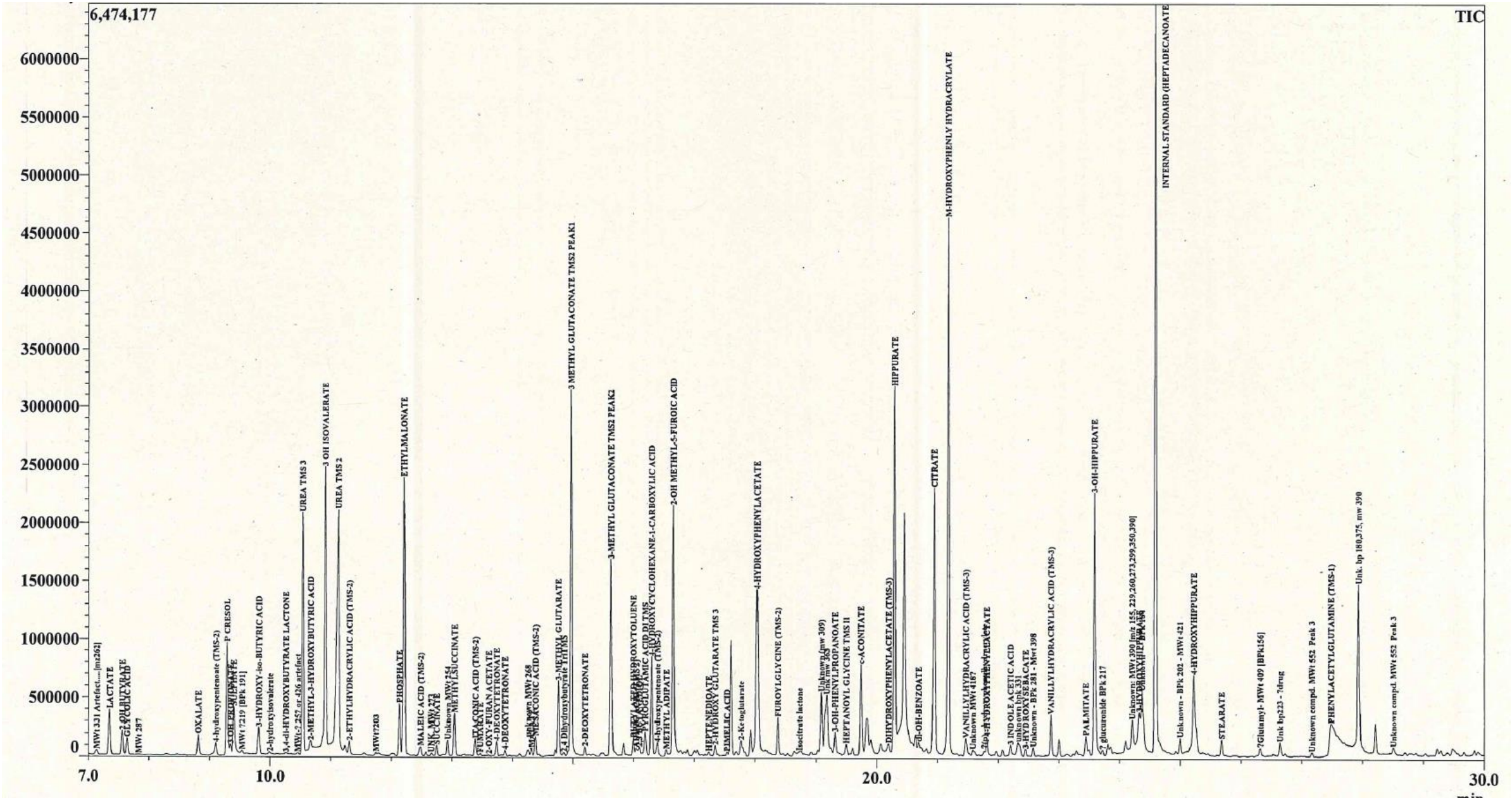
The 3-Methylglutaconic Acidurias – Newer categorization

- **Primary** (catabolism of leucine)
  - 3-methylglutaconyl –CoA hydratase deficiency (AUH gene, Type 1 MGA) – increased 3-hydroxyisovaleric acid in addition to 3MGA
  - 3-HMG-CoA lyase deficiency (HMGCL gene) –3-MGA + increased 3-hydroxy-3-methylglutarate (3HMG) and usually 3-hydroxy IVA

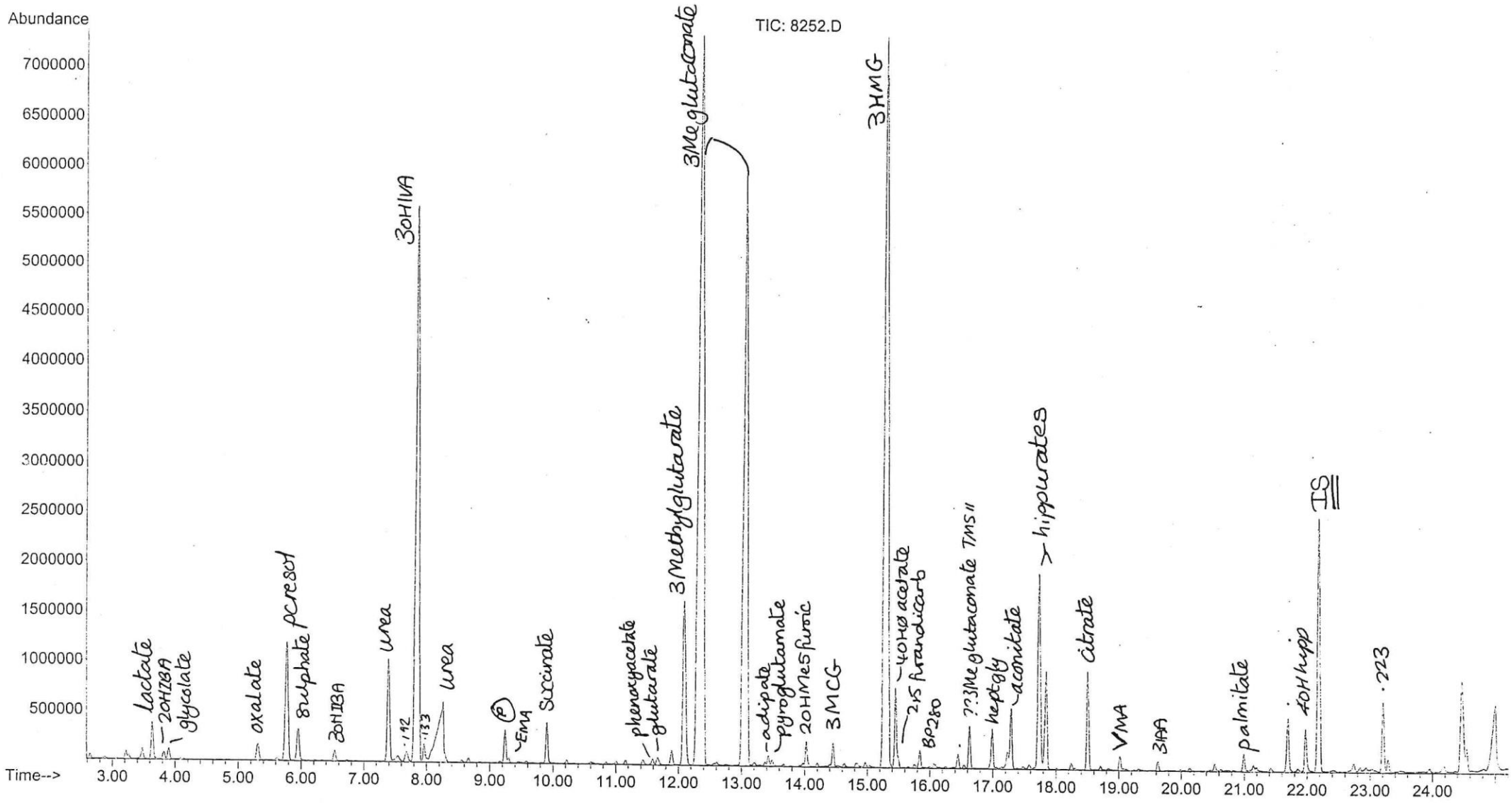
These disorders should be distinguishable from each other and from secondary causes

- **Secondary** (mechanism of production unclear but related to mitochondrial dysfunction, often due to phospholipid metabolism and hence mitochondrial membrane dysfunction)
  - Barth Syndrome (TAZ gene, MGA Type 2)
  - Costeff syndrome (OPA3 gene, MGA Type 3)
  - MEGDEL Syndrome (SERAC1 gene)
  - THEM70 deficiency (THEM70 gene)
  - DCMA syndrome (DNAJC19 gene, MGA Type 5)
  - CLPB gene defects (Type 7A and 7B)
  - Various others, e.g. ECHS1 deficiency, mtDNA depletion (POLG, SUCLA2), MELAS, other mitochondrial disorders, CPS1 deficiency, Smith-Lemli-Opitz,

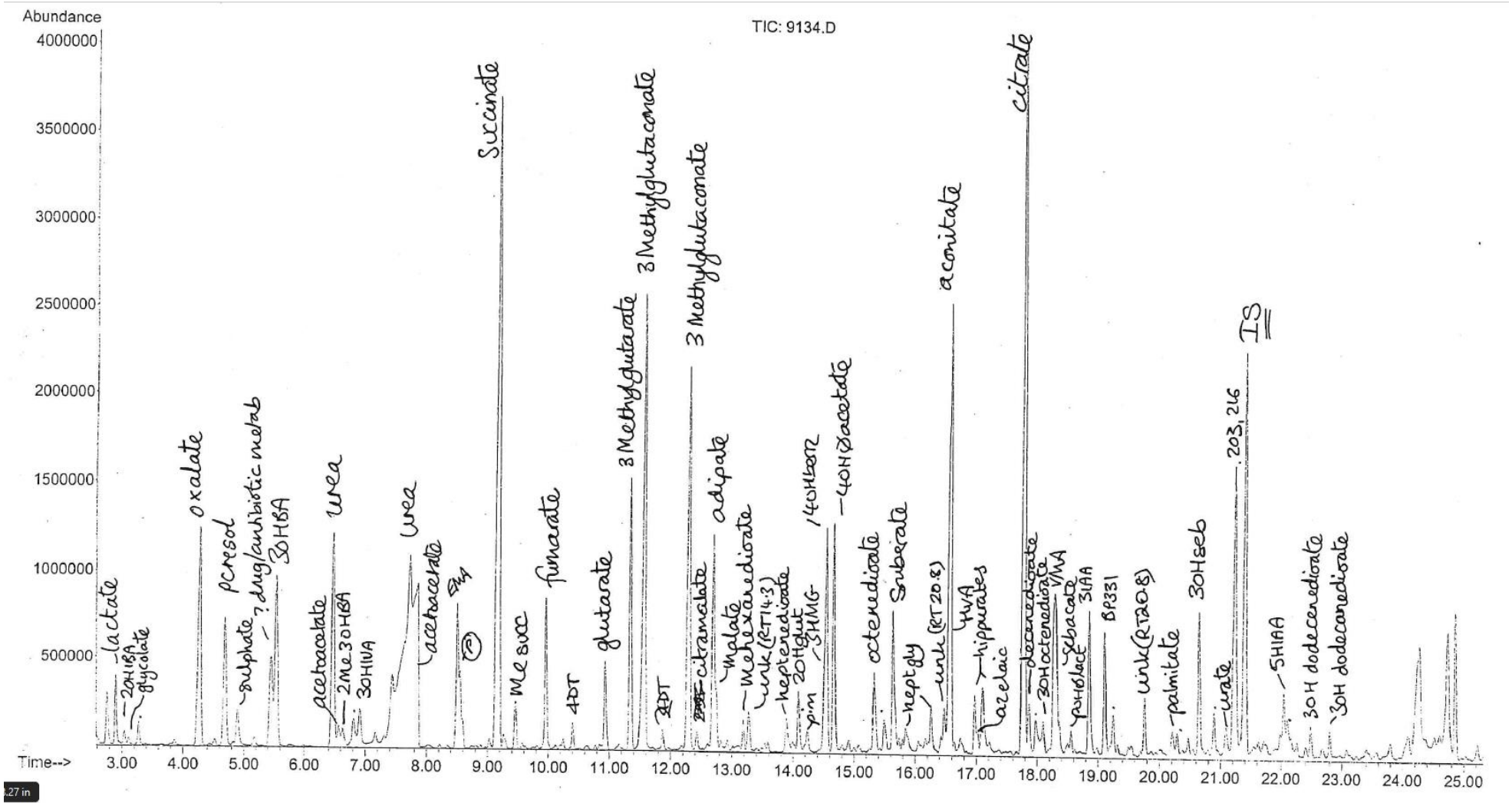
Chromatogram 1: 3-Methylglutaconyl-CoA hydratase deficiency



