Galactosaemia and Immunoreactive Trypsin

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Raised serum or dried bloodspot immunoreactive trypsin (IRT) may occur in:-

- Occlusion of pancreatic ductules/ducts
- Hyperconcentration of ductular fluid (for example in Cystic Fibrosis where the Cystic Fibrosis Transmembrane Regulator (CFTR) is defective)
- Exocrine pancreatic inflammation/necrosis
• In infancy raised serum or bloodspot IRT is a characteristic finding in Cystic Fibrosis

• Used as the basis of newborn screening programmes for CF
IRT is not ideal!

- Newborns have higher values of IRT than adults.
- Values decrease to adult levels by about 6 wks of age.
- Most babies with CF (~98%) will have raised values of IRT on days 5-8.
• When tested on days 5-8 there is overlap between unaffected babies and CF babies.

• Therefore a second step is added to the screening process. This can be a second IRT, a DNA test or a combination of both.

• In CF babies the IRT remains elevated when retested at approximately 4 wks of age.
Two Stage IRT Screening

Day 6-14 sample

IRT in singleton

IRT less than 60µg/L
Report CF Not Suspected

IRT greater than 60µg/L
Average less than 70µg/L

Average greater than 70µg/L

Repeat Blood Sample Requested

Blood spot tested for common mutation, Clinical referral & Sweat test if indicated

Average greater than 60µg/L

Re-assay in duplicate

Average less than 60µg/L

Report CF Not Suspected

Assay in duplicate
IRT day 5-8

IRT <60 ug/L

CF NOT SUSPECTED

IRT >60 ug/L

Repeat in duplicate

IRT <70 ug/L

IRT >70 ug/L

Mutation Analysis
DF 508

Negative
DF 508 not detected

If initial IRT <90 ug/L
Second card for IRT day 27
Second IRT <60 ug/L
Probable CF
Report to paediatrician
CF not suspected
Second IRT >60 ug/L
1 mutation detected
Second card for IRT day 27
29 mutation panel including DF 508
2 mutations detected

1 or 2 DF 508 mutations detected
Second IRT <60 ug/L
Probable carrier
Report to paediatrician
Second IRT >60 ug/L

IRT-DNA-IRT Protocol

IRT >70 ug/L

IRT >60 ug/L
Non-CF causes of raised IRT

Faecal contamination of the bloodspot card

ΔF508 heterozygotes
Hypoxic insult to pancreas
Renal insufficiency
Congenital heart disease
Nephrogenic DI

Spina bifida
Gastroschisis
Viral infections
Trisomies 13, 18

Mechanisms not always clear!
Serum IRT in galactosaemia

15/19 galactosaemias had raised IRT
• Raised IRT was not seen in other inherited metabolic disorders with renal Fanconi syndrome, including those with liver damage (Wilson’s, tyrosinaemia type 1) – intracellular trapping of phosphate is not a feature of these disorders

• However number of cases very small
Serum IRT pre and post treatment in galactosaemia

- Pre galactose free diet.
- Post galactose free diet

99th centile
sIRT Pre and post treatment

- Routine PKU/CHT/CF screen (E. Anglia)
  A and B already acutely ill in hospital.
  C and D at home.

- Pre galactose free diet.

- Post galactose free diet

![Graph showing sIRT levels over age (days)]
## Biochemical results

<table>
<thead>
<tr>
<th>Patient A</th>
<th>At diagnosis</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>325</td>
<td>139</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>2648</td>
<td>1378</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>467</td>
<td>139</td>
</tr>
<tr>
<td>Phe (µmol/L)</td>
<td>357</td>
<td>normal</td>
</tr>
<tr>
<td>Tyr (µmol/L)</td>
<td>923</td>
<td>523</td>
</tr>
<tr>
<td>Met (µmol/L)</td>
<td>90</td>
<td>normal</td>
</tr>
<tr>
<td>Amino aciduria</td>
<td>gross</td>
<td>normal</td>
</tr>
<tr>
<td>IRT (µg/L)</td>
<td>181</td>
<td>normal</td>
</tr>
</tbody>
</table>
## Biochemical results

<table>
<thead>
<tr>
<th></th>
<th>At diagnosis</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient B</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino aciduria</td>
<td>Gross</td>
<td>Mild</td>
</tr>
<tr>
<td>IRT (µg/L)</td>
<td>175</td>
<td>Normal</td>
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<tr>
<td><strong>Patient D</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino aciduria</td>
<td>Gross</td>
<td>Normal</td>
</tr>
<tr>
<td>IRT (µg/L)</td>
<td>130</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Patient E</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino aciduria</td>
<td>Gross</td>
<td>Normal</td>
</tr>
<tr>
<td>IRT (µg/L)</td>
<td>200</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Large number of the more common mutations are located in the nucleotide binding folds of the protein.

Normal function is dependent on the adequate supply of ATP for phosphorylation of the regulator domain and allosteric binding of ATP to the nucleotide domains. Also $\text{Na}^+/\text{K}^+$ ATPase required to maintain the electrochemical gradient for $\text{Cl}^-$ to exit the apical membrane.
According to “Scriven”

“Modest inhibition of mitochondrial ATP synthesis, even with small changes in tissue ATP, will disrupt transport processes.”

Do conditions which depress the ATP pool affect the action of CFTR and mimic the pathophysiology in CF?
Babies in intensive care

Day 1 Guthrie’s undertaken
(paper chromatography of amino acids)

Observed that babies with raised alanine
(usually related to hypoxia) had raised IRTs.
Unexplained lactic acidosis

Female infant aged 4m
Failure to thrive, steatorrhoea, renal tubular acidosis

Raised IRT, normal sweat test, negative for common CF mutations

Plasma lactate = 5.5 mmol/l
CSF lactate = 4.2 mmol/L
Age 9m – viral gastroenteritis triggered seizures and encephalopathy

Raised IRT persisted

CT scan: Mild diffuse atrophy affecting brain stem, cerebellum and cerebrum
Respiratory chain enzyme complexes normal (muscle biopsy)

Fibroblast pyruvate dehydrogenase assay repeatedly just below the reference range (?PDH heterozygosity)
Summary

Serum IRT concentrations in untreated galactosaemia are comparable to those seen in CF.

Values may be normal prior to development of acute symptoms and signs.

IRT rapidly declines with dietary treatment.

The decline mirrors improving amino aciduria rather than acute markers of liver disease.
Is energy-deficit the common link between the observed renal absorption defect and the raised sIRT in galactosaemia?