Neonatal Biochemistry
Investigation for Inherited Metabolic Disorders (IMDs)

Anne Green
Birmingham Children’s Hospital

Leeds May 2005
• Overview of how IMDs present
  – cases

• Range of Disorders & Incidence
  – disorders presenting in the newborn

• Approach to Investigation
Presentation of IMD

• Intrauterine
  – HELLP
  – AFLP
• **HELLP**
  - Haemolysis
  - Elevated Liver Enzymes
  - Low Platelets

• **AFLP**
  - Acute Fatty Liver of Pregnancy
    - severe liver dysfunction
    - thrombocytopenia
LCHADD

• Long chain 3-hydroxy acyl CoA dehydrogenase deficiency
  - defect of mitochondrial fat oxidation (1990)
  - hypoketotic hypoglycaemia
  - metabolic collapse (esp. liver)
  - death
  - other problems include:
    - myopathy and cardiomyopathy
    - retinitis pigmentosa
    - FTT with diarrhoea and vomiting
  - common mutation G1528C
HELP/AFLP and LCHADD
(Wilcken et al, 1993)

• 11 pregnancies in 5 mothers (HELP)
• 6 LCHADD babies

In pregnancies where foetus has LCHADD, the frequency of pre-eclampsia related conditions is high
HELLP/AFLP and LCHADD
(Ibdah et al 1996)

• 16 families with LCHADD
  - 12 different mutations
  - G1528C found in 50% of mutant alleles
  - 11 women had AFLP
  - All these women were carrying foetuses with G1528C (5 homozygous, 6 heterozygous)

• LCHADD G1528C mutation associated with AFLP
Fatty Acid Oxidation Defects in the Foetus which can cause AFLP/HELLLP

- LCHADD
- MCADD
- Carnitine Palmitoyl transferase type 1
- SCADD
Case 1

1 month
- presented to A&E with hypoglycaemia
- collapse → ITU Died
- post mortem fatty acid changes in liver/kidney
- LCHADD diagnosed P.M. (skin fibroblasts)
Case 1

• Mother 35/40 HELLP

• Cord blood diagnosis LCHADD (acyl carnitines)

• Treatment - MCT
Investigation of HELLP and AFLP

- **Mother**
  - organic acids (urine)
  - carnitine, acyl carnitines (blood)
  - glucose, lactate, (free fatty acids, 3-hydroxybutyrate)
  - +/- LCHADD DNA

- **Baby**
  - organic acids (urine)
  - carnitine, acyl carnitines (blood)
  - LCHADD DNA
  - ? fibroblasts fat oxidation (esp if baby dies)
Presentation of IMD

• Intrauterine
  • HELLP
  • AFLP

• Birth
  – Hydrops
  – Dysmorphism
Fetal and Neonatal Hydrops

• Hydrops
  – IMDs are present in approx. 1-2% of non-immune hydrops fetalis

• Investigate if unexplained, familial or history of still-births/neonatal deaths/spontaneous abortions
Investigation for Neonatal/Foetal Hydrops

– skin (chromosomes & ? enzymes)
– blood- haemoglobinopathies
– placenta - histology
– urine (IMD) (amniotic fluid)
– liver/muscle- histology/biochemistry

– www.metbio.net
Presentation of IMD

• Intrauterine
  • HELLP
  • AFLP

• Birth
  – Hydrops
  – Dysmorphism
IMD & Dysmorphism in the neonate

- Menkes
- Zellwegers and Z like (Perox disorders)
- Lysosomal
  - GM2, ML2, MPS, Multiple sulphatase
- Congenital hypothyroidism
- Maternal PKU
- CDGS
- Cholesterol synthesis defects
- GA II
- Sulphite/xanthine oxidase
- Congenital lactic acidoses
- Mevalonic kinase
Presentation & IMD

• Intrauterine
• Birth

• SUDI
SUDI cases 1999/2000 - age at death
Sudden Unexpected Death in Infancy (SUDI)

- Explained SUDI
  - infection (respiratory, CNS, GI)
  - cardiovascular
  - accident/trauma
  - metabolic

- SIDS
  - no cause of death is found after a thorough post mortem examination
Investigation of SUDI

• Non accidental injury
  – radiology exam
  – forensic investigation option

• Infection

• Metabolic
Sudden Unexpected Death in Infancy

- Working Group RCPath & RCPCH (Sept 2004) Baroness Helena Kennedy
SUDI
Immediate Specimen Collection

• Blood
  – blood culture
  – blood spots - IMD
  – blood - lithium heparin Chromosomes
  – Blood – serum Toxicology