Chromatogram 2: 3-HMG-CoA lyase deficiency



Chromatogram 3: Barth Syndrome – excretion may be very variable from very clearly elevated to normal



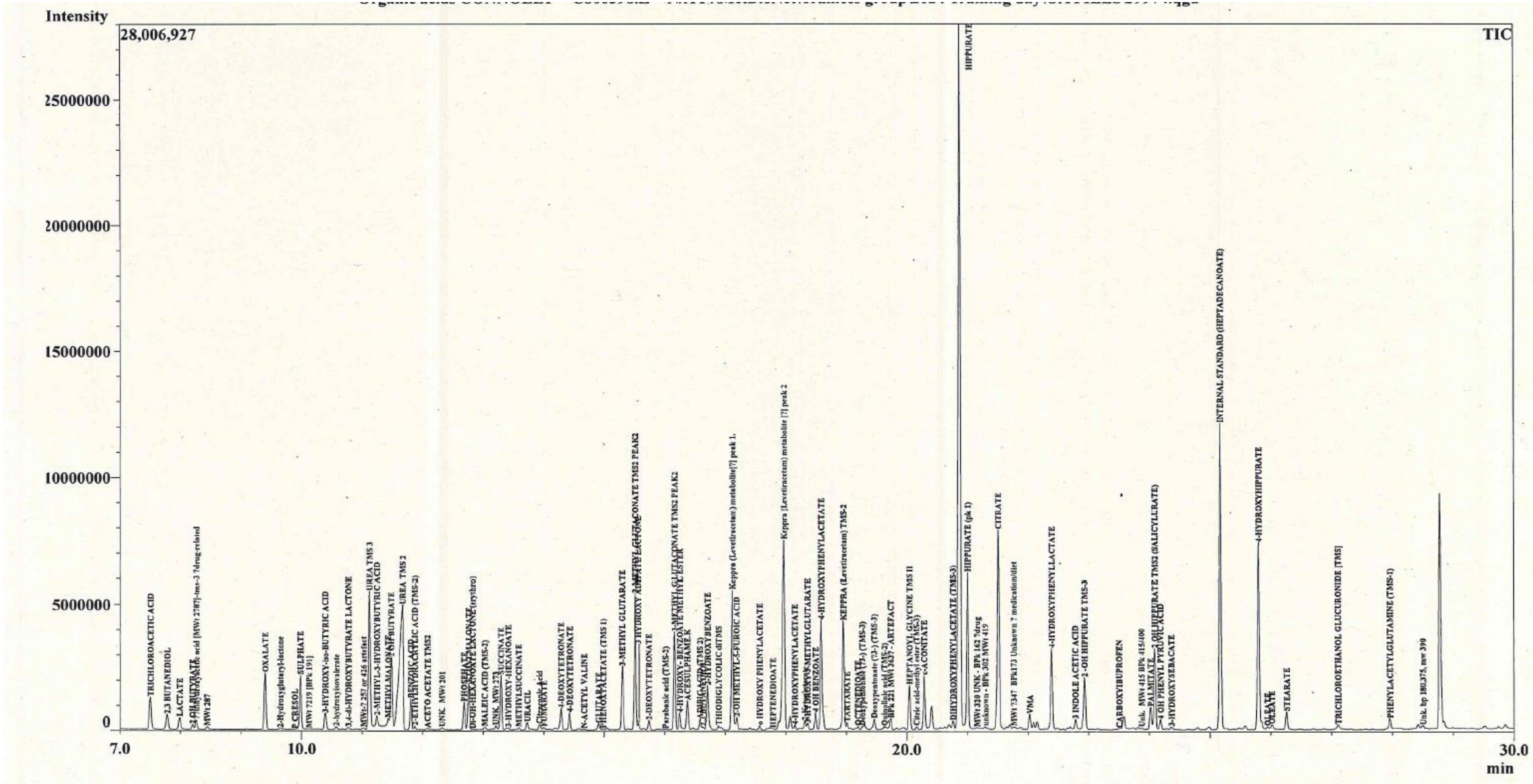
Follow Up

- Acylcarnitines
  - Possibly raised C5-OH in 3MGA-hydratase (some patients have had clearly elevated C5-OH, our patient had borderline raised C5-OH and C6DC)
  - Clearly raised C5-OH and C6DC in 3-HMG CoA lyase

- Cardiolipin (monolysocardiolipin/cardiolipin ratio) - ? Barth Syndrome
  - Especially relevant if patient is male, with short stature / FTT, neutropenia, cardiomyopathy (and FH of affected males on maternal side)
  - Barth is probably the single most common cause of secondary MGAuria so it makes sense to consider it early on

- Other tests – NH3, amino acids, lactate, blood gases, sterols / 7DHC, VLCFA
- Genetics – some causes only diagnosable by genetics

Chromatogram 4: Quiz 1- (4 month old baby girl)



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What is significant in the chromatogram and what diagnoses are potentially relevant?

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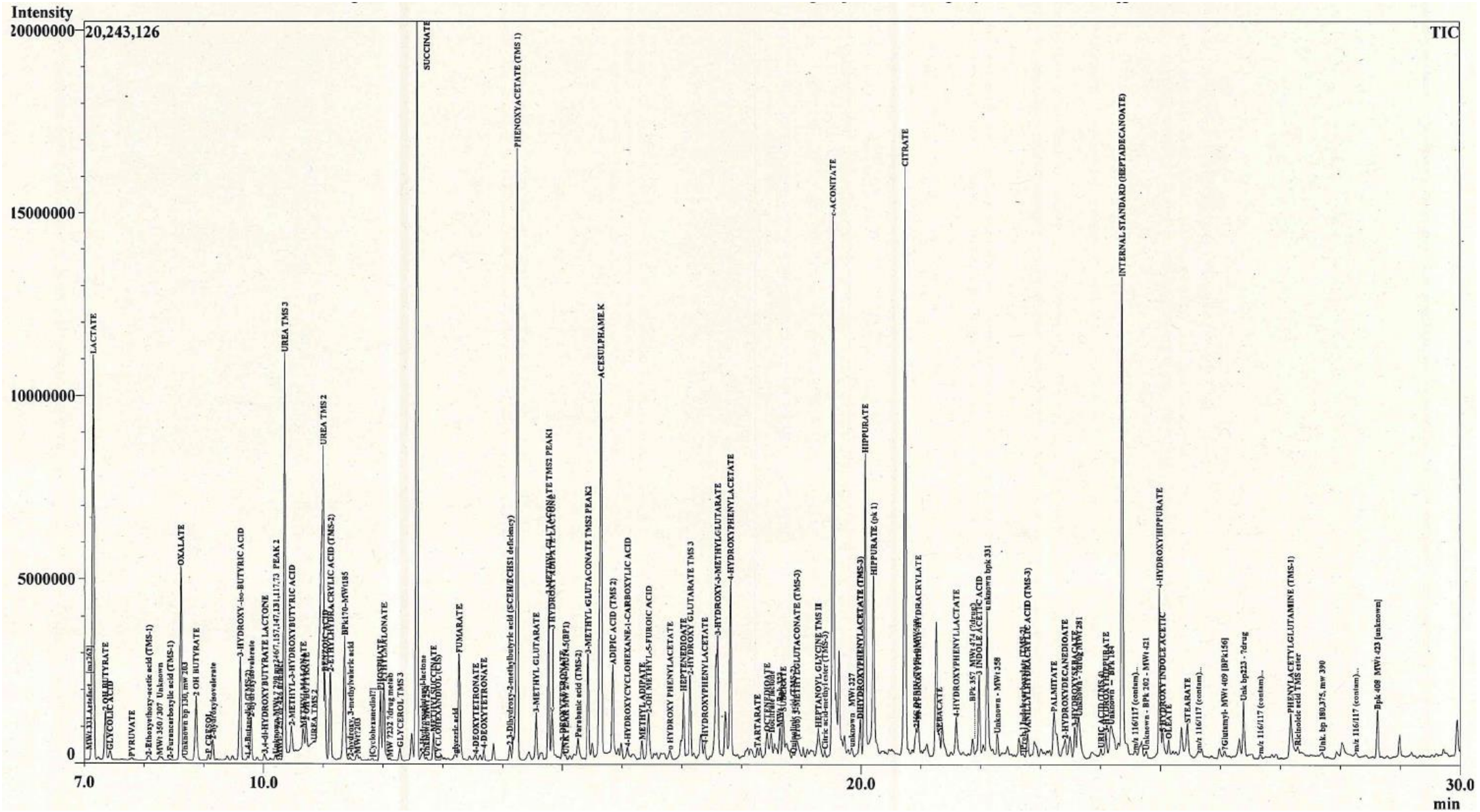
Patient Info

- 4 month old baby girl
- First urine sample clinical details = “investigation of development”
- Further info obtained; - In first few months of life experienced poor feeding and weight gain, choking episodes, breathing issues, repeated admissions for respiratory infections and noted to have low neutrophils -stabilised once given daily GCSF injections
- Tonic seizures started at around 4 months
- Floppy but with spasticity in legs
- No developmental progress – little awareness of her surroundings
- On home oxygen
- Rapid WGS sent + metabolic tests (OA, AA, acylcarn etc)

3-Methylglutaconic Aciduria Type 7B

- Rapid exome sequencing came up with diagnosis of 3-MGA –type 7B (compound heterozygous for missense mutations in CLPB gene)
- CLPB (caseinolytic peptidase B homolog) has ATPase activity and regulates folding of proteins in mitochondria

Chromatogram 5: Quiz 2 -(3 yr old male)



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What do you think about this one?

