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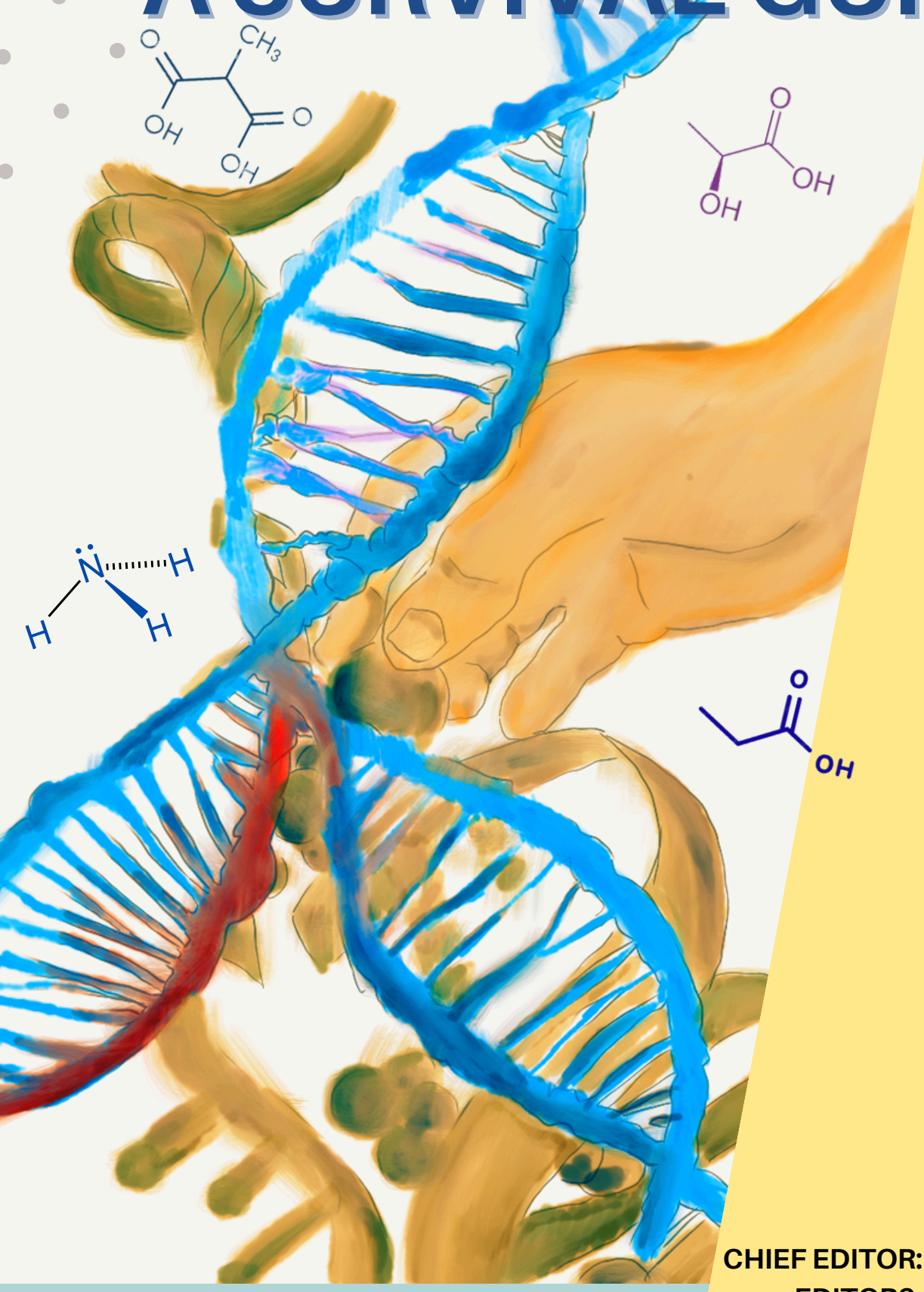
INBORN ERRORS OF METABOLISM
A SURVIVAL GUIDE



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Inborn Errors of Metabolism

A Survival Guide

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Acknowledgement

This survival guide could never have been completed without the knowledge and practical experience that we acquired throughout our professional careers in providing care for patients and families affected by inborn errors of metabolism (IEMs). The management of IEMs involves many healthcare professionals from multiple disciplines. We would like to thank our valued colleagues who have worked with us all these years including chemical pathologists, biochemists and molecular geneticists from the Institute for Medical Research (IMR) and Genetic Laboratory, Hospital Tunku Azizah; dieticians from the Dietetic and Food Service Department**,** Hospital Kuala Lumpur (HKL); pharmacists from the Pharmacy Department, HKL; all doctors, nurses and allied healthcare professionals; patient support and advocacy groups, pharmaceutical partners and everyone who has helped us in one way or another in ensuring that our patients get the best possible medical care. We also thank all the patients, parents and caretakers who have entrusted us with their care. Lastly, we are grateful to Miss Dhania Camelia Ngu for helping us to design the cover pages.

