

# Pitfalls of Acylcarnitine Analysis

Anthea Patterson  
Biochemical Genetics

St James  
University Leeds



# Complications Acylcarnitine Measurement and Interpretation

- Analytical problems
- Low C0
- Cord blood



# Analytical Problems

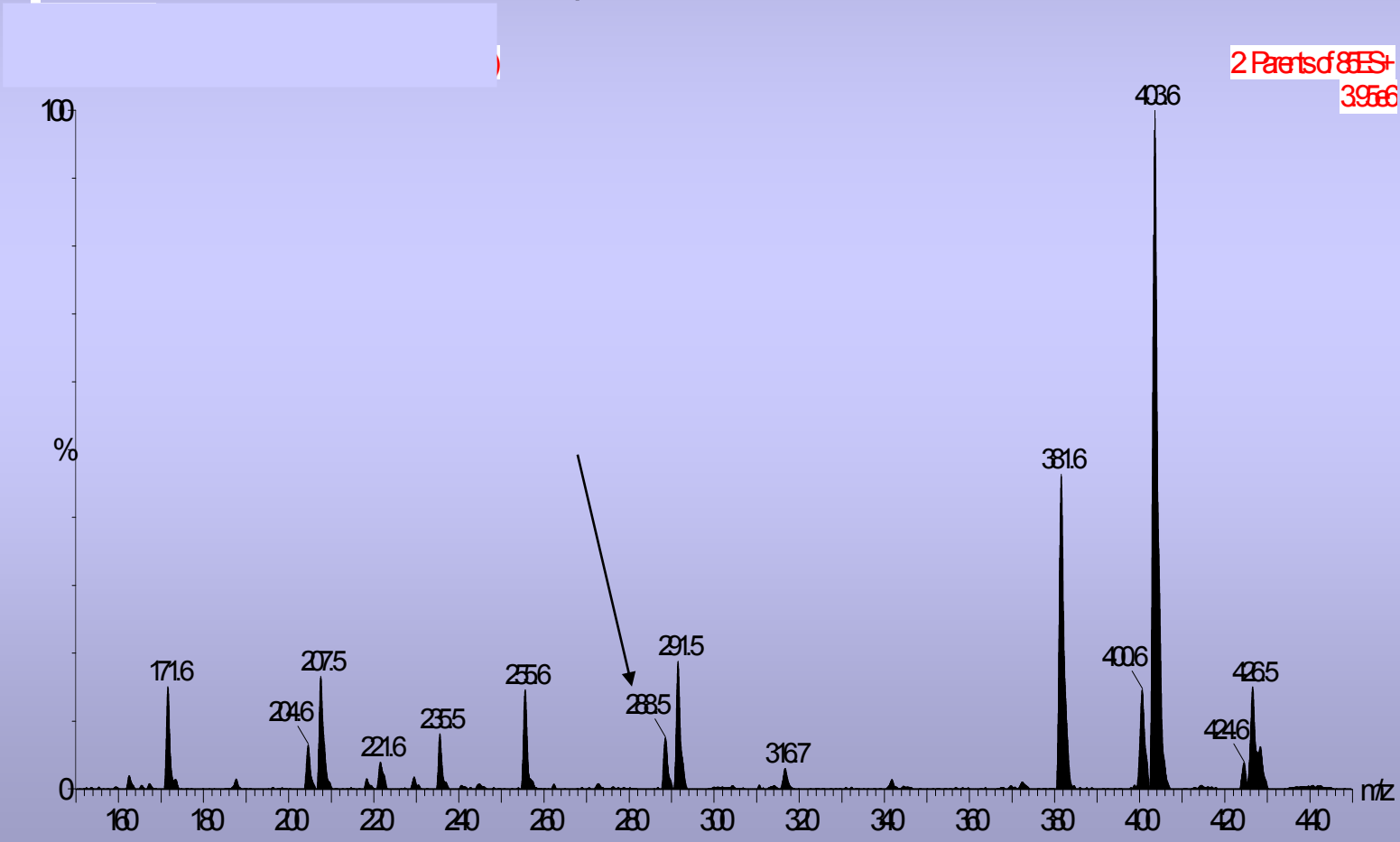
## Analytical interference

- Isobaric interference
- General contamination



# Baby AD

Octanoyl Carnitine = 1.5  $\mu\text{mol/l}$   
Free carnitine = 10  $\mu\text{mol/l}$   
(C10:1 C6 not elevated)