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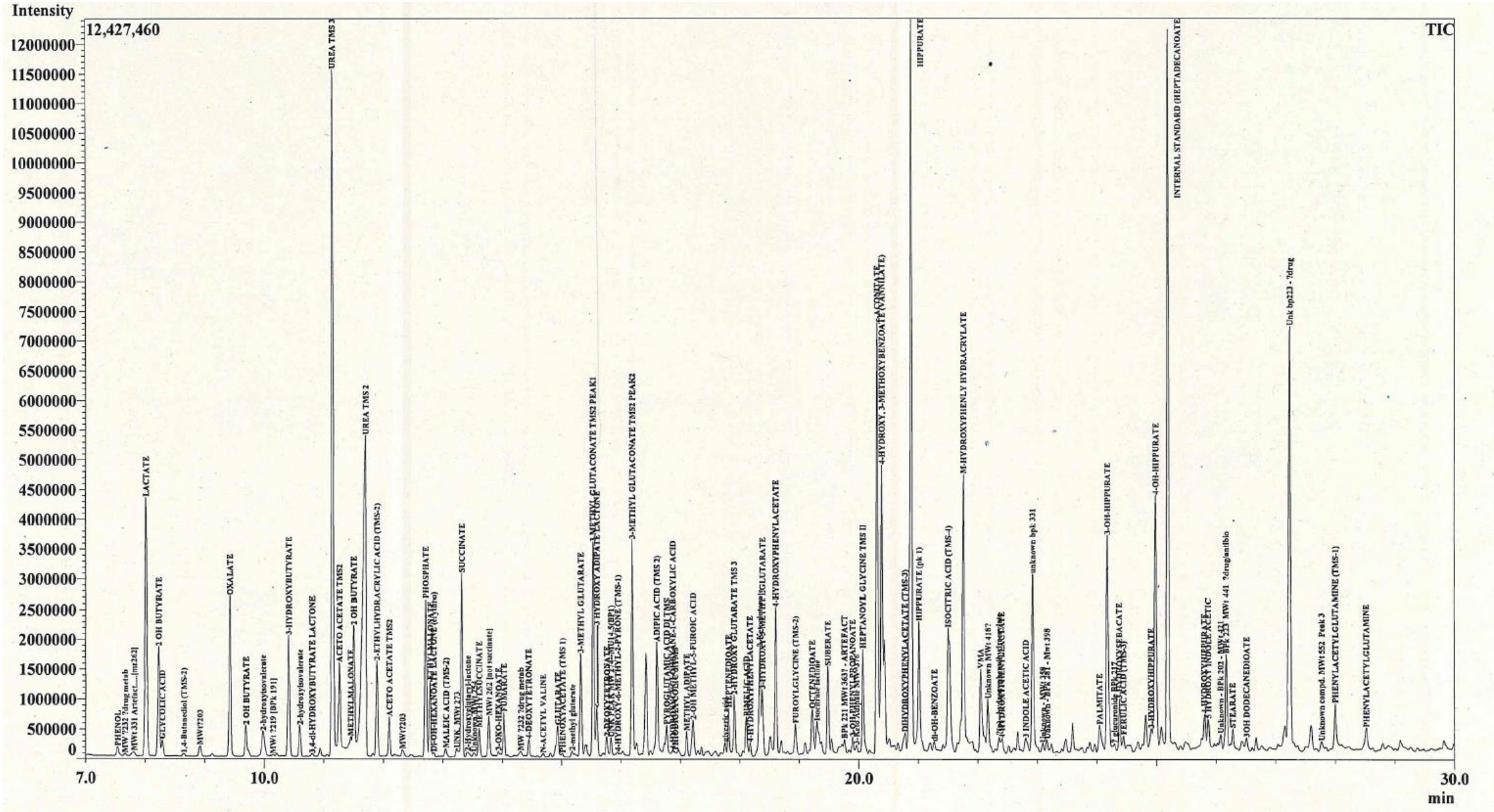
Patient Info

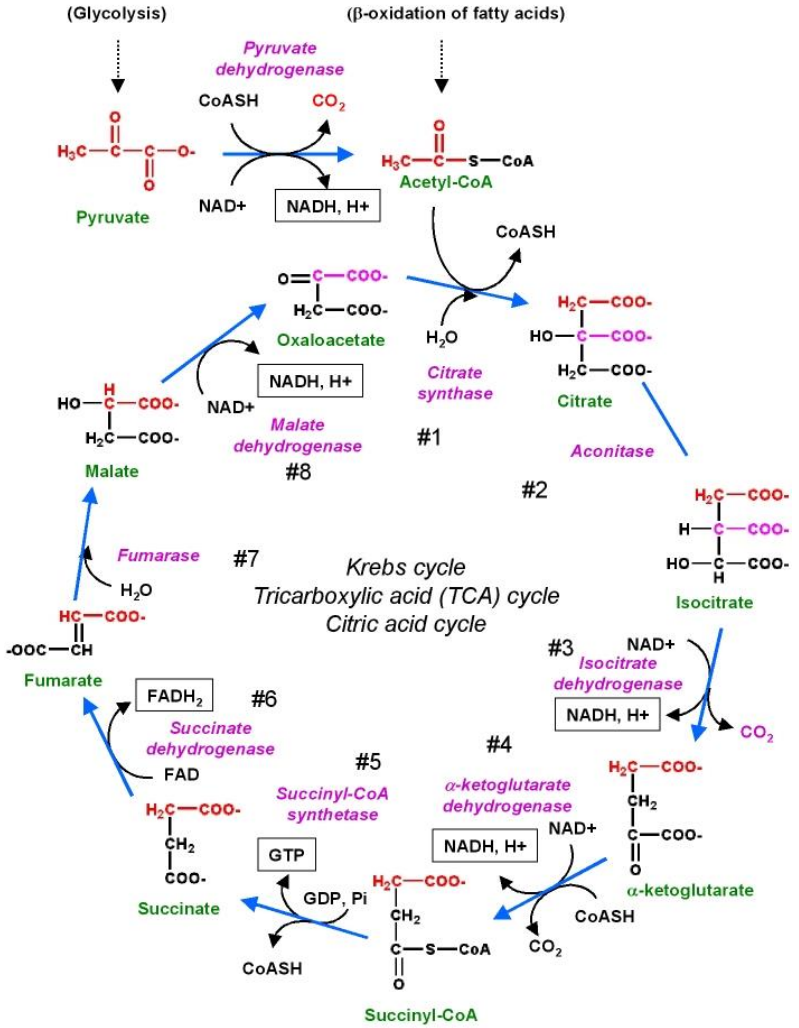
- 3 yr old male
- “global developmental delay”
- OA –reported as having raised 3-MGA and fumarate – likely metabolic
- Referred to metabolic consultant - became apparent he was having repeated episodes of hypoglycaemia
- Cholesterol 7.6 mmol/L (2.8-6.0)
- Triglycerides 13.2 mmol/L (0.4-2.1)
- Lactate 8.9 mmol/L (<1.8)

- Confirmed genetically as GSD 1a

Chromatogram 6 - GSD 1b



Interpreting TCA (Tricarboxylic Acid) / Kreb's Cycle Intermediates



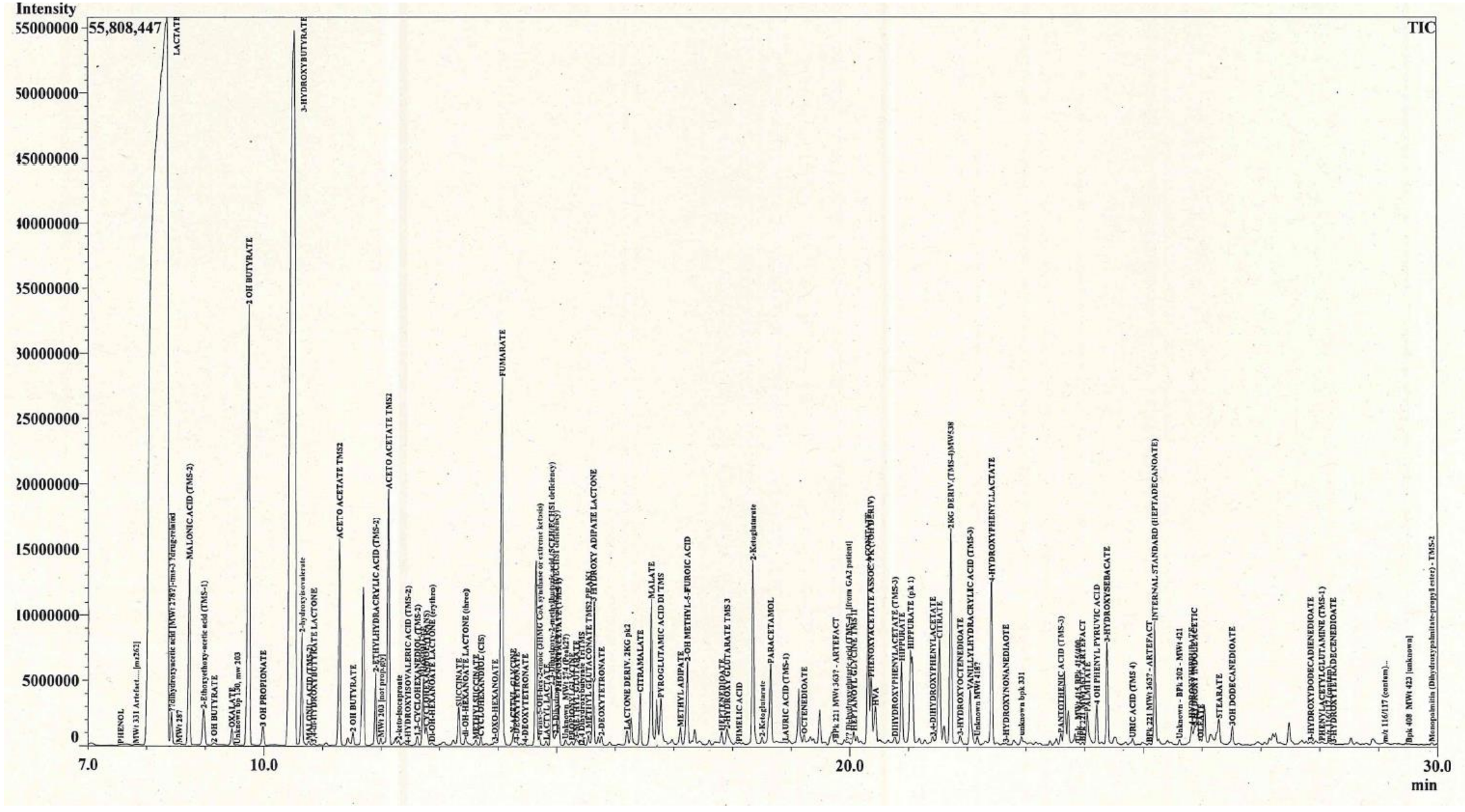
TCA (Tricarboxylic Acid) / Kreb's Cycle Intermediates

- Common to see increased fumarate and 2-ketoglutarate in neonates up to about 1 month of age – due to metabolic immaturity
- How to spot when there may be more to it?
  - If persistent beyond 2 months of age (assuming not a sick prem)
  - Is there any history of acute collapse? If so may be transient / secondary – start by getting a repeat >48 hrs post collapse / resusitation
  - If MALATE is also present this ALWAYS suggests a cause other than immaturity even in a very young baby
  - Is excretion of FUMARATE or 2- KETOGLUTARATE disproportionately increased over others?

Mitochondrial Patient

- 3/12 baby girl
- History of poor growth, poor tone, head lag, dev delay, not smiling, not fixing or following
- Presented to local hospital after 2 days of vomiting and poor feeding
- Found to be acidotic - lactate initially 14 mmol/L , always around 10-14.
- No hypoglycaemia, no cardiovascular issues, no suspicion of sepsis

