IMD and Newborn Screening

Specialist Portfolio Tutorial

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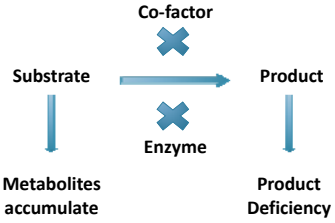
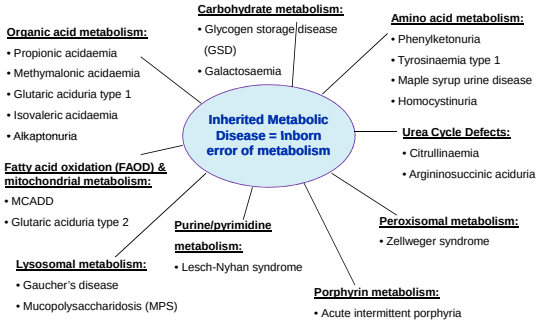
Inherited Metabolic Disease

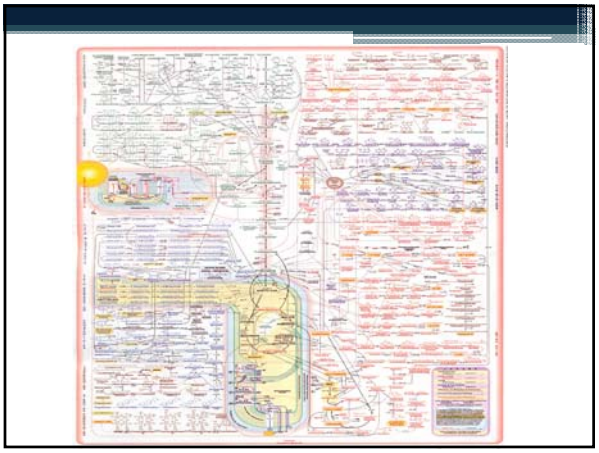
What is IMD?

- Gene mutations – prevent synthesis of protein or cause synthesis of abnormal protein
  - Generally protein = enzyme
  - Results in ↓ catalytic activity of enzyme in synthetic pathway

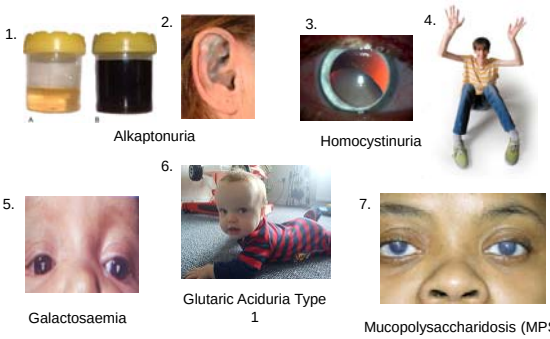


What is IMD?





Clinical presentation



Clinical presentation

Disorder	Odour
Untreated PKU	Mouse/animal-like
IVA, GA II	Acrid (sweaty feet)
Tyrosinaemia Type 1	Cabbage
	Rancid Butter
Fish odour syndrome (Trimethylaminuria)	Fish-like
Maple Syrup urine disease (MSUD)	Maple syrup

Clinical presentation

- Related to toxic metabolite accumulation and/or effects to the distal pathway.

Often non-specific/ not suggestive of particular disease:	Onset of symptoms (disease-dependent):	Other Associations
▫ Lethargy ▫ Hypotonia (floppy baby – energy deficiency) ▫ Vomiting ▫ Fits/seizures (encephalopathy) ▫ Poor feeding (energy deficiency) ▫ Irritability	▫ Fasting ▫ Increased exercise ▫ Infection ▫ Changes in carb/protein intake ▫ GSD – Hypoglycaemia when fasting ▫ Galactosaemia – Following galactose ingestion ▫ FAODs – Fasting, infection	▫ Family History ▫ Consanguinity ▫ Hx multiple miscarriages ▫ Unexplained death (Anything unexplained or unexpected)