# Clinical Referral

- Family contacted by local consultant
- MCAD protocol followed
- Samples taken
- Information given

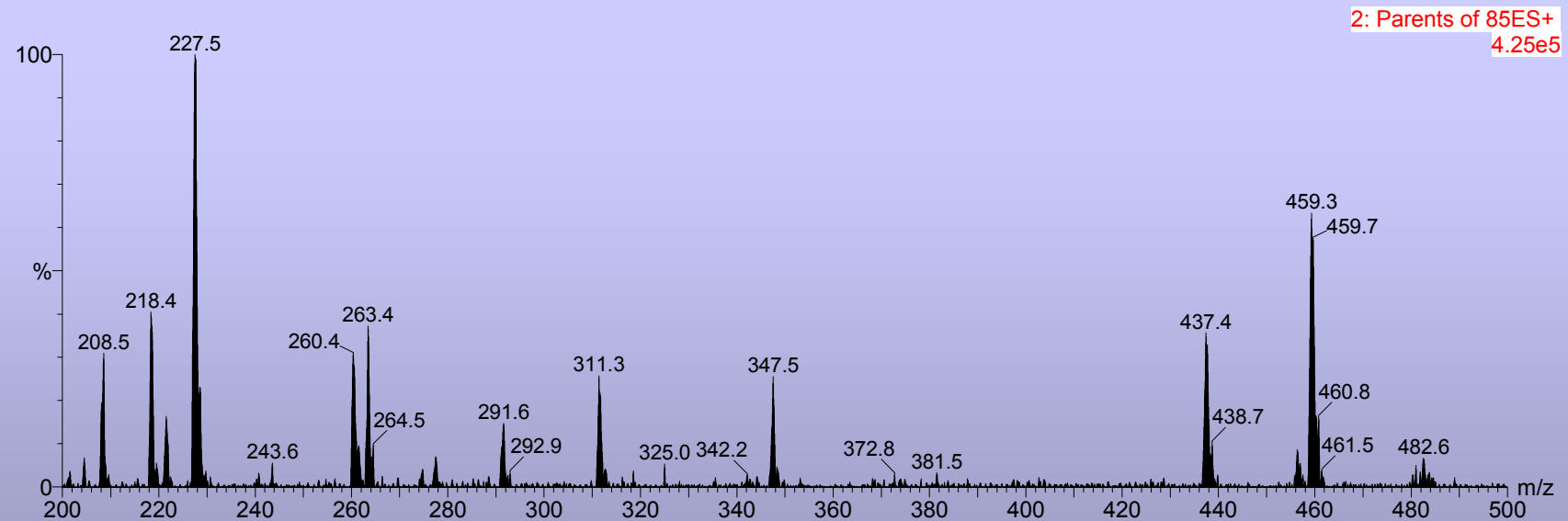


# Follow Up Investigations

- Octanoyl carnitine = 0.15  $\mu\text{mol/l}$
- Free carnitine = 22.7  $\mu\text{mol/l}$
- Organic acids = NAD