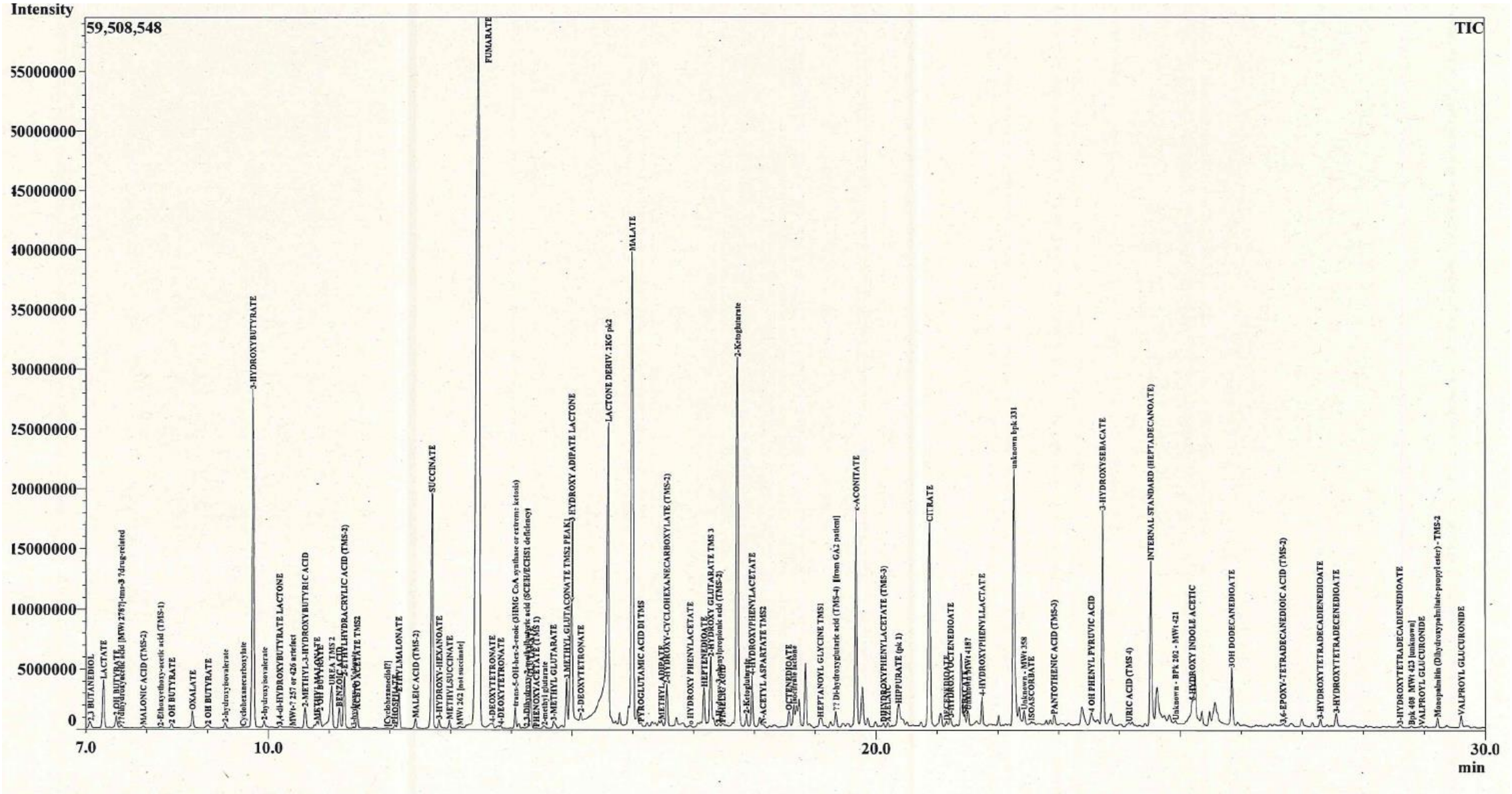
Chromatogram7:



NDUFAF6 Gene

- Complex 1 assembly factor which results in low complex 1 activity – i.e. respiratory chain disorder
- Causes a Leigh-like syndrome – neurodevelopmental delay, regression, dysarthria, dysphagia, hypotonia, dystonia, epilepsy, respiratory problems – severity can be variable between neonatal onset and slow onset at a few years of age (with near normal lactate)

So, - what about this one? -Chromatogram 8: Quiz 3 –(9 month old girl)



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How would you interpret this one?

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Patient Info

- 9 month old girl
- Presented to local DGH with “deranged LFTS, vomiting, hypoglycaemia…”
- Acylcarnitine - “ketolytic and lipolytic picture, nothing specifically diagnostic”
- Plasma Amino acids: Only abnormality of note was glutamine of 910 umol/L (Ref. 279-695) - (nothing else suggesting specific UCD, lysine low normal)
- Organic Acids- “VERY marked fumarate and marked malate and 2-ketoglutarate excretion, plus ketosis and appropriate dicarboxylic aciduria”
- Is this a mitochondrial respiratory chain defect? Fumarate hydratase?

Ongoing…

- Was admitted for further investigation / optimisation of management of hypoglycaemia
- LFTs improved significantly once being managed for hypoglycaemia (most recent effectively normal)
- Had a VERY RAISED GLUTAMINE of 1549 µmol/L at one point during admission which caused a bit of a stir – but NH3 and repeat glutamine normal when checked

- Whole genome sequencing came back with diagnosis of…

Phosphoenolpyruvate carboxykinase deficiency (PEP-CK deficiency) – PCK1 gene (cytosolic)

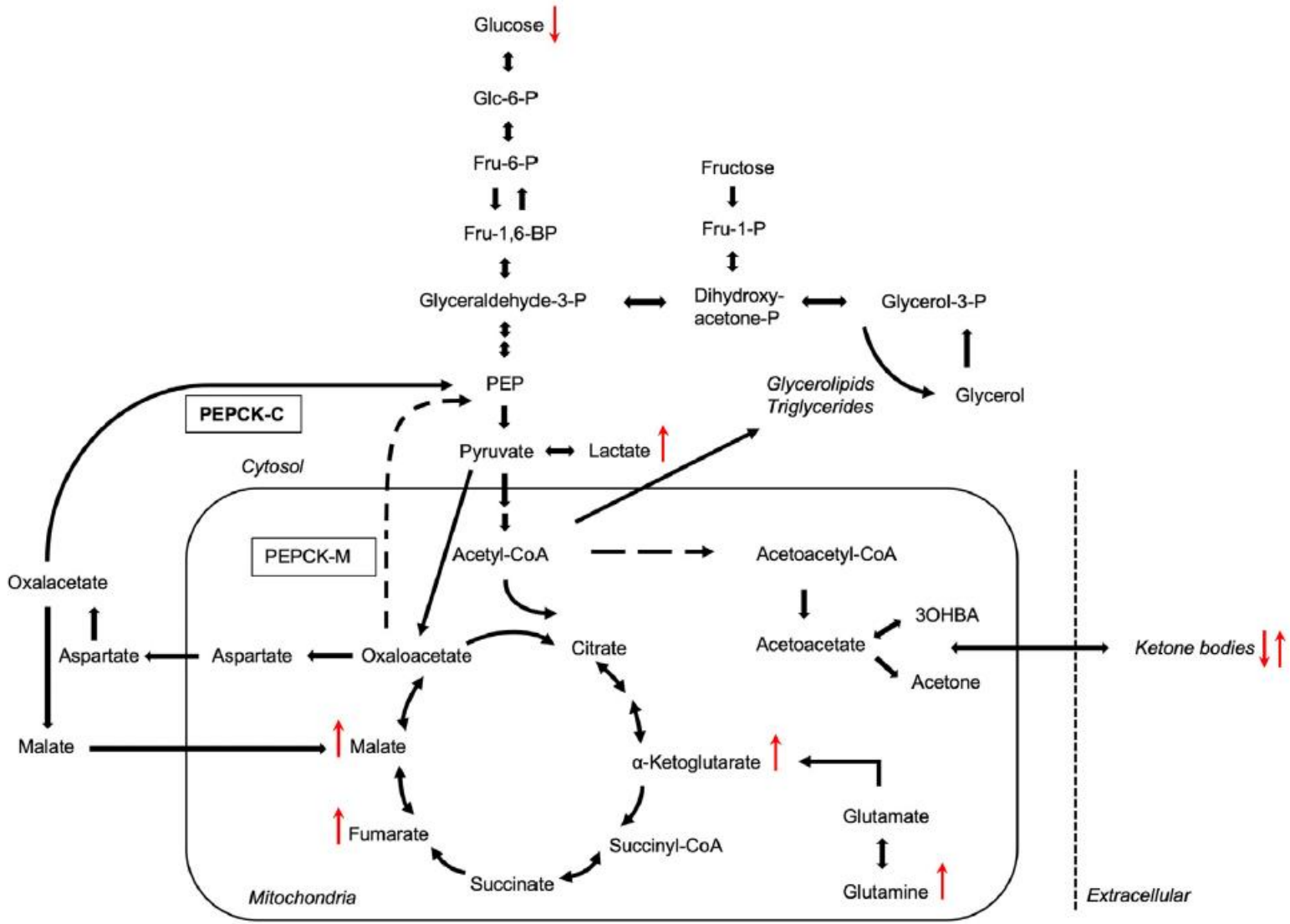
Compound heterozygous for;

c.961+2T>C -affects splice site, null allele predicted

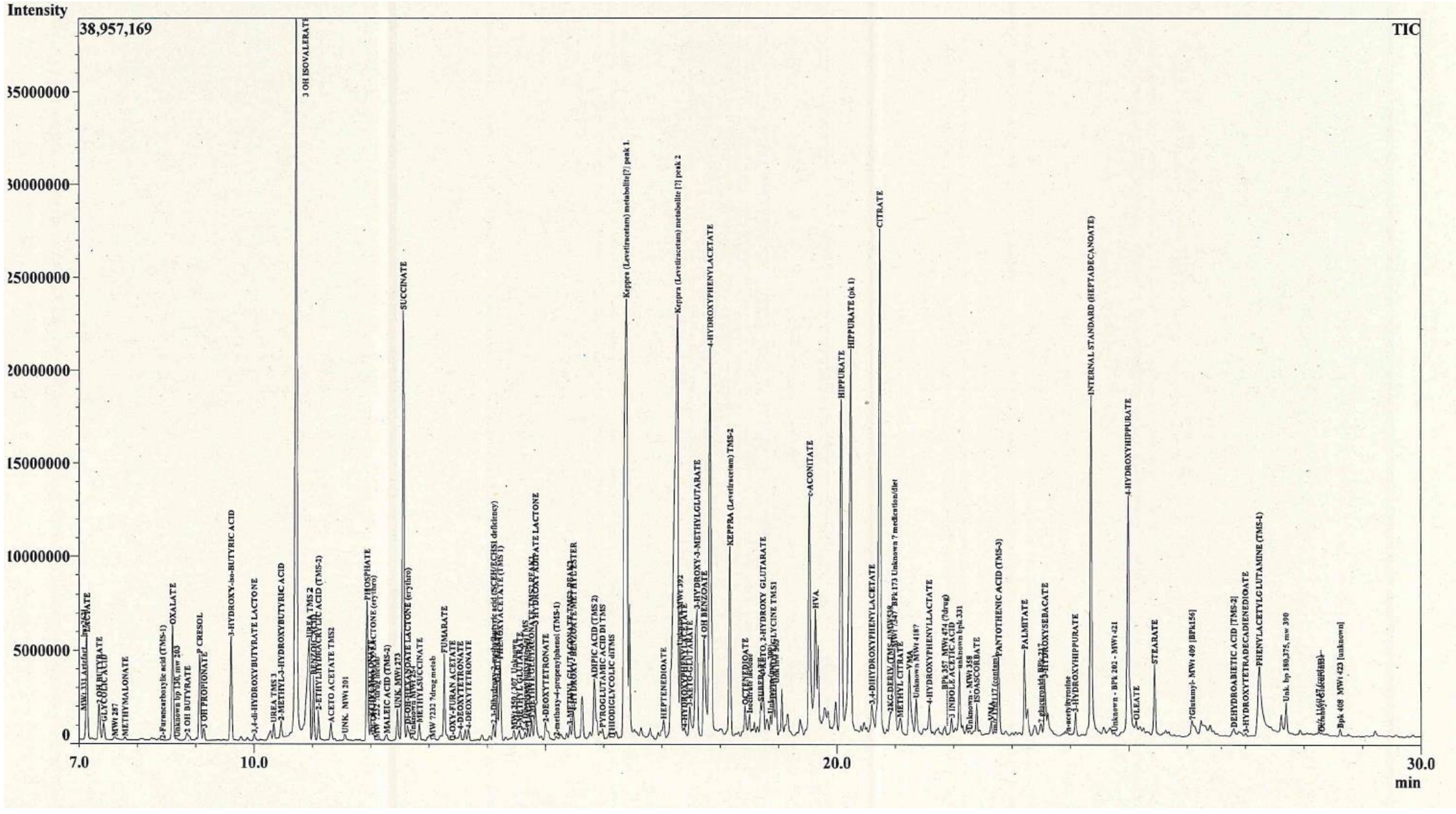
c.204del, p.(Leu69Ter) -predicted to elicit nonsense mediated decay and result in null allele

PEP-CK deficiency

- Very rare disorder of gluconeogenesis
- PEP-CK is the key enzyme and rate controlling step in gluconeogenesis catalyzing the conversion of oxaloacetate into phosphoenolpyruvate
- There is cytosolic form and a mitochondrial form of the enzyme (but no known cases of latter)
- Common mutation in Finnish population and so most patients in the literature are Finnish and homozygous or compound heterozygous for one mutation


Glucose
Glc-6-P
Fru-6-P
Fru-1,6-BP
Glyceraldehyde-3-P
Fructose
Fru-1-P
Dihydroxy-acetone-P
Glycerol-3-P
Glycerol
Glycerolipids Triglycerides
PEP
PEPCK-C
Pyruvate
Lactate
Cytosol
PEPCK-M
Acetyl-CoA
Acetoacetyl-CoA
3OHBA
Acetoacetate
Acetone
Ketone bodies
Oxalacetate
Aspartate
Aspartate
Oxaloacetate
Citrate
Malate
Malate
α-Ketoglutarate
Glutamate
Fumarate
Succinyl-CoA
Succinate
Glutamine
Mitochondria
Extracellular

Chromatogram 9: Quiz No 4 (3 month old baby boy)



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What's notable about this chromatogram and what tests would you want to do next?