• Nasopharyngeal aspirate & swabs

• Urine Tox & IMD
Biochemical Investigations

• Amino acids (blood & urine)
• Organic acids (urine)
• Acyl carnitines (blood spot)
Metabolic Investigations
Quantitative plasma amino acids

• All show similar abnormalities
  – grossly increased glutamine, glycine, alanine and proline consistent with acute collapse

  – taurine, aspartate, serine, ornithine increased consistent with haemolysis/autolysis
Quantitative plasma amino acids ($\mu$M)

Upper limit of normal indicated by shaded area
Acyl carnitines in SUDI cases

- blood spots +/- plasma
- analysed by tandem mass spectrometry
Acyl carnitine results in SUDI

• most specimens show
  – increased free carnitine
  – increased short chain acyl carnitines
  – decreased long chain acyl carnitines
Metabolic Investigations (if indicated)

- Cultured fibroblasts
  - enzymes
  - DNA
IMD diagnoses in SUDI cases

- Carnitine transporter
- LCHADD - 2
- Citrullinaemia
Sick Neonate

- Well at birth/no signs or symptoms
- Family History
  - Consanguinity
  - Sibling illness/death
- Presentation
  - Non specific (e.g., poor feeding, hypotonia)
  - Symptoms relate to feeding
- Clues
  - Smell
  - Hair/skin
  - ‘Biochemical’ features
Presentation in the neonate

- Hypoglycaemia
- Acid base disturbance
  - Metabolic acidosis
  - Respiratory alkalosis
- Liver dysfunction/organomegaly
  - Jaundice
  - Hepatitis
  - Liver failure
- Neurological dysfunction
  - Seizures
  - Hypotonia
  - Conscious level ↓
  - Encephalopathy
Case 2

- Consanguinous parents, 2 older siblings both well
- 1 Sib died aged 4 days – no Dx
- Normal birth 38/40
- Well until 26 hours – jittery, not feeding
- 40h – seizures, floppy
- 45h ‘hiccups’, required ventilation
Case 2 (cont’d)

• Acid base normal
• Plasma ammonia normal
• Liver enzymes normal
• Calcium, Magnesium, Glucose normal
• Hb & FBC normal

• Lactate 3.5mmol/l (sl increase)
Case 2 (cont’d)

- Amino acids (urine)
- Organic acids
  - Increased urine glycine
Glycinuria

- Bacterial (Hippuric acid)
- Valproate therapy
- Organic acid disorder eg MMA, PA, IVA
- Non ketotic hyperglycinaemia
- Iminoglycinuria
- Prolinaemia / Hydroxyprolinaemia
- Atypical persistent hyperglycinaemia
Metabolic investigation

- csf glycine: 205 µmol/l (<20)
- plasma glycine: 1626 µmol/l (<700)
- csf:plasma glycine ratio: 0.12 (<0.03)

Consistent with non-ketotic hyperglycinaemia
Progress

• ventilation withdrawn
• died 24h later
• liver biopsy taken
  – glycine cleavage enzyme undetectable

• ante-natal diagnosis possible
CSF quantitative amino acids

- 0.5ml clear csf in plain bottle (non traumatic)

- Glycine
  - present at low concentrations (<20 µM)
  - non ketotic hyperglycinaemia
    - looking for high concentrations (usually >50 µM)

- Serine
  - present at low concentrations (approx 40 µM)
  - serine deficiency disorders
    - looking for low concentrations (usually <20 µM)
Case 3

- 1st child unrelated caucasian parents
- 38/40 – well for first 24h
- 28h – jittery (plasma glucose 4mmol/l)
- 40h – grunting respiration → NNU
  peripheral shut down
Case 3 ( cont’d)

- CSF glucose & protein – normal
- Na,K Ca ,Mg – normal
- Started antibiotics ( infection screen awaited )
- 50h – convulsions  Rx phenobarb
- 62 h – unresponsive, hyperventilating
- 72h- further convulsions & Bradycardias

- 74h – DIED ? Sepsis
Case 3 cont’d

• PM
  – Fatty liver

– Urine
  • Glutamine, alanine & citrulline +++
  • ASA anhydrides
  • Orotic acid ++
Numbers represent enzyme defects in disorders of urea cycle:

1. Carbamoyl phosphate synthetase
2. Ornithine carbamoyl transferase
3. Argininosuccinate synthetase
4. Argininosuccinate lyase
5. Arginase
6. N-acetylglutamate synthetase
Plasma (retrieved retrospectively) $\uparrow$ ASA + anhydrides $\uparrow$ Glutamine, alanine and citrulline