Thank you all!

Preface

Inborn errors of metabolism (IEMs) make up a large group of rare disorders caused by inherited deficiencies or absence of proteins that have enzymatic, carrier, receptor, or structural roles. IEMs which now sum up to more than 1,500 disorders are often presumably only comprehensible to a few metabolic specialists. IEMs also receive little attention in medical training and daily practice, and are considered as a complicated field of medicine by many healthcare professionals. Failing to make a timely diagnosis of a treatable IEM in an ill patient can have tragic consequences and should not happen in this era of genomic medicine which increasingly emphasizes on personalised and targeted therapies. Therefore, we need to address the gaps in knowledge amongst healthcare professionals across multiple disciplines. With this in mind, coupled with our own experience in managing IEMs in the Malaysian setting over many years, this survival guide aims to provide practical guidance to the healthcare professionals in our country in managing IEMs. Healthcare professionals from the paediatric fraternity are the primary target readers for this survival guide, but healthcare professionals in adult disciplines may also find it useful.

In this survival guide, IEMs are classified into three large groups based on the size of molecules ("small and simple" or "large and complex") and their implications in energy metabolism. Disorders in each group share similarities in their pathophysiological basis and clinical diagnostic approach. The key clinical phenotypes, diagnostic approach and treatments for all the three groups and their representative disorders will be presented in a simple yet clear format. We hope this structural approach will help healthcare professionals to understand IEMs in an easier and non-intimidating way. Where it is relevant, biochemical pathways, radiological images and clinical photographs are also included for better illustration. We have included Malaysian data whenever it is available. Nonetheless, as a reminder, this survival guide is not a comprehensive textbook. Healthcare professionals are advised to refer to other comprehensive reference textbooks and currently available online resources if you require in-depth information.

We hope this brief guide will serve as a useful guide for all healthcare professionals whenever you encounter a patient in whom the diagnosis of IEM should rightfully be considered. We hope that the insights gained will translate into tangible changes in practice in your respective places of work.

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June 2024
Kuala Lumpur, Malaysia

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Chapter 1

DIAGNOSIS AND MANAGEMENT OF INBORN ERRORS OF METABOLISM: AN INTRODUCTION

Inborn errors of metabolism (IEMs) are biochemical genetic disorders that result from the deficiency of enzymes, membrane transporters, or other functional proteins.

IEMs are individually rare but cumulatively common. According to a pilot study conducted by the Institute for Medical Research, Ministry of Health Malaysia in 2006 that screened >30,000 newborns from 11 major public hospitals in Malaysia for 27 inborn errors of amino acid, organic acid and fatty acid metabolism using tandem mass spectrometry on dried blood spot samples, the incidence of IEMs was one in 2916 newborns [Zabedah 2016].

Children with IEMs may present with either acute overwhelming sickness or a prolonged, smouldering illness. For the former, rapid diagnosis is vital to limit morbidity and mortality. For the latter, diagnosis is important for the initiation of appropriate clinical care.

"Red flags" that should prompt IEM investigations:

- Neonates with unexplained sepsis-like appearance, overwhelming or progressive diseases without evidence of infection, particularly after normal pregnancy and birth.
- Recurrent episodes of vomiting, ataxia, seizures, lethargy, altered consciousness, particularly when preceded by vomiting, fever, infections or fasting.
- Patients with unexplained symptoms and signs of metabolic acidosis, hyperammonemia or hypoglycaemia.
- Severe hypotonia.
- Severe global developmental delay, especially with loss of skills.
- History of being severely symptomatic and needing longer to recover with benign illnesses (e.g., upper respiratory tract infection).
- Unusual dietary preferences (e.g. protein or carbohydrate aversion).
- Subtle neurological or psychiatric abnormalities in older children or adolescents.
- Epileptic encephalopathy.
- Movement disorder (e.g. dystonia).
- Hepatomegaly, cirrhosis, liver failure, cholestatic jaundice.
- Dysmorphic syndromes (e.g. coarse facial features).
- Hypertrophic cardiomyopathy.
- Skin signs (e.g. ichthyosis, light sensitivity).
- Eye abnormalities (e.g. cataract, corneal opacities, pigmentary retinopathy).
- Chronic muscle weakness with pain.
- Renal stone disease in children.
- Renal tubular disease in children.
- An unusual smell from skin or urine: sweaty feet, burnt maple syrup, etc.
- Neuroimaging abnormalities.