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Patient Info…

- 3 month old baby presenting with seizures

- Plasma Acylcarnitines

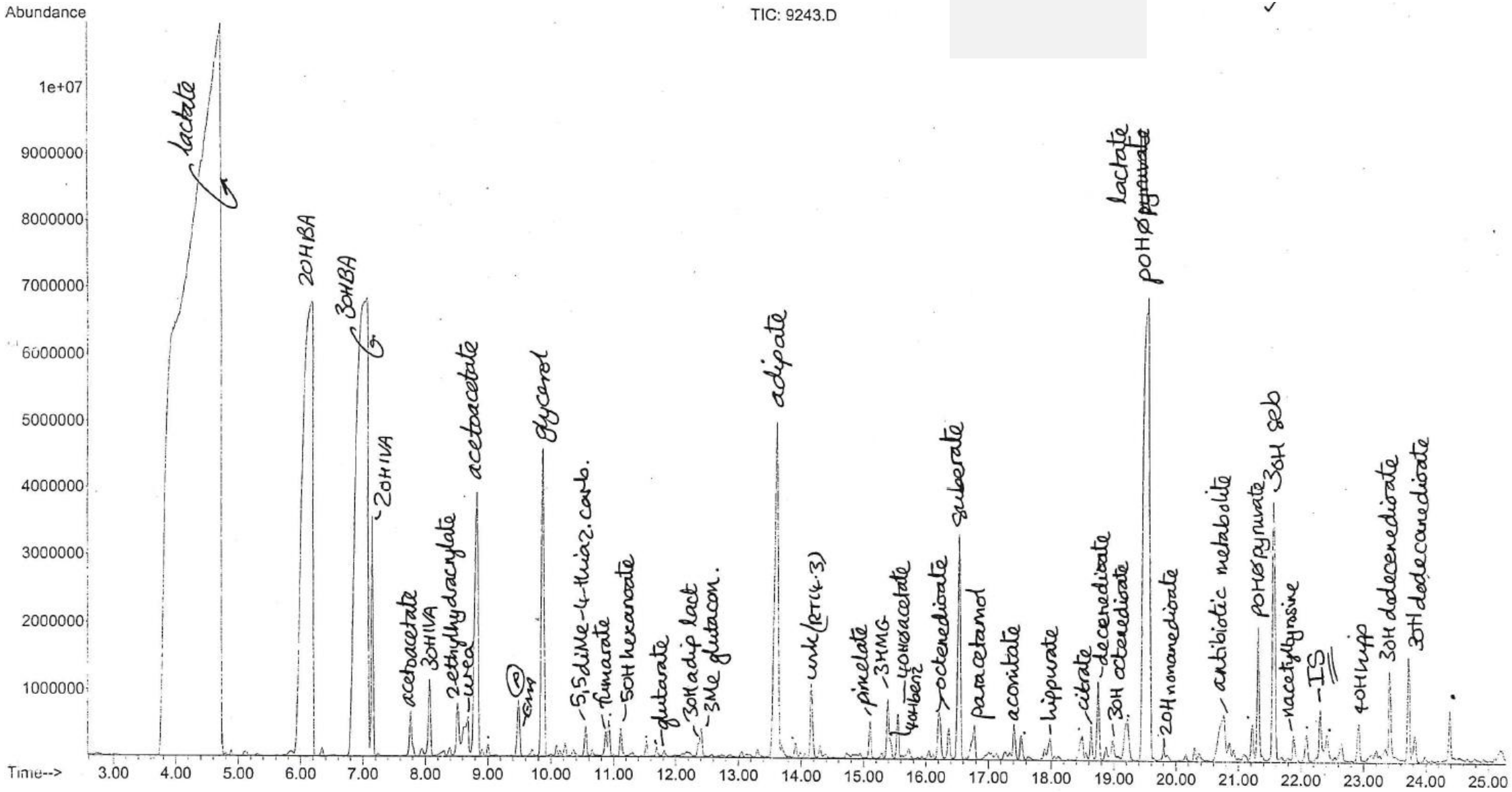
  C3 = 2.07 umol/L (<1.3)

  C5-OH = 0.18 umol/L (<0.06)

- What Test do you want to do next?

- Biotinidase activity = 0.0 U/L (ref. 2.5-10.5)

Chromatogram 10: Quiz No 5 (1 day old baby)



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What's the possible diagnosis here?

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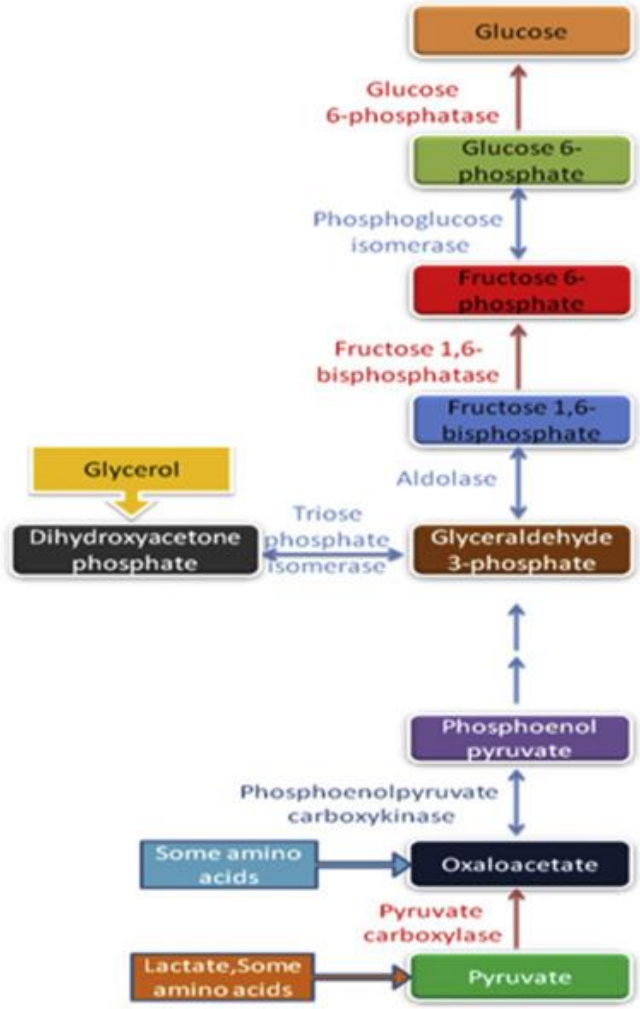
Further info on patient…

- Marked lactate, ketones and glycerol in OA
- Collapsed 1 day old baby presenting with glucose < 1 mmol/L
- This urine sample taken within few hours of collapse
- Urine organic acids on sample taken around 12-24 hrs post presentation – completely normal!
- What test do you want to do next?
- Fructose-1,6-bisphosphatase deficiency confirmed enzymatically

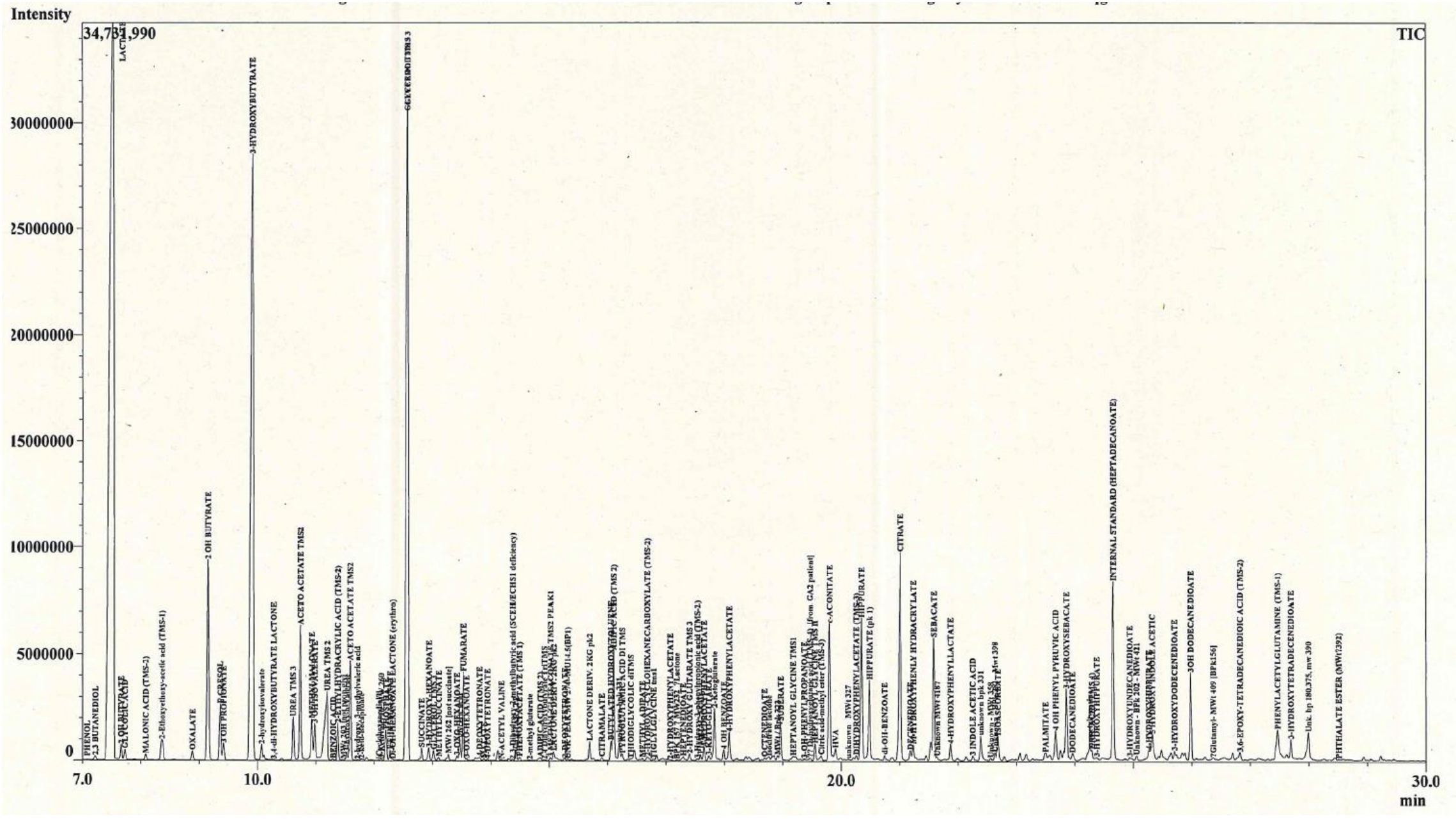
Fructose-1,6-bisphophatase Deficiency –disorder of gluconeogenesis

Gluconeogenesis inhibited at fructose-1,6-bisphosphate – backing up back down pathway causes:

- glycerol production from glyceraldehyde-3-phosphate and
- lactate from pyruvate



Chromatogram 11 -Quiz No 6 (3 yr old girl)



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What do you think about this one? What might be the diagnosis here?

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Further patient details

- 3 yr old girl
- Previously fit and well
- Went to forest play centre with grandparents –on way home became lethargic, sleepy and difficult to wake
- Taken to ED and found to be hypoglycaemic and acidotic
  - Glucose 1.2 mmol/L
  - Lactate 6.4 mmol/L (0.9-1.8)

- Treated with lots of glucose and fluid bolus
- By next morning was back to normal self
- A diagnosis of fructose-1,6-bisphosphatase was suggested on basis of organic acids
- But genetics and enzyme assay negative for Fructose-1,6-bisphosphatase deficiency
- Transpired child had had a slushy type frozen drink while at the play centre

“Slushy Drink Effect”

- Predominantly seen in children < 4 yrs old
- Typically are previously well children with no particular history of hypoglycaemia
- Will become hypoglycaemic and acidotic within 30 mins-1 hr of consuming a so-called slushy type drink containing glycerol (which is an ingredient used to stop them freezing)

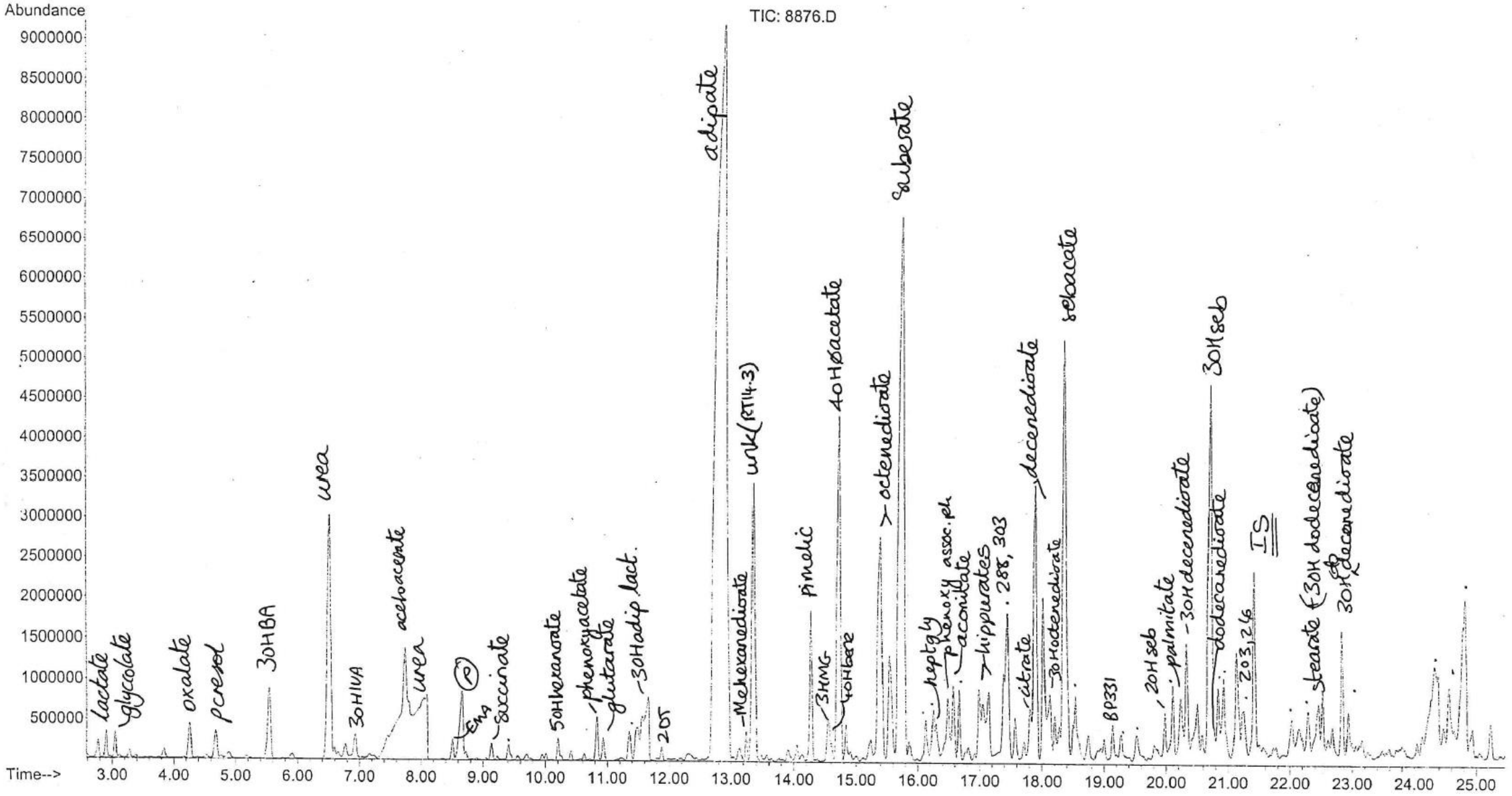
Fatty Acid Oxidation Disorder and Organic Acids

- Very useful for diagnosis of;
  - **MCAD**
  - **MADD** / Glutaric aciduria type 2 (classic forms)
- Because;
  - Have patterns specific to the disorder
  - Are always abnormal even when well

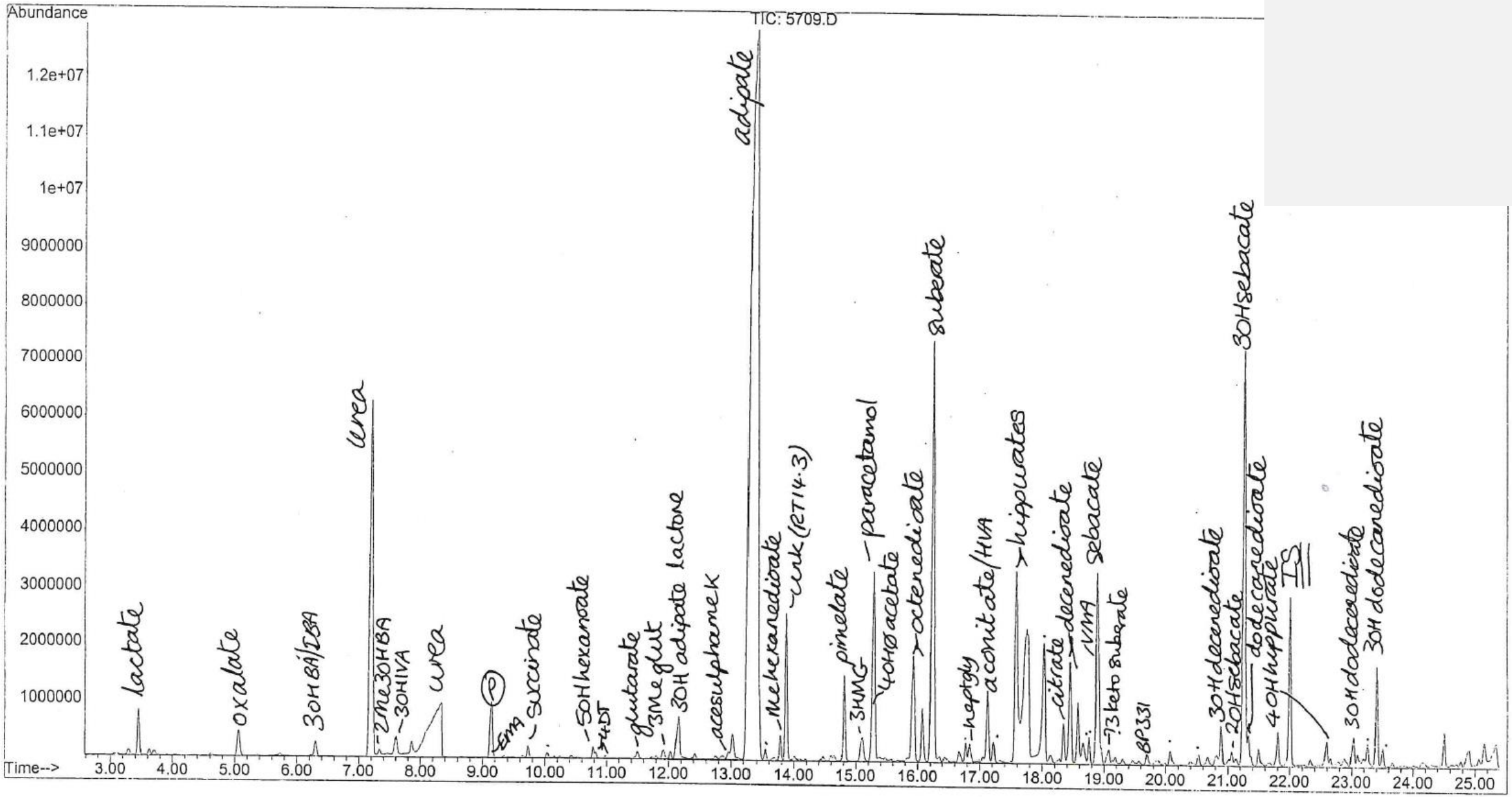
- Much less helpful for;
  - **Primary Carnitine deficiency**
  - **VLCAD**
  - **CPT2**
  - **CACT**
- Because;
  - Patterns are non-specific (may all look the same) and will likely be completely normal when patient not in crisis state / not catabolic

- **LCHADD / MTP –** Has a very specific pattern that is essentially diagnostic in samples from classic patients in crisis, - but a non-crisis sample can be entirely or virtually normal

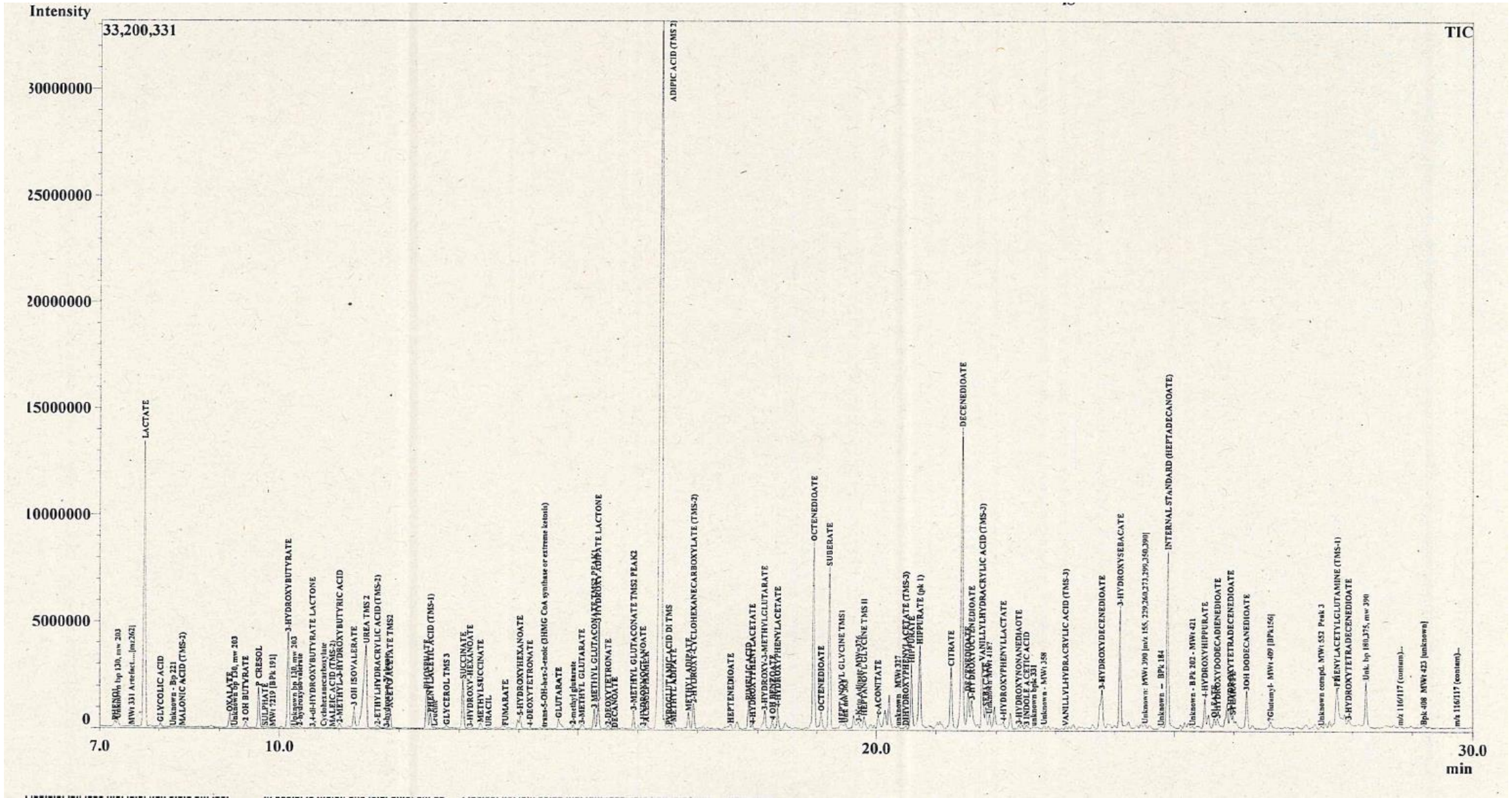
Chromatogram 12: Quiz 7a



Chromatogram 13- Quiz 7b



Chromatogram 14- Quiz 7c



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What is / are the diagnoses for these 3 profiles?

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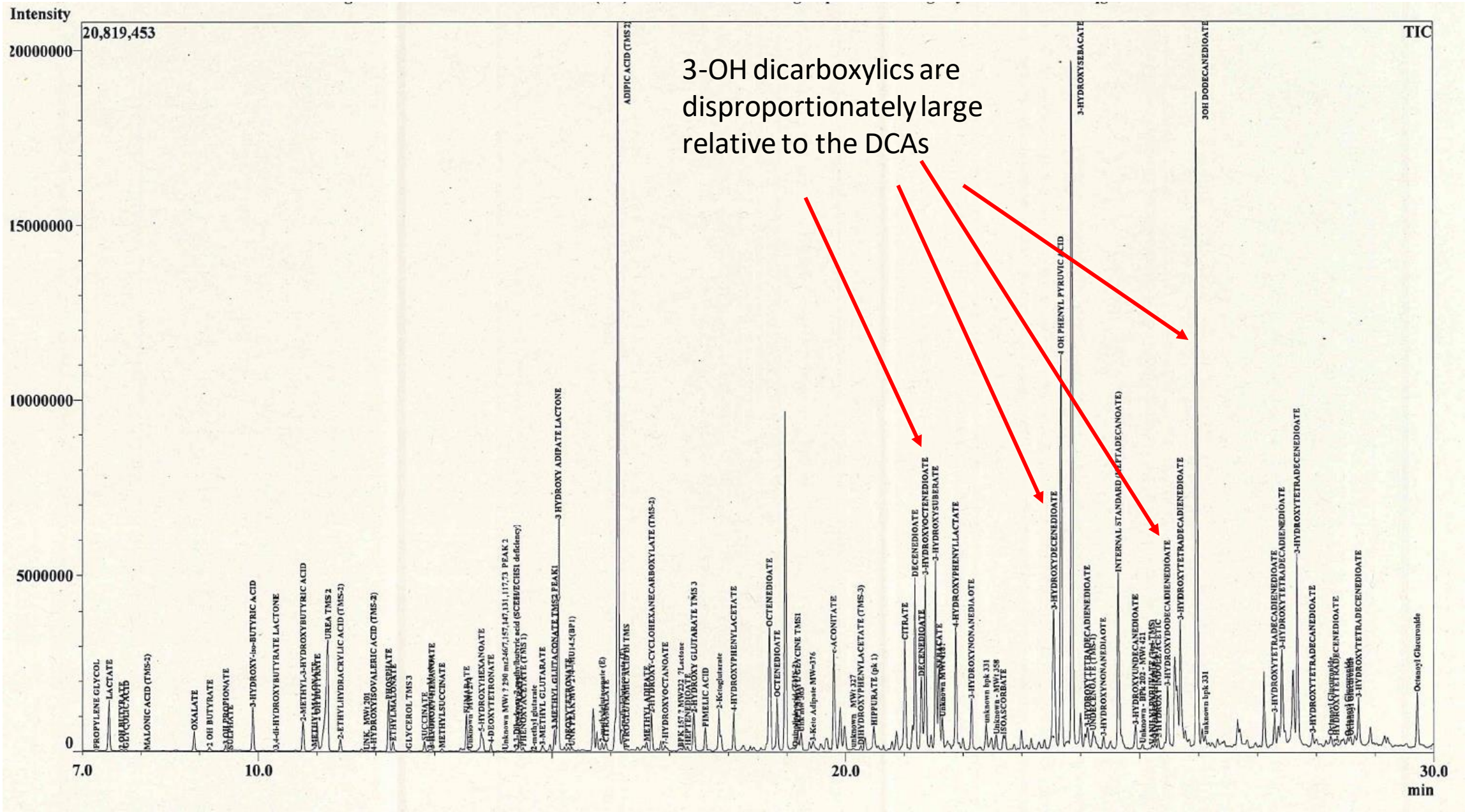
Answers…

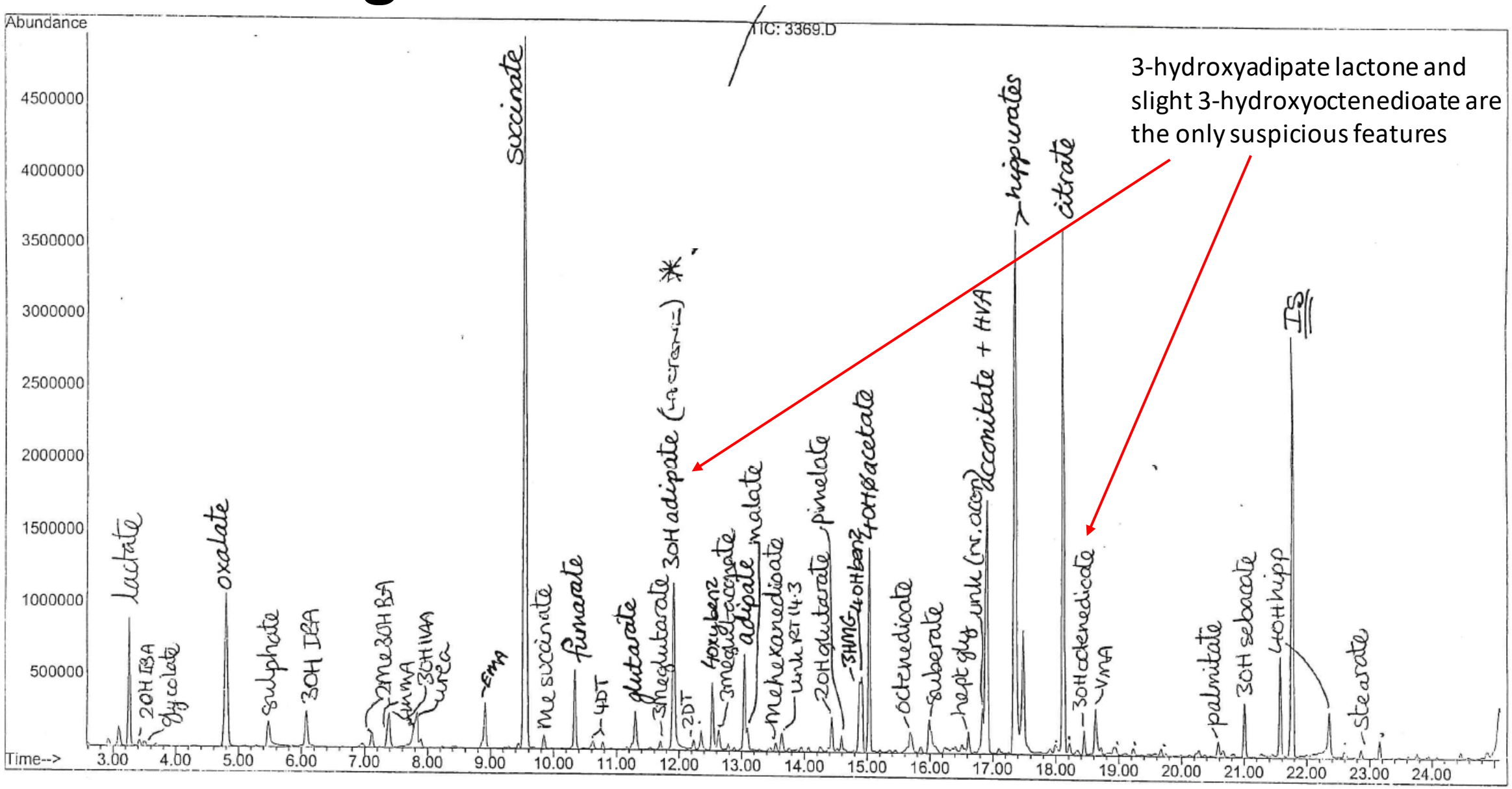
- Chromatogram 12 = PCD
- Chromatogram 13 = CPT2 deficiency
- Chromatogram 14 = VLCAD deficiency

You can’t meaningfully tell them apart – all that can be said from these chromatograms is that there is a very disproportionate dicarboxylic aciduria, significant decenedioate excretion, & strong suspicion of a fatty acid oxidation disorder that needs prompt attention

Chromatogram 15 -Crisis LCHADD



Chromatogram 16 -Non-crisis LCHADD

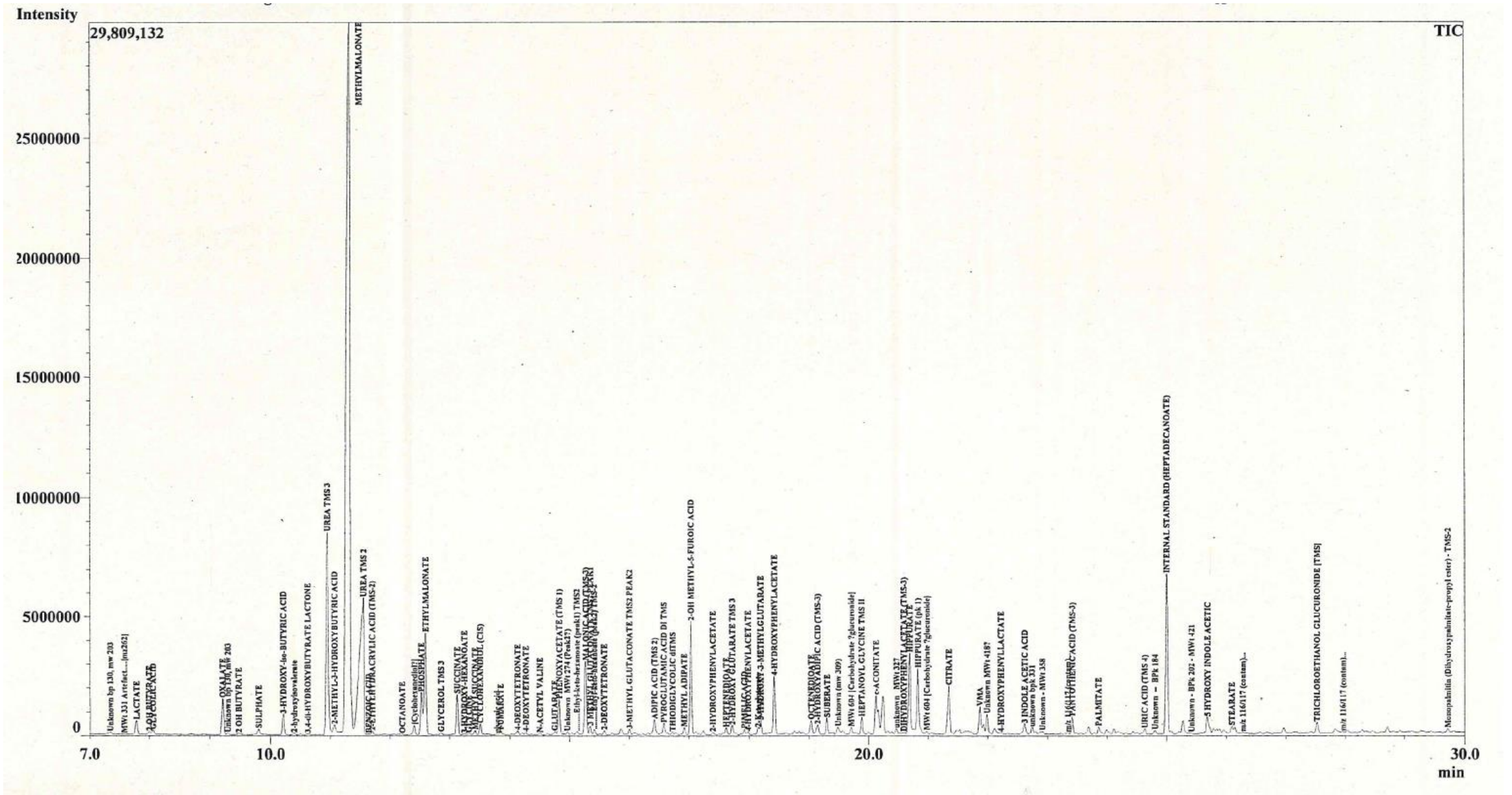


3-hydroxyadipate lactone and slight 3-hydroxyoctenedioate are the only suspicious features