8

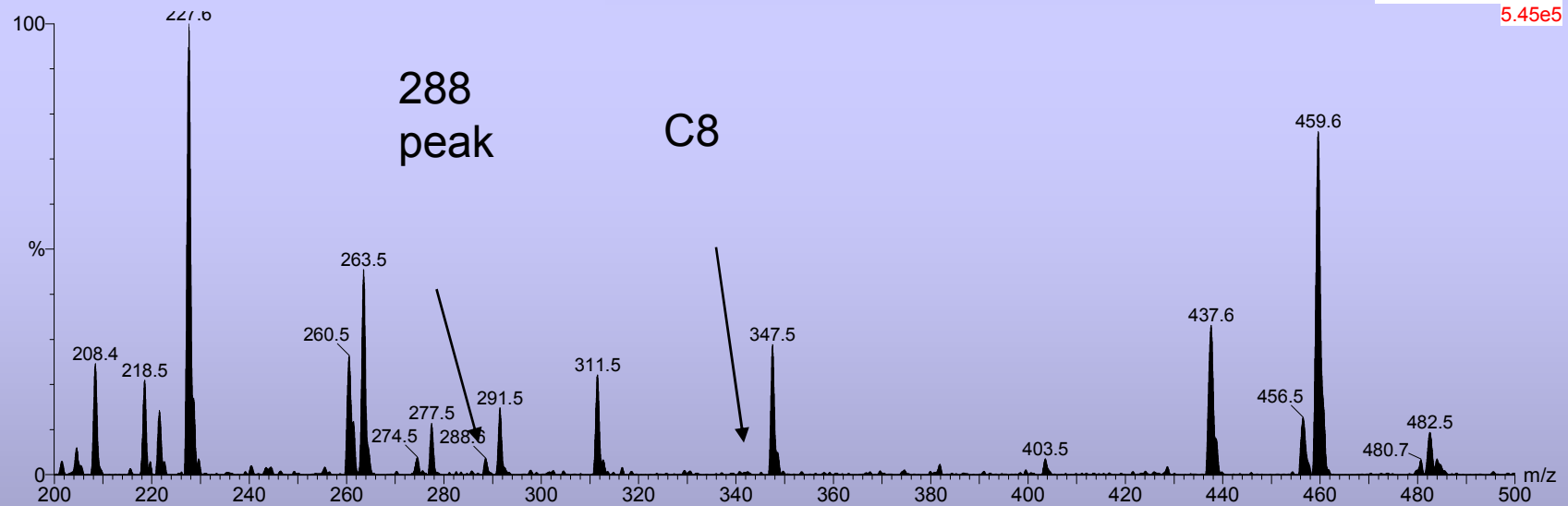
9241



# Screening Specimen: Derivatised

Octanoyl carnitine = 0.09  $\mu\text{mol/l}$

Free carnitine = 14.2  $\mu\text{mol/l}$



# ? Source of Contamination

- 4 blank spots from various parts of the cards.
- 288 ion present on blank  
C8 = 1.1, 1.0, 0.96, 0.5





# Action

Derivatise all elevated C8 specimens prior to referral.



# Two Case From Sheffield

BR

Male, 29/40, SCBU

Day 5 dbs:

C8 = 1.53  $\mu\text{mol/l}$  (1.6, 1.27/1.72)

C0 = 25  $\mu\text{mol/l}$

Acylcar full scan underiv:

atypical, C5:1 increased in addition  
to C8,

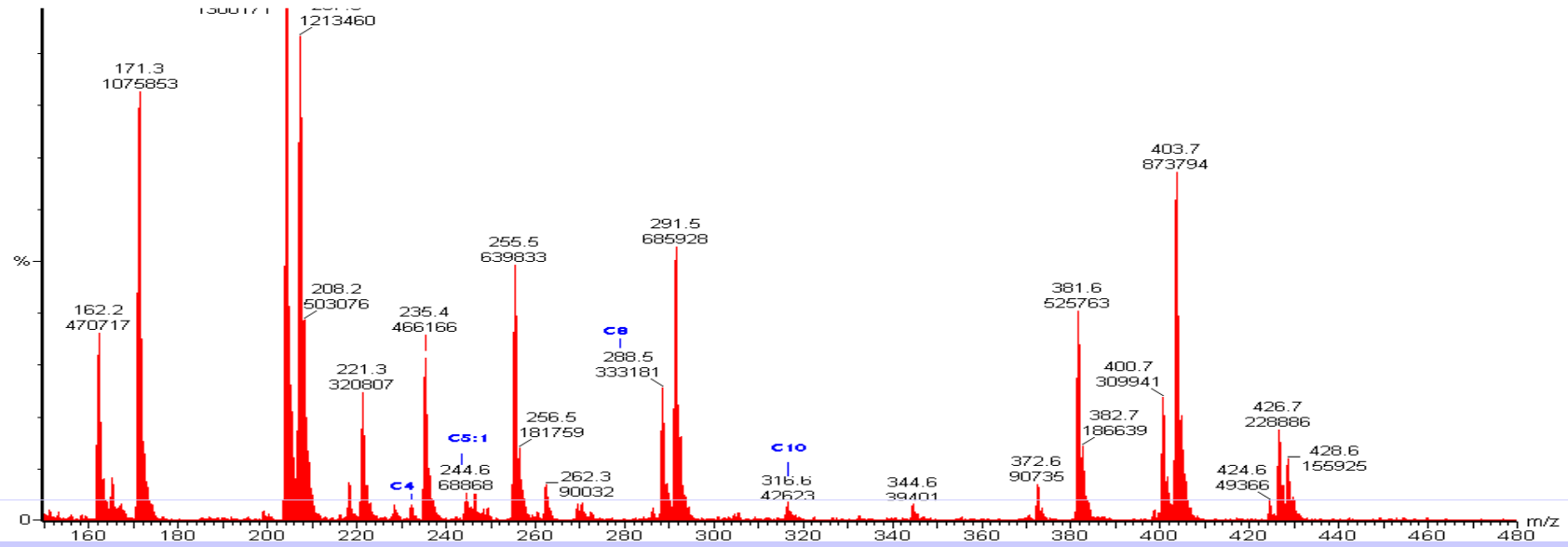
C6 and C10:1 normal



23-Dec-2004

Parents of 85ES+  
1.30e6

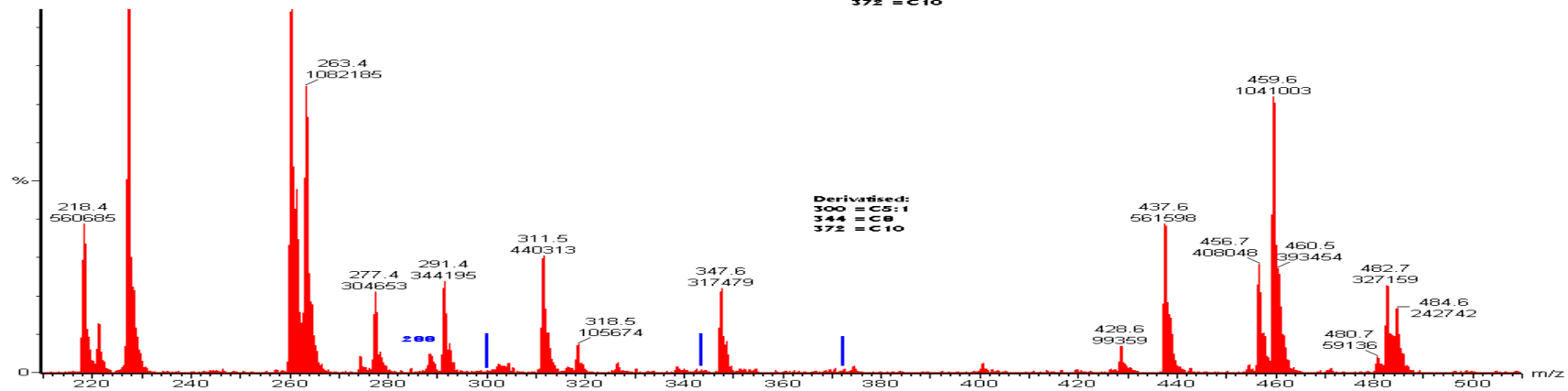
UNDERIVATISED



23-Dec-2004

Parents of 85ES+  
1.45e6

Derivatised:  
344 = C8  
300 = C5:1  
372 = C10



# Sheffield

- Contamination on blank card
- All samples (7) from SCBU over 2 week period reviewed – all samples and blank card normal
- SCBU discarded all stored cards and improved storage conditions

