Neonatal Biochemistry

Investigation for Inherited Metabolic Disorders (IMDs)

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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

HELLP/AFLP and LCHADD
(Wilcken et al, 1993)

- 11 pregnancies in 5 mothers (HELLP)
- 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/HELLP

- 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

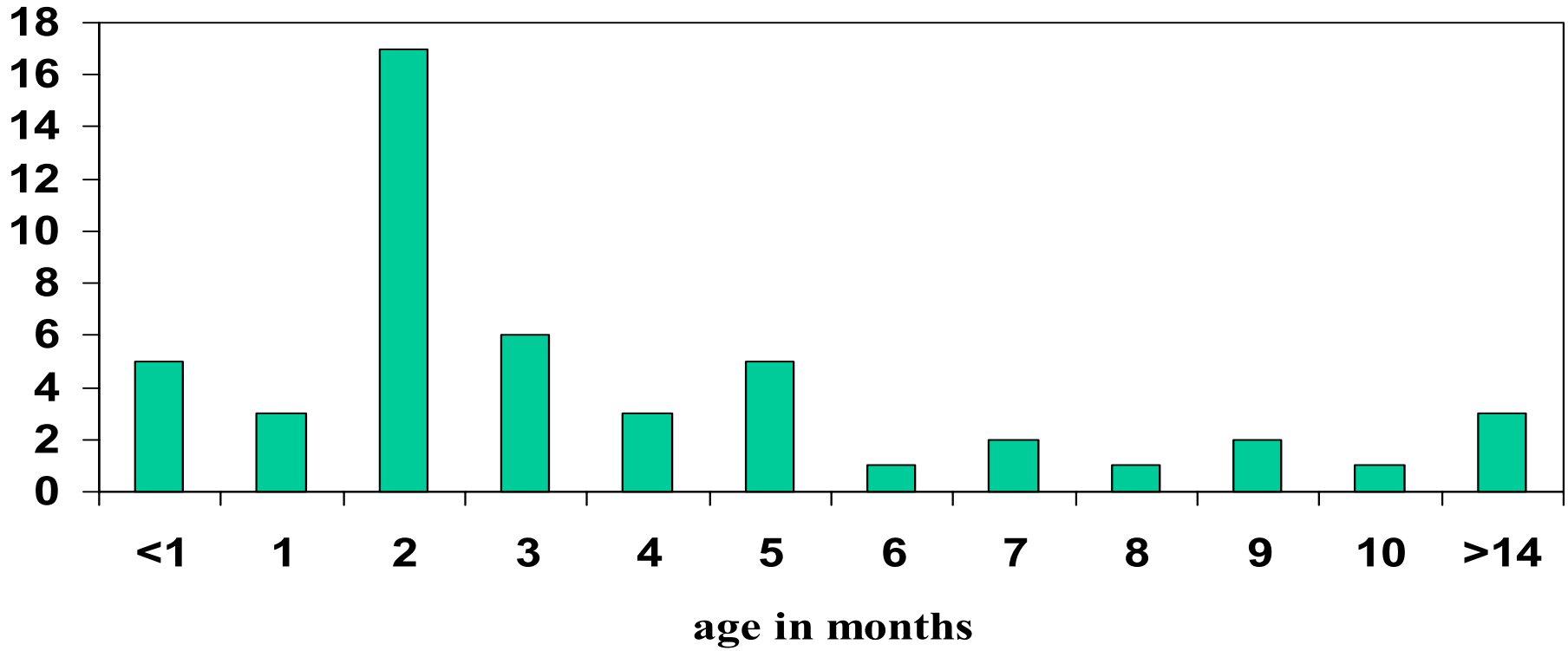
- Menkes
- Zellwegers and Z like (Perox disorders)
- Lysosomal
  - GM2,ML2,MPS,Multiple sulphatase
- Congenital hypothyroidism
- Maternal PKU
- CDGS
- Cholesterol synthesis defects
- GA II
- Sulphite/xanthine oxidase
- Congenital lactic acidoses
- Mevalonic kinase