**DIAGNOSIS - Argininosuccinic aciduria**

ASA lyase deficiency confirmed in cultured fibroblasts
Argininosuccinic Aciduria

Neonatal onset
• Lethargy/poor feeding
• Vomiting
• Hypotonia
• Hepatomegaly
• Seizures
• Coma
Case 4

• Previous sib died at 2 days – no diagnosis
• Age 4 days
  – 24 hour history of poor feeding and excessive sleeping
  – totally breast-fed
  – on admission
    • convulsing
    • hypothermic
    • hypoglycaemic (lab glucose 0.7 mmol/l)
Case 4 - metabolic Ix

• urine organic acids
  – dicarboxylic aciduria
  – glycine conjugates (C6,C8)

• plasma carnitine
  – total 13 mmol/l (23-60)
  – free 4 mmol/l (15-53)
Case 4 - metabolic Ix

• DNA
  – G985 homozygote

Δ - medium chain acyl CoA dehydrogenase (MCAD) deficiency
MCAD deficiency

• commonest fatty acid oxidation disorder
  – approx 1 in 10,000 births in UK

• peak age of presentation 12-18m
  – 25% die during first attack

• readily treatable

• fasting tolerance improves with age
Case 4 - family history

• previous sib died at 2 days

• Coroner’s PM
  – neonatal infection due to prolonged rupture of membranes; no metabolic Ix

• tissue obtained for DNA analysis (retrospective)
  – G985 homozygote
Lessons

• importance of family history
• relationship between symptoms and feeding
• MCAD can present in the neonatal period (especially if breast fed)
Range of Disorders

• Intermediary metabolism ‘intoxication’
  – Amino acids
  – Organic acids
  – Urea cycle
  – Carbohydrates
  – Purines/pyrimidines
Range of Disorders

• Intermediary metabolism ‘intoxication’
  – Amino acids
  – Organic acids
  – Urea cycle
  – Carbohydrates
  – Purines/pyrimidines

• Energy production (liver, heart, muscle, brain)
  – Mit resp chain/electron transport disorders
  – Fat oxidation
  – Gluconeogenesis
  – Glycogen storage
### IMD Diagnoses at BCH*
#### 2000 - 2004

*excludes sibling diagnoses & at risk cases*

<table>
<thead>
<tr>
<th>Disease Type</th>
<th>0-7 days</th>
<th>7-14 days</th>
<th>15-28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCD</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NKH</td>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Fat Ox</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Org acid</td>
<td>3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>AA (Tyr &amp; MSUD)</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Galactosaemia</td>
<td>6</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Zellweger</td>
<td>2</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Elect Tr chain</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSD</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLO</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>20</strong></td>
<td><strong>17</strong></td>
<td><strong>11</strong></td>
</tr>
</tbody>
</table>
- NKH
- UCD
- Galactosaemia
- Fat oxidation
- Organic acids
Approach to Investigation

• Newborn screening
• Sib testing
• Clinical Presentation
Newborn Screening

- **Current universal screening**
  - PKU*
  - Congenital hypothyroidism
  - Duchenne (Wales)
  - Cystic Fibrosis (some areas only)
  - Sickle Cell disorders

- Plus MCADD in ~50% UK

- *plus other amino acids - MSUD, tyrosinaemia in some areas
Importance of Sib Testing

• When to test
  – need to start treatment for baby
  – when does biochem abnormality appear?

• How to test
  – Metabolite
  – Enzyme
  – DNA
Approach to Investigations

• 1st line test
  – ‘CLUES to further tests

• 2nd line tests
  – Metabolites

• 3rd line tests
  – Enymes
  – DNA
First stage Investigations

• **BLOOD**
  - Calcium
  - Glucose
  - Blood count
  - Blood gases
  - Sodium, potassium
  - Liver function
  - Urea, uric acid
  - Lactate
  - Ammonia
  - FFA
First stage Investigations

• URINE
  – Appearance & Smell
  – Colour
  – pH
  – Reducing substances/glucose
  – Ketones
  – Ferric Chloride
  – DNPH
  – Cyanide Nitroprusside
  – TLC MMA
Metabolites

- Amino acids (urine & plasma)
- Organic acids (urine)
- Acyl carnitines (blood)

- +/-vlcfa
- **Discuss with Lab**
Summary

• Consider IMD
  – Intrauterine
  – SUDI
  – Sick baby

• Commonest disorders
  – Intermediary metabolism
  – Energy metabolism

• Staged approach to Ix