First-line investigations

First-line investigations

- Should be performed in every child with an acute illness in whom a metabolic disorder is a possibility
  - Glucose
  - Ammonia
  - LFTs
  - Lactate
  - CK
  - FBC
  - Coag
  - Blood gases
  - Urine ketones
  - Urate
  - Cholesterol

Hypoglycaemia
Hyperammonaemia
Hyperbilirubinaemia
Lactic acidosis

Hypoglycaemia

Definition

Venous plasma glucose <2.5 mmol/l

Whipple's triad:
- Plasma glucose low
- Clinical features typical of hypoglycaemia
- Symptoms resolve when glucose administered

Clinical features

- Adrenergic
  - Pallor, anxiety, sweating, tachypnoea tremor, weakness, nausea and vomiting.
- Neuroglycopenic
  - Jitteriness, hunger, abdominal pain, apnoea, headache, confusion, feeding problems, visual disturbances and convulsions and coma.
- Neonate (Non-specific)
  - Irritability, lethargy, hypotonia, feeding problems, cyanosis, apnoea/tachypnoea, hypothermia, pallor

Causes

Endocrine
Hyperinsulinism Adrenal insufficiency Hypopituitarism Growth Hormone Deficiency Hypothyroidism
Metabolic
Disorders of Fatty Acid Oxidation and Carnitine Transport Disorders of Carbohydrate Metabolism Disorders of Organic Acid Metabolism Disorders of Gluconeogenesis
Other Causes
Neonatal complications: prematurity, birth asphyxia, congenital heart defects, infants of diabetic mother – secondary hyperinsulinism Drug Related: insulin, alcohol, aspirin, chemotherapy Liver and multi-organ failure Sepsis, Gastroenteritis
Idiopathic ketotic hypoglycaemia
The most common cause for hypoglycaemia in children after the neonatal period is idiopathic ketotic hypoglycaemia. This is usually precipitated by an often relatively mild illness.

Investigation

- Need to collect samples at time of hypoglycaemia/prior to glucose administration

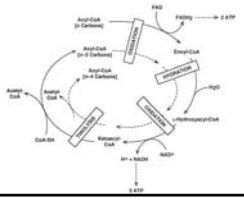
Analyte	Minimum volume and common preservative
Glucose 3 OH butyrate Free Fatty Acids Lactate	2ml Fluoride Oxalate
Insulin Cortisol Growth Hormone Amino Acids	3ml Lithium Heparin
Acyl carnitines	1ml Lithium Heparin or 2-3 blood spots collected on a Guthrie Card

3. Collect first passed urine sample for -
Ketones
Reducing substances
Organic acids
Toxicology screen (only required if there is a clinical suspicion of factitious or accidental illness).

Fatty acid oxidation defects

- Mitochondrial β oxidation of FA is a major source of cellular energy during fasting, prolonged exercise or illness
- Lypolysis – long-chain FA released from stored triglyceride

- FA activated to acyl-CoA esters and then undergo β-oxidation
- Chain-length specific enzymes
  - VLCAD
  - MCAD
  - SCAD
- Identify by
  - Plasma/DBS acylcarnitines
  - Urine organic acids



Hyperammonaemia

Definition

- Reference intervals are age dependant:

Premature neonate	<150 µmol/L
Term neonate	<100 µmol/L
Child/adult	<40 µmol/L

- Immediate attention:
  - Children >150 umol/L
  - Neonates >200 umol/L
- Second sample to confirm
- Repeat in 4 hours to assess trend

Clinical Features

- Ammonia
  - Respiratory stimulant - acts on respiratory centre in the brain stem - characteristic respiratory alkalosis
  - Neurotoxic – neurological symptoms

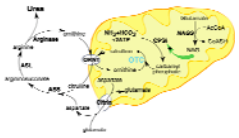
- Neonatal
  - Tachypnoea
  - Lethargy
  - Vomiting
  - Convulsions
  - Encephalopathy
- Child/adult
  - Vomiting
  - Feeding difficulties
  - Failure to thrive
  - Neurological signs
  - Developmental delay