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

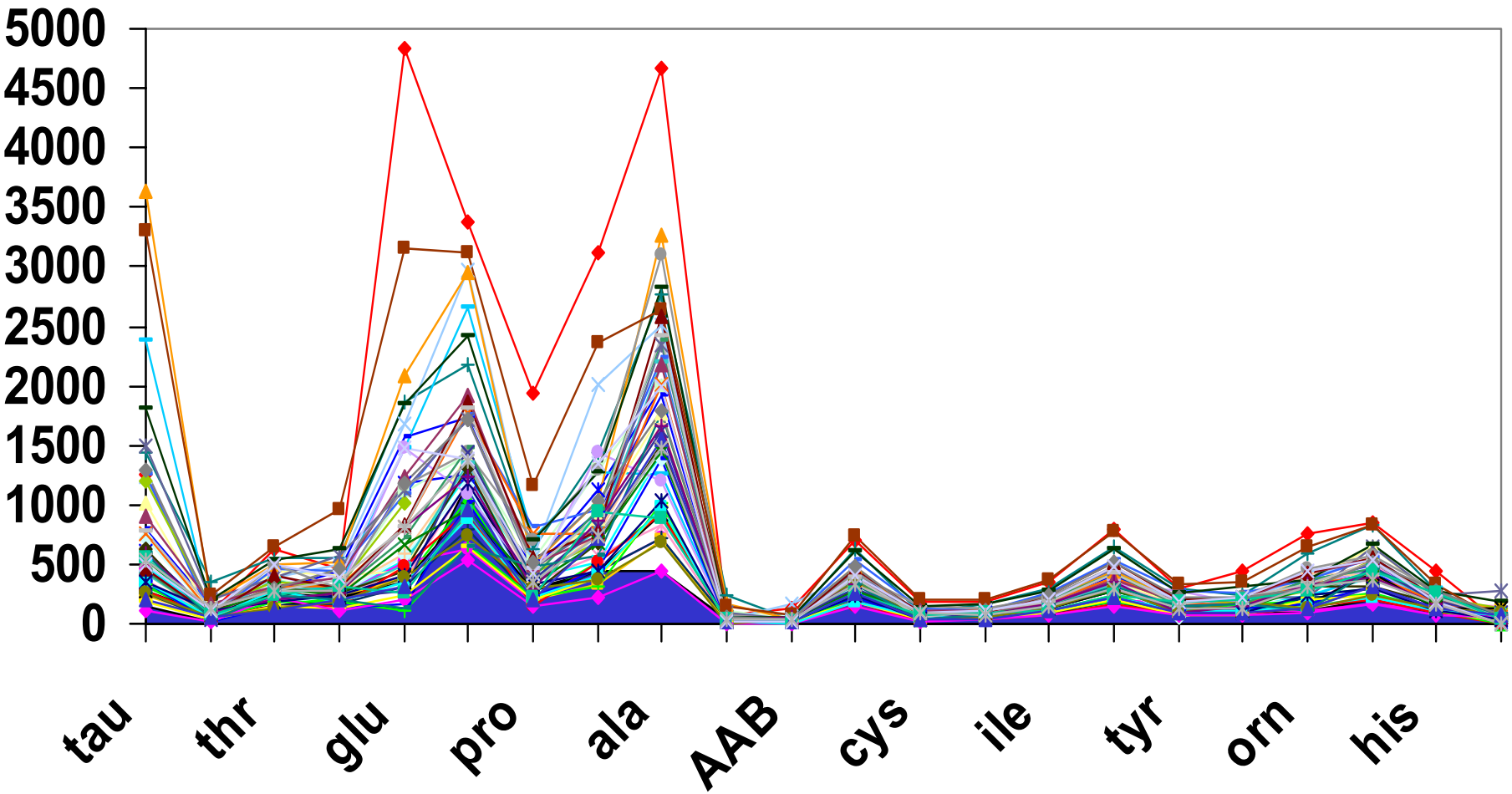
- Amino acids (blood &urine)
- Organic acids (urine)
- Acyl carnitines(blood spot)

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 (μM)

Upper limit of normal indicated by shaded area



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NHS

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 (eg 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 ↓
  - Encepaholopathy

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)