# Contamination Problems

- Microtitre plates.  
New batch of Greiner plates- Every specimen on run had C8 of  $\sim 1.3 \mu\text{mol/l}$ . Changed to Corning plates.
- Transfer plates
- Pipette tips.
- Instrument failures – Backup essential



# Isobaric Compounds

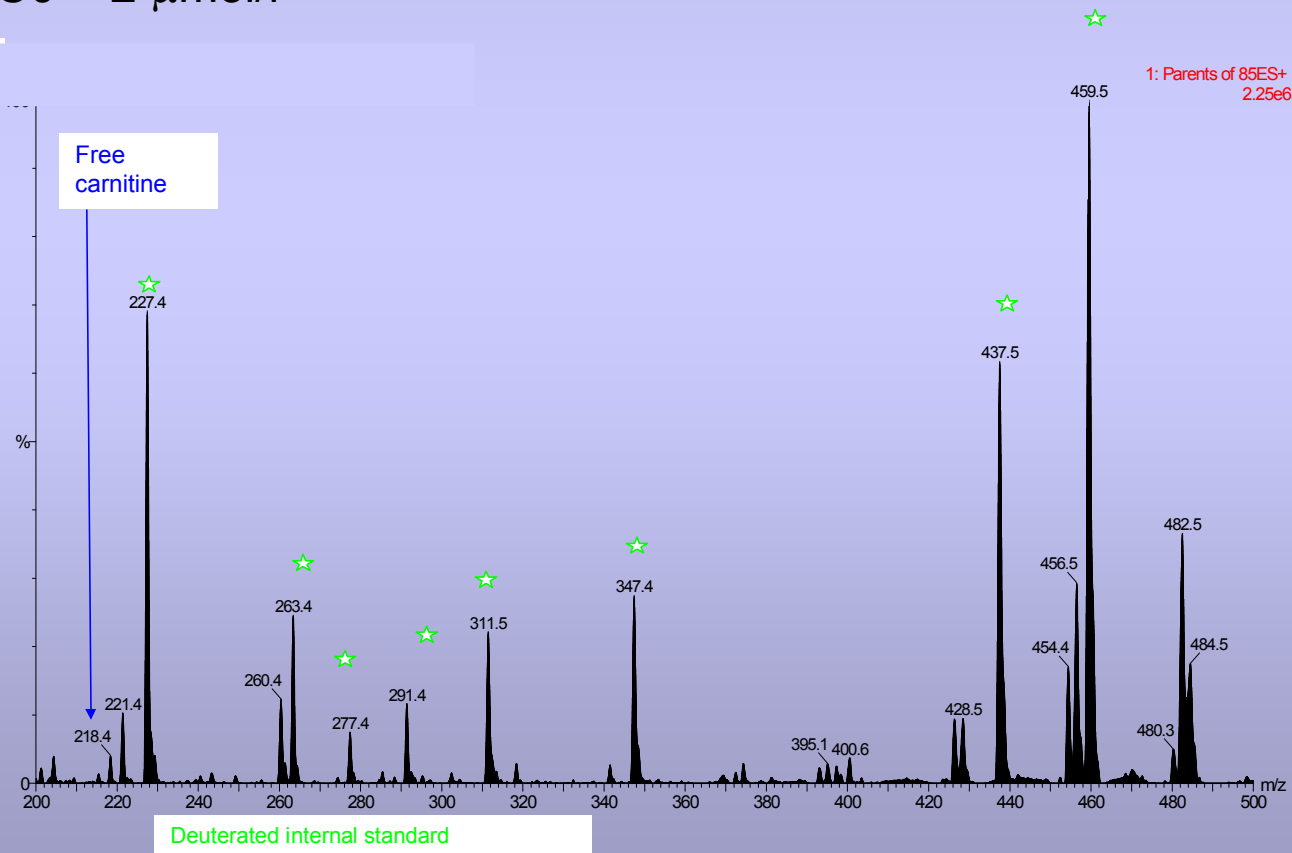
Acylcarnitine	MRM (butyl)	MRM (underiv.)	Disorder
C0	218 > 85	162 > 85	PCD
<b>C2</b>	<b>260 &gt; 85</b>	204 > 85	<b>(Glutamate)</b>
C3	274 > 85	218 > 85	MMA; PA
C4	288 > 85	232 > 85	EMA;SCAD; GA2
C5:1	300 > 85	244 > 85	PA; BKT
C5	302 > 85	246 > 85	IVA; GA2
<b>C4-OH</b>	304 > 85	<b>248 &gt; 85</b>	(Ketosis)
C6	316 > 85	260 > 85	GA2 (MCAD)
<b>C5-OH</b>	318 > 85	<b>262 &gt; 85</b>	Biot;IVA;BKT;3HMG
<b>C8</b>	344 > 85	288 > 85	MCAD / <b>[?]</b>
<b>C3-DC</b>	<b>360 &gt; 85</b>	<b>248 &gt; 85</b>	Malonic Aciduria
<b>C8-OH</b>	<b>360 &gt; 85</b>	304 > 85	(Metab Crisis)
C10:1	370 > 85	314 > 85	MCAD
C10	372 > 85	316 > 85	GA2
<b>C4-DC</b>	374 > 85	<b>262 &gt; 85</b>	[MMA]
<b>C5-DC</b>	<b>388 &gt; 85</b>	276 > 85	GA1 ; (GA2)
<b>C10-OH</b>	<b>388 &gt; 85</b>	332 > 85	(Metab crisis)
C12:1	398 > 85	342 > 85	[B-oxidn]
C12	400 > 85	344 > 85	(B-oxidn]