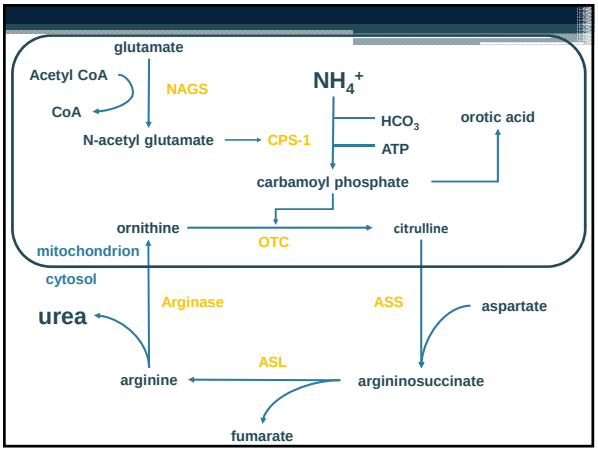
Causes

DEFECTS OF THE UREA CYCLE	OTHER METABOLIC DISORDERS
• Inherited Deficiencies of : - N-Acetyl Glutamate Synthase (NAGS) - Carbamoyl Phosphate Synthase (CPS) - Ornithine Transcarbamylase (OTC) - Argininosuccinate Synthase (Citrullinaemia) - Argininosuccinate Lyase (Argininosuccinic Aciduria) - Arginase (Argininaemia)	• Organic Acidurias • Disorders of Fatty Acid Oxidation • Others : - HHH Syndrome - Lysinuric Protein Intolerance - Hyperinsulinaemic Hyerammonaemia - Ornithine Aminotransferase Deficiency (neonatal form) - Mitochondrial Respiratory Chain Defects - Pyruvate Dehydrogenase Deficiency - Citrin Deficiency (Citrullinaemia Type II) - Congenital lactic acidosis
ACQUIRED	**OTHER**
• Liver Failure / Impairment • Urinary Tract Infection • GI Bacterial Overgrowth • Drugs e.g. valproate, chemotherapy • TPN • Sick Baby - e.g. asphyxia, sepsis • Reyes Syndrome	• Artefactual Increase : - poor specimen quality / haemolysis - difficult venepuncture - skin contamination - contaminated tube - delayed analysis • Transient Hyperammonaemia of the Newborn

Urea cycle disorders

- Ammonia excreted via the urea cycle
- Caused by a deficiency in any of six classical enzymes
  - Carbamyl phosphate synthetase — CPS 1 def.
  - N-acetyl glutamate synthetase — NAGS def.
  - Ornithine transcarbamylase — OTC def.
  - Argininosuccinic acid synthetase — Citrullinaemia
  - Argininosuccinic acid lyase — ASAciduria
  - Arginase — Argininaemia





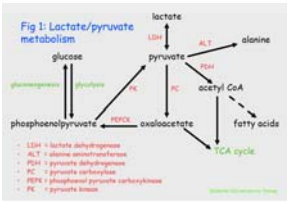
Treatment

- Protein and energy intake
  - Stop protein intake
  - Give high energy intake (i.v glucose +/- insulin)
- Remove ammonia
  - Haemodialysis or CVVH (>500 µmol/L)
  - Sodium benzoate
  - Sodium phenylbutyrate
  - Arginine
  - Citrulline
  - Carbaglu

Lactic acidosis

Lactate

- Produced by anaerobic metabolism of pyruvate
- Used as substrate for gluconeogenesis
- Excess lactate produced by peripheral tissues is transported to the liver
  - converted to pyruvate and subsequently glucose
  - utilised in fatty acid synthesis
- Concentrations are generally stable
  - production = utilisation



Causes – Non specific

- Hypoxia/hypoperfusion
  - Hypovolaemia, septic shock, cardiogenic shock, asphyxia, severe anaemia
- Systemic disease
- Liver disease, Renal failure, DM, Seizures
- Other causes of increased muscle activity
  - Exercise, struggling infant
- Drugs/toxins
  - Carbon monoxide, salicylates/paracetamol, methanol/ethanol/ethylene glycol

Causes - IMD

- Consider when
  - persistently elevated (> 3mmol/L)
  - + hypoglycaemia +/or hyperammonaemia
  - Glycogen storage disorders
  - Organic acid disorders
  - FAOD
  - Disorders of pyruvate metabolism
    - Pyruvate dehydrogenase def.
  - Disorders of gluconeogenesis
    - Pyruvate carboxylase def.
  - Respiratory chain defects
    - Especially if hypotonia

Investigations

- Should be directed by clinical history
- Consider
  - Acylcarnitines
  - Organic acids
  - Urate
  - CK
  - Glucose
  - FFA:3OHB
  - Muscle biopsy

Hyperbilirubinaemia

Definition

- Physiological jaundice is the most common clinical sign in the neonate
- During first week of life
  - 30-70% healthy term
  - Almost all preterm infants
- T.bilirubin generally <200 umol/L (unless preterm)
- Conj. generally <20 umol/L
- Levels peak around 3-4 days and return to normal by day 7-10

Definition

- Features suggesting a pathological cause include:
  - Early jaundice (<3 days)
  - Jaundice >14 days
  - T.Bili >200 umol/L
  - Conj. Bilirubin >20 umol/L
  - Rapid increase in bilirubin (>100 umol/L/day)
  - Jaundice in sick neonate
- Early jaundice is most likely due to a haemolytic cause
  - G6PD deficiency, PK deficiency
  - Blood group incompatibility
- Prolonged jaundice, persisting after 10-14 days should be investigated

Investigations

T.Bili >50 umol/L in term neonate >14days of age

Conjugated (>20 umol/L)	Unconjugated
Infection	Breast feeding
Biliary atresia	Hypothyroidism
A1AT def.	Infection
Galactosaemia	G6PD def.
Tyrosinaemia	Crigler-Najjar

Causes

Bile duct abnormalities	Endocrine disorders	Inherited and metabolic disorders
Biliary atresia **Choledochal cyst** **Inspissated bile** Caroli disease Gall-stones Spontaneous perforation of bile ducts Neonatal sclerosing cholangitis	**Hypopituitarism** **Hypothyroidism** **Hypoadrenalism**	**α1-Antitrypsin deficiency** **Alagille's syndrome** **Galactosaemia** **Cystic fibrosis** **Neonatal haemochromatosis** **Bile acid synthesis disorders** **Tyrosinaemia** **Progressive familial intrahepatic cholestasis** **Gauchers disease** Neimann–Pick type C Wolmans disease Peroxisomal disorders Congenital disorders of glycosylation Dubin–Johnson syndrome Rotor syndrome Aagenaes syndrome Citrin deficiency Fatty acid oxidation disorders Mitochondrial disorders Transaldolase G3D IV Mevalonic acidemia Hereditary Fructose Intolerance
Infections	**Toxic**	
Septicaemia **Urinary tract infection** **TORCH infections (toxoplasmosis, rubella, CMV, herpes viruses)** **Human Herpes virus-6,** **Varicella-zoster** **HIV, Hepatitis B** Echo, **Adeno**, Coxsackie-virus Parvovirus, EBV	**Parenteral nutrition** Chloral hydrate Foetal alcohol syndrome	
Vascular disorders	**Chromosomal disorders**	**Miscellaneous**
Perinatal asphyxia **Budd–Chiari syndrome** **Multiple haemangiomata** Congestive heart failure	Trisomy 21, 13, 18 Turner syndrome	**Haemophagocytic lymphohistiocytosis** ARC syndrome (Arthrogryposis, renal tubular dysfunction and cholestasis)

Investigations

- Blood
  - Acylcarnitines
  - VLCFA
  - Amino acids
  - TFTs
  - Gal-1-PUT
  - A1AT
  - G6PD
- Urine
  - Amino acids
  - Reducing substances
  - Organic acids

Specialist metabolic investigations

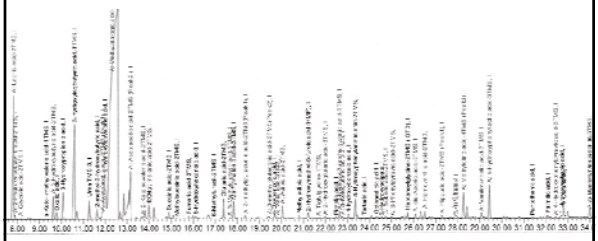
Specialist metabolic investigations

- Organic acids (urine)
- Amino acids (plasma/urine/CSF)
- Acylcarnitines (plasma/DBS)
- Urine reducing substances
- Intermediary metabolites
- Glycosaminoglycans/Oligosaccharide (Urine)
- Gal-1-PUT (erythrocytes)

- Specific enzymes
  - Leukocyte
  - Skin
  - Fibroblasts
  - Liver
- Mutation analysis

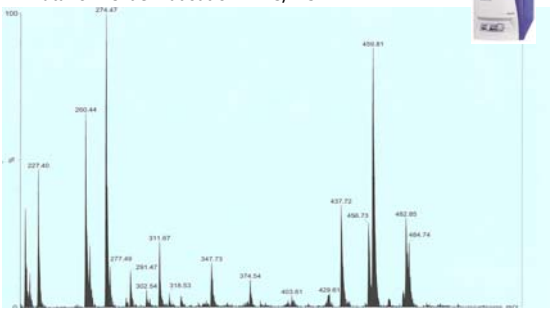
Organic acids

- Liquid-liquid extraction - GCMS



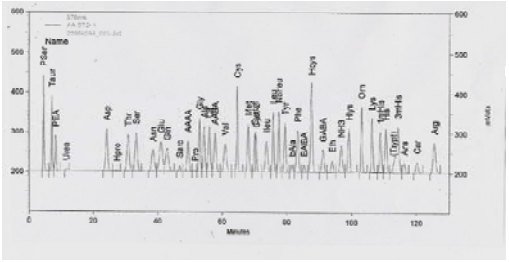
Plasma acylcarnitines

- Butanol HCl derivatisation – MS/MS

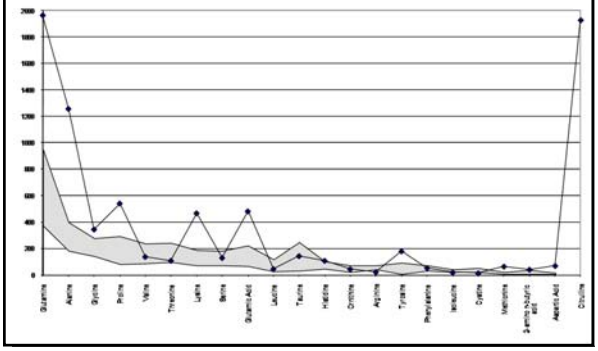


Amino acids

- Ion-exchange chromatography



Plasma amino acids



Newborn Screening

NBS - Definition

- *"A population-based public health programme applied to infants to reduce morbidity, severity or mortality of certain biochemical disorders using blood samples from newborns."*

- **Aims:**
  - **Early detection** of pre-symptomatic babies
  - Enable early treatment to **improve health outcome**
  - **Reduce anxiety** caused by uncertainty over symptoms before clinical diagnosis made

Screening Criteria

- Must be a common and serious disease
- Natural history well understood
- Accurate and reliable screening test available
  - Simple, safe, agreed policy for diagnosis
- Effective and acceptable treatment available
- Affordable/cost-effective screening test, follow up and treatment.