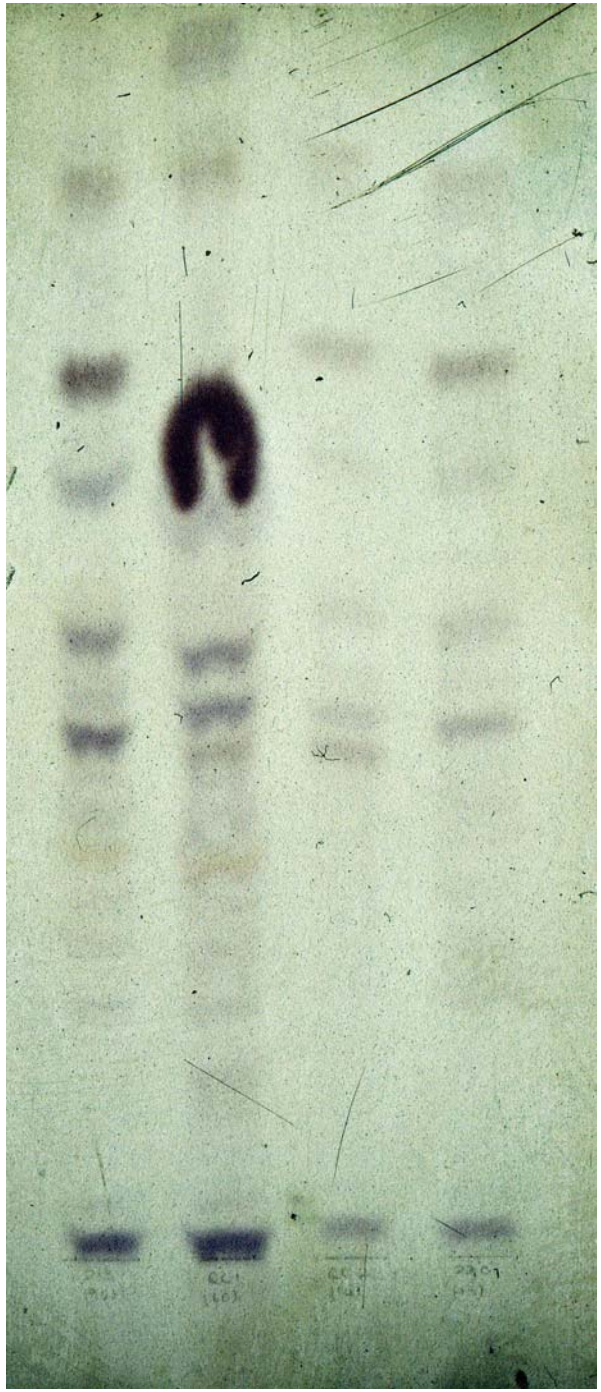
- Acid base normal
- Plasma ammonia normal
- Liver enzymes normal
- Calcium, Magnesium ,Glucose normal
- Hb & FBC normal

- Lactate 3.5mmol/l (sl increase)

Case 2 (cont'd)

- Amino acids(urine)
- Organic acids

- Increased urine glycine



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Glycinuria

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

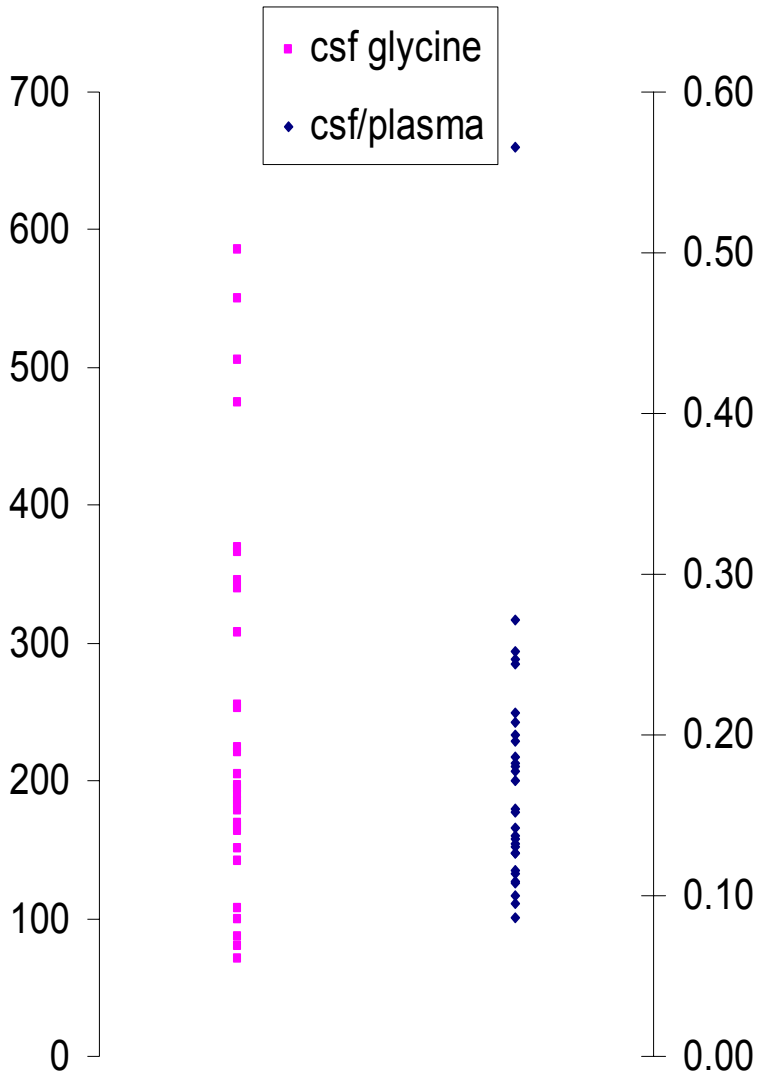
Metabolic investigation

- 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

csf/plasma glycine ratio in NKH



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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