# Problems With Carnitine Depletion



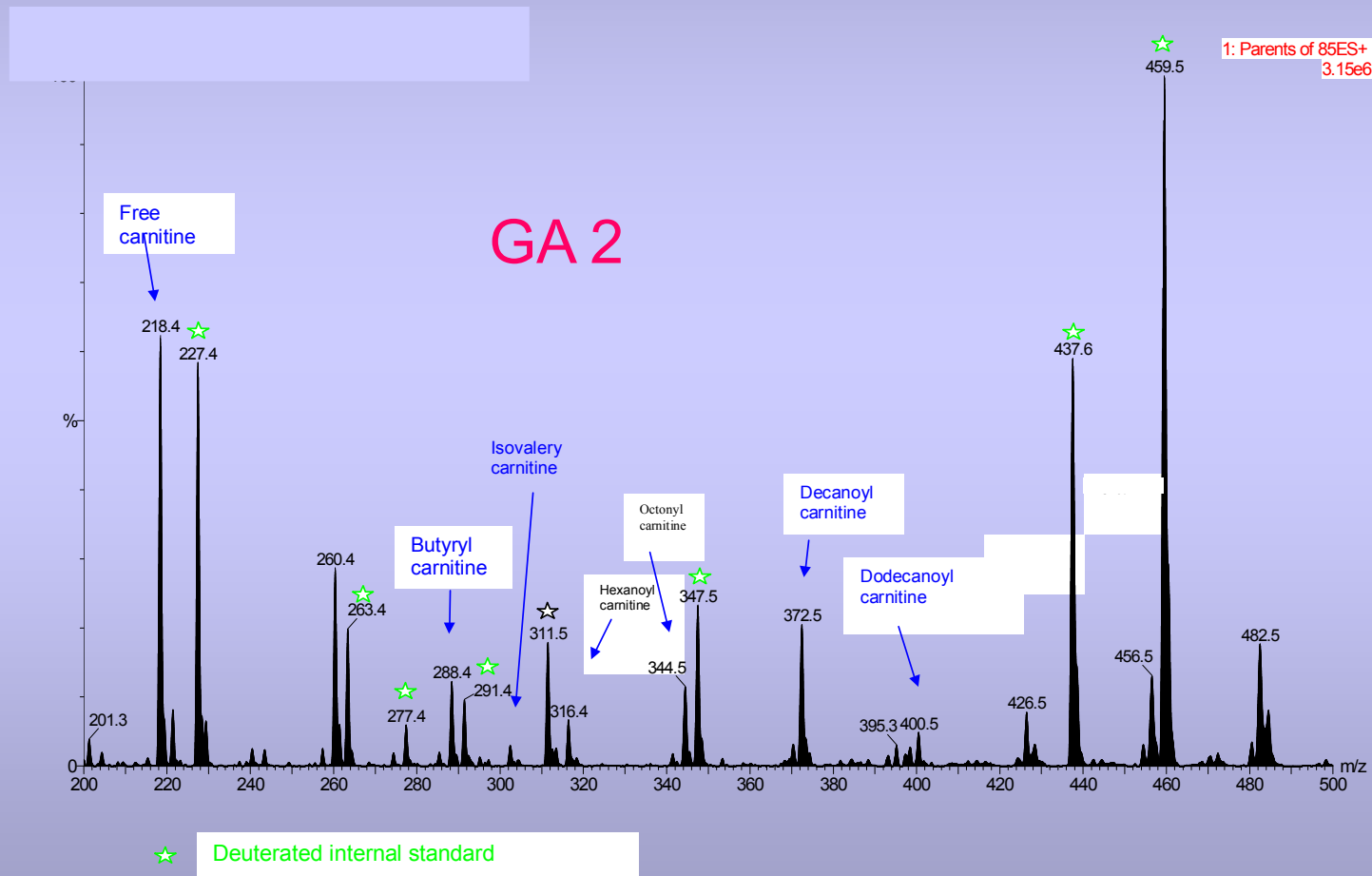
# Initial Specimen

- VF 14 years
- Collapse, Coma
- C0 = 2  $\mu\text{mol/l}$





# Post carnitine supplementation



# Low Free Carnitine

- Included in the MCADD protocol.  
(To prevent erroneously low C8 due to carnitine depletion.)
- Free carnitine  $\leq 2.0$  (mean of analysis in triplet )



# Carnitine Transporter Deficiency

- 4 cases: Infant
- 4 cases : Maternal

Mean CO at screening:  
1.05  $\mu\text{mol/l}$



# Clinical importance of CTD diagnosis

## **Maternal CDT.**

- No cardiomyopathy
- All have myopathy ( aches and pains)
- Easy fatigability

## **Babies**

- All on supplements – all doing well
- One baby – withdraw from Rx. Wg loss and FTT
- Recommended symptomatic improvement



# Cord Blood Analysis

- Joint project, Manchester and Leeds
- Cord blood was collected from births in high IEM prevalence areas