Screening process

- Following consent, capillary sample (4 drops) obtained by heel-prick
  - 5-8 days of age
  - NB: False neg/pos results if too early or post-transfusion
- Blood spotted on to request card and sent to NBS laboratory

- Positive results usually communicated to parents before the baby is 3 weeks old
  - Clinical referral process begins
  - NB: Positive screen is <u>not</u> final diagnosis (biochemical abnormalities can sometimes be transient)

What is screened for?

- **<u>Standard NBS programme</u>**
  - Phenylketonuria (PKU)
  - Congenital hypothyroidism (CHT)
  - Sickle cell disease (SCD)
  - Cystic fibrosis (CF)
  - Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)

All babies screened for in England, Wales, Scotland and NI

Phenylketonuria (PKU)

- **Phenylalanine hydroxylase deficiency:** Unable to metabolise phenylalanine:

  Phenylalanine → Tyrosine
- Affects approximately 1 in 10,000 babies in UK
- Inherited condition – carriers not identified
- Untreated babies develop serious, irreversible, mental disability (Phe passes B-B barrier)
- **Clinical features:** Blonde hair, blue eyes (Tyr needed for melanin)
- **Screening Test:**
  - ↑ Phenylalanine by MS-MS
- **Treatment:**
  - Strictly controlled diet (low Phe content) - prevents disability
  - Should be start by 21 days of age

Congenital Hypothyroidism (CHT)

- **Reduced function of thyroid gland from birth:** Inadequate thyroxine
- Affects approximately 1 in 3,000 babies in UK
- 1 in 10 cases are inherited – carriers not identified
- Untreated babies develop serious, permanent, physical and mental disability
- **Clinical features:** Generally lethargic, slow feeding etc…
- **Screening Test:** ↑DBS TSH (and ↓FT4) by immunoassay
- **Treatment:**
  - Early institution of thyroxine tablets - prevents disability
  - Should start by 21 days of age

Cystic Fibrosis (CF)

- **Mutation in CF transmembrane conductance regulator (CFTR) gene:** regulates transport of Cl^- ions and H_2O across cell membranes
- Affects approximately 1 in 2,500 babies in UK
- Inherited condition – some carriers identified.
- **Clinical features:**
  - Thick secretions from membranes that produce mucus, sweat, saliva and digestive enzymes
  - Poor digestion (exocrine pancreatic insufficiency): Steatorrhea, FTT
  - Lung disease (infections)
- **Screening Test:**
  - ↑DBS Immunoreactive trypsinogen (IRT) by immunoassay
  - DNA analysis for CF mutations, Sweat testing
- **Treatment:** May improve health, cannot prevent progression of condition
  - **Diet** - ↑Energy, fat-sol vits, essential FAs
  - **Medication** - Panc. Supplements, antibiotics, inhaled/aerosol t'ment
  - **Physiotherapy** – Remove mucus

Sickle cell disease (SCD)

- **Mutation in β-globin gene of Hb** – produces Sickle Cell Hb (HbS)
- Affects approximately 1 in 2,000 babies in UK
- Inherited condition – some carriers identified
- **Clinical features:**
  - Red blood cells become sickle shaped
  - Pain, tissue damage, infection and even death
- **Screening Test:**
  - HbS by cation-exchange HPLC and Iso-electric focussing (confirmation)
- **Treatment/management:**
  - Immunisations (e.g. pneumococcal vaccine), antibiotics (prophylactic)
  - Parent education, genetic counselling
  - Treatment should be started by 2 months of age
  - Early treatment improves health and prevents death

MCADD

- **Medium-chain acyl-CoA dehydrogenase deficiency**
- Commonest FAOD - 1 in 10,000
- Peak age clinical presentation 12-18 months
  - 25% die during first attack
- Screening began Oct 2003
- **Clinical Presentation:**
  - Normally follows excessive period of fasting
  - Lethargy, nausea, vomiting
  - Acidosis, hyperammonaemia
  - Hypoketotic hypoglycaemia → FFA:3OHB >2 (able to liberate free fatty acids but unable to oxidise them)