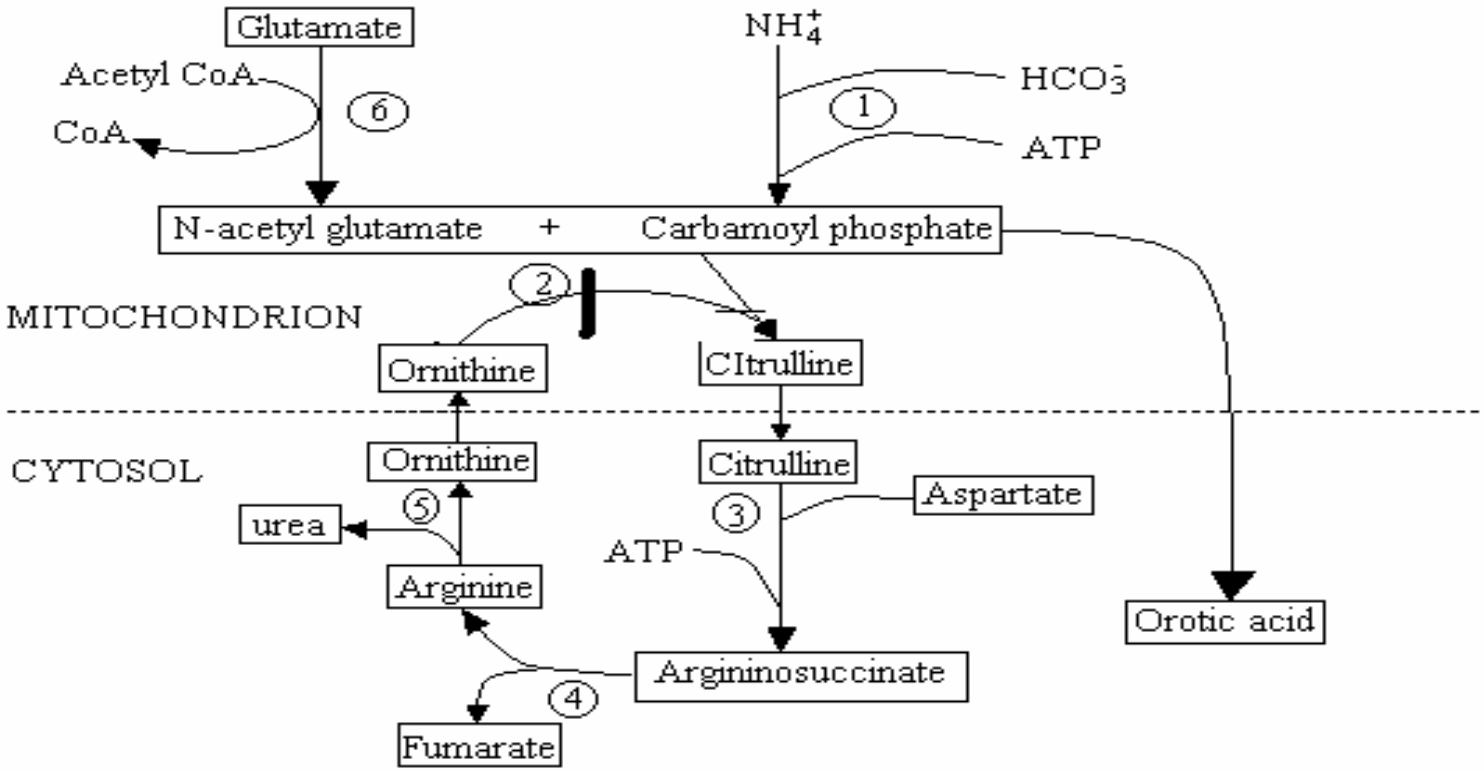
Case 3 (cont'd)

- 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

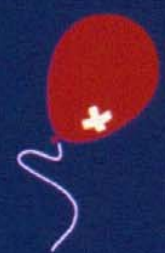
(1)	Carbamoyl phosphate synthetase	(4)	Argininosuccinate lyase
(2)	Ornithine carbamoyl transferase	(5)	Arginase
(3)	Argininosuccinate synthetase	(6)	N-acetylglutamate synthetase

Plasma (retrieved restrospectively)

↑ ASA + anhydrides
↑ Glutamine, alanine and citrulline

DIAGNOSIS - Argininosuccinicaciduria

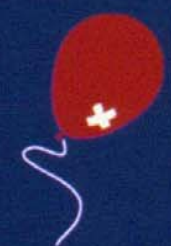
ASA lyase deficiency confirmed in cultured fibroblasts



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

	0-7 days	7-14days	**15-28 days**
UCD	7		
NKH	6	2	3
Fat Ox	3	1	1
Org acid	3		2
AA (Tyr& MSUD)	1	2	
Galactosaemia		6	1
Zellweger		2	3
Elect Tr chain		2	
GSD		2	
SLO			1
Total	20	17	11

- 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

- Ist 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