Patient Info

- 3 month old baby
- No clinical details provided with organic acid request – was reported as suspicious with recommendation to investigate further if clinical features of FAOD
- No acylcarnitine request received
- Some months later fibroblast fatty acid oxidation request received with information that mother had had HELLP syndrome
- (HELLP = haemolysis, elevated liver enzymes and low platelets. Life threatening complication of pregnancy. In mothers with HELLP syndrome there is a higher incidence of LCHADD in the foetus)
- LCHAD deficiency was confirmed on fibroblast FAO studies

Chromatogram 17 – Quiz 8 (6 month old boy)



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What's of note in this profile and what tests do you want to do next?

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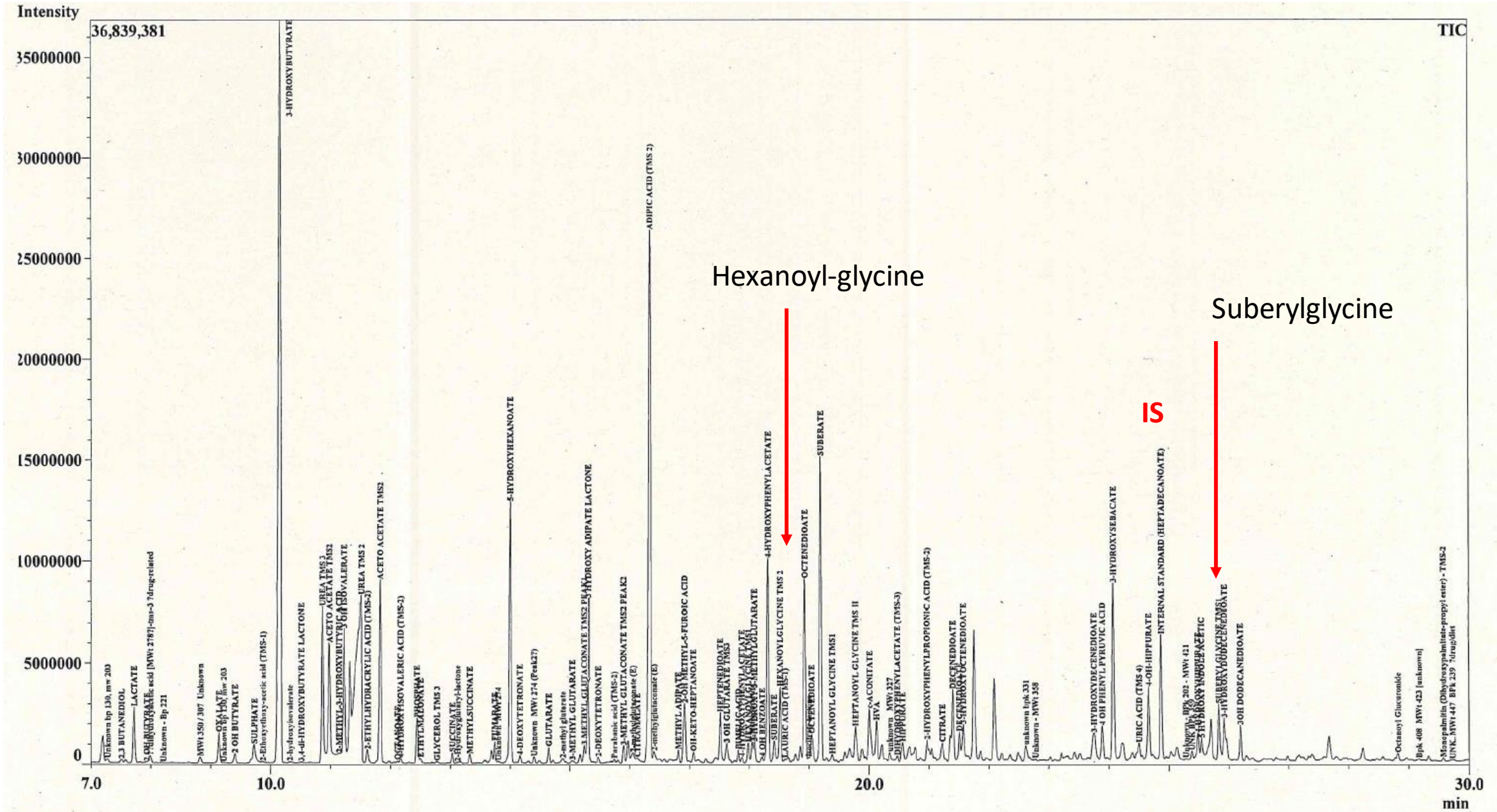
Patient Details

- 6 month old baby boy
- Septic ileus and refeeding syndrome, ? underlying metabolic cause
- Quant urine MMA 1329 umol/mmol creatinine (ref < 8)
- Total homocysteine 6 umol/L (<18)
- Plasma Vit B12 not low

- DBS Acylcarnitines – NSA including normal C3 (0.22 umol/L, ref <3.6)

- Raised Methylmalonic acid and mild / moderate malonic acid – with *normal* C3 acylcarnitine = Combined Malonic and Methylmalonic Aciduria (CMAMMA) = ACSF3 gene defect
- ACSF3 codes for an AcylCoA synthetase for MA and MMA – so do not make the MA-acylCoA or MMA-acylCoA (hence lack of raised C3)

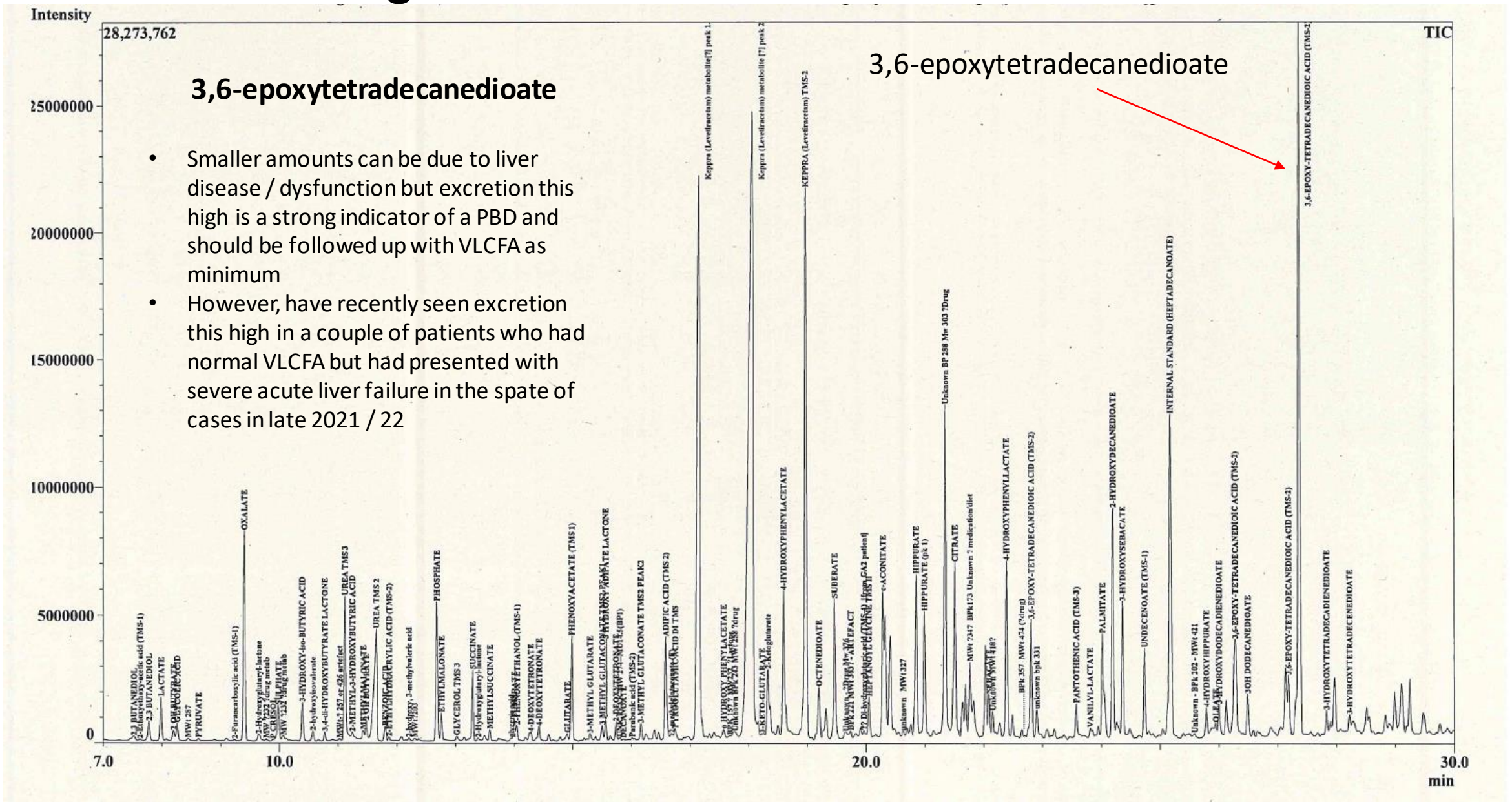
Chromatogram 18 - Crisis MCADD



Crisis MCAD profile

- This sample came from a 6 day old baby who presented in crisis with metabolic acidosis and floppiness - ? Organic acid disorder
- NBS sample taken on Day 5 just prior to presentation
- Results from screening and diagnostic samples became available on same day
- Very unusual to see a crisis profile now, in the era of newborn screening
- But we shouldn't forget what they look like though, or the extent to which patients can be ketotic
- Some crisis profiles will have a more or less proportionate DCA, others will be disproportionate but still with significant ketosis – but the hexanoyl, suberyl and phenylpropionylglycine gives it away (NB don't forget PPG not seen in neonates)
- Not uncommon for clinicians to express surprise at diagnosis because "we dipsticked the urine and it was strongly positive for ketones"

Chromatogram 19 – Severe Peroxisomal Biogenesis Disorder / Classical Zellweger



3,6-epoxytetradecanedioate

- Smaller amounts can be due to liver disease / dysfunction but excretion this high is a strong indicator of a PBD and should be followed up with VLCFA as minimum
- However, have recently seen excretion this high in a couple of patients who had normal VLCFA but had presented with severe acute liver failure in the spate of cases in late 2021 / 22

Summary

- It can be difficult to interpret non-specific findings such as 3-methylglutaconic aciduria or raised TCA cycle intermediates – but knowing the range of possibilities and the context of the patient can help you to point clinicians in the right direction for confirmation / further investigation
- Some of these disorders are only going to be fully diagnosable by genetics
- Beware small children with recent history of going to a play centre or similar
- MCADD patients can go surprisingly ketotic – we just don't see it much anymore