MCADD

- **Biochemical features:**
  - Plasma/DBS AC by MS-MS: ↑C8 (octanoyl), C6, ↑ratio C8/C10
  - Urine OA: C6-C10 dicarboxylic acids & glycine conjugates
- **Treatment:**
  - Avoid fasting
  - Dietary management during inter-current illness
    - Emergency regime
    - High caloric supplement
    - IV 10% dextrose if feeds not tolerated
  - Advice on exercise
    - Uncooked cornstarch

Expanded NBS Programme

- 6 centres - Leeds, Manchester, Sheffield, Birmingham, Guy's St Thomas and Great Ormond Street
- Pilot screening until 31st March 2014 for further 5 rare conditions (already screened for in USA and across Europe):
  - Maple syrup urine disease (MSUD)
  - Homocystinuria (HCU)
  - Isovaleric acidaemia (IVA)
  - Glutaric aciduria Type 1 (GA1)
  - Long chain hydroxyl acyl CoA dehydrogenase deficiency (LCHADD)
- Data collection on medical care received by babies who screen positive
  - Health economic analysis completed
  - Inclusion of GA1, HCU and MSUD (in England) currently supported by UK National Screening Committee – public consultation imminent

MSUD

- Amino acid disorder – unable to metabolise branched chain amino acids (Leucine, Isoleucine, Valine)
- Incidence – 1:116,000
- Coma and permanent brain damage if untreated
- **Enzyme defect:** Branched chain α-oxoacid dehydrogenase
- **Clinical features:** Poor feeding, vomiting, excessive sleepiness
- **Screening test:** Leucine
- **Treatment:** Low protein, branched chain AA-restricted diet

HCU

- Amino acid disorder – unable to metabolise homocysteine
- Incidence – 1:144,000
- **Enzyme defect:** Cystathioneβsynthase
- **Clinical features:** Without treatment - severe short-sightedness, lens dislocation, learning difficulties, osteoporosis
- **Screening test:** Methionine (confirm by plasma AAs and total plasma homocysteine)
- **Treatment:** Low protein, lysine-restricted diet plus carnitine

IVA

- Organic acid disorder – unable to metabolise leucine
- Without treatment can lead to coma and permanent brain damage
- Incidence – 1:155,000
- **Enzyme defect:** Isovaleryl CoA dehydrogenase
- **Clinical features:** Vomiting, excessive sleepiness, hypotonia, rapid breathing
- **Screening test:** Isovaleryl carnitine (confirm by plasma AC and urine OA)
- **Treatment:** Low protein diet plus carnitine and glycine

GA1

- Amino acid disorder – unable to metabolise lysine/tryptophan
- Brain damage at ~9 months without treatment
- Incidence – 1:110,000
- **Enzyme defect:** Glutaryl-CoA Dehydrogenase
- **Clinical features:** Vomiting, irritability, hypotonia, breathing difficulties
- **Screening test:** Glutaryl carnitine (confirm by plasma acylcarnitines)
- **Treatment:** Low protein, lysine-restricted diet plus carnitine

LCHADD

- FAOD causing defective fat metabolism (βoxidation)
- Associated with hypoglycaemia during fasting/infection
- Incidence – 1:220,000
- **Enzyme defect:** Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency
- **Clinical features:** Poor feeding, irritability, excesssive sleeping, vomiting, hypotonia etc…
- **Screening test:** C16 hydroxyacylcarnitine (confirm by plasma AC, urine OA, DNA analysis
- **Treatment:** Low fat diet and emergency regimen during illness (high sugar drinks)

Further Reading

- Books:
  - Neonatology and Laboratory Medicine, Chpt 10 (Anne Green, ACB Venture publications 2003)
- Useful links:
  - National Metabolic Biochemistry Network (MetBioNet):
    - http://www.metbio.net/metbioHome.asp
    - Best practice guidelines → MetBio guidelines
  - NBS Website:
    - http://newbornbloodspot.screening.nhs.uk/professionals
  - Expanded NBS Website:
    - http://www.expandedscreening.org/site/home/start.asp