- 25,000 samples over two years
  - High C3s
  - Maternal diagnoses
  - False negatives



# Problems with early C3s

	CB	Day 1	Day3	screening	Organic acids
	C3 μmol/l	C3 μmol/l			
Corb Blood Study	<b>7.8</b>		<b>6.4</b>	<b>0.8</b>	No abnormality detected @3 day
Diagnostic specimen/sib with PA	<b>9.2</b>	<b>52</b>			Pattern consistant with PA @1day

Leeds diagnostic ref : 0.3 – 2.6  
 CDC screening cut off, mean: 7



# Early diagnosis GA2

		C3	C4	C5	C6	C8	C10	C10:1	C5DC	C12	C14	C16
Cord blood	<b>17.2</b>	<b>0.84</b>	<b>0.67</b>	<b>0.73</b>	<b>0.09</b>	<b>0.08</b>	<b>0.12</b>	<b>0.1</b>	<b>0.03</b>	<b>0.27</b>	<b>0.5</b>	<b>2.28</b>
24 hrs old	<b>22.7</b>	<b>1.01</b>	<b>12.0</b>	<b>3.63</b>	<b>3.44</b>	<b>5.34</b>	<b>3.91</b>	<b>0.63</b>	<b>0.91</b>	<b>4.97</b>	<b>3.16</b>	<b>8.09</b>



# Maternal diagnosis

- 2, Carnitine Transporter Deficiency
- 1, MCAD
- 1, MCC Deficiency



# The End