When to consider IEMs in adults?

It is important to note that some IEMs can present in adulthood, either with slowly progressive onset disorders, such as lysosomal diseases, or with episodes of acute decompensation, characteristic of acute porphyria attacks or urea cycle disorders. In addition, due to major improvements in the diagnosis and treatment of IEMs that have led to an increasing number of affected patients reaching adulthood, it has become crucial for healthcare professionals to recognize these diseases in adults.

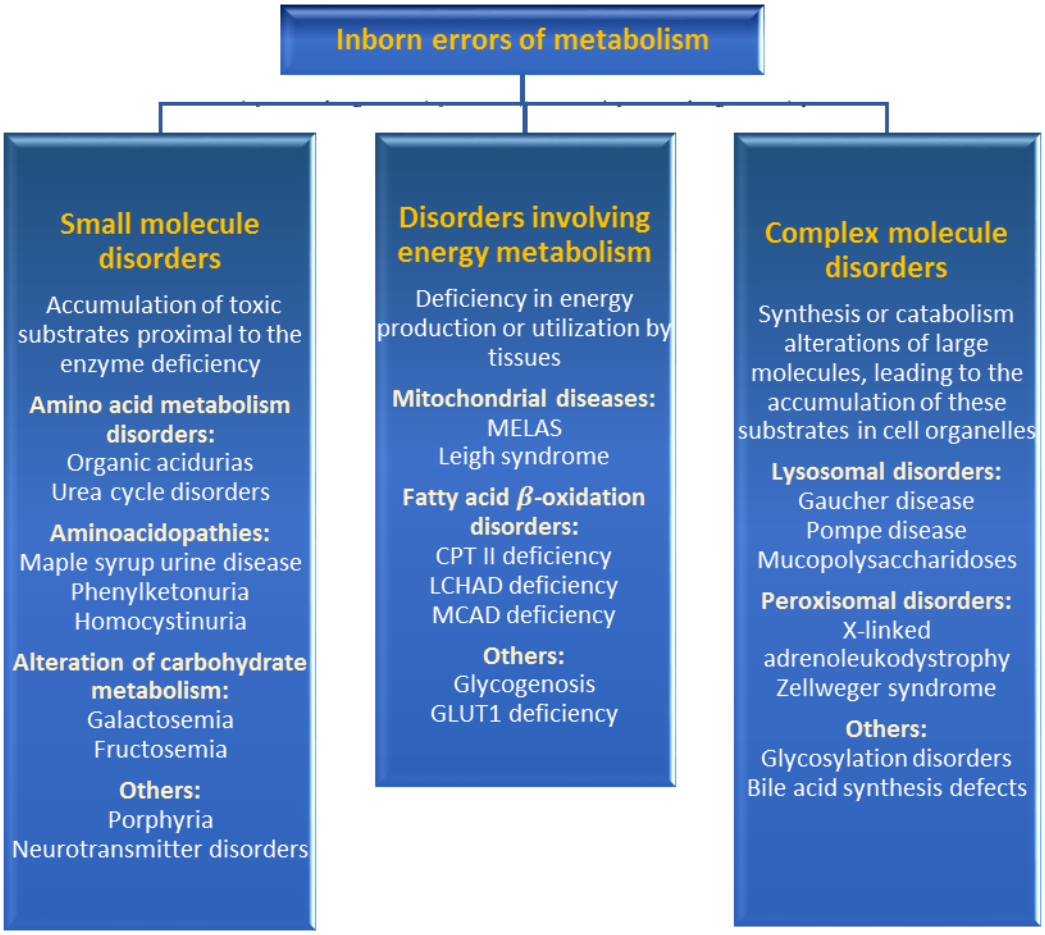
Classification of IEMs

While the most recent international classification of IEMs encompasses >1500 disorders, from a clinical point of view, all IEMs can be classified into 3 groups based on clinical diagnostic perspective and pathophysiological approach.

Group 1: Small molecule disorders

Group 2: Disorders involving energy metabolism

Group 3: Complex molecule disorders



Chapter 2

GROUP 1: SMALL MOLECULE DISORDERS

There are two subgroups in small molecule disorders defined by whether the clinical phenotype primarily results from an acute or progressive "intoxication" caused by accumulation of toxic compounds proximal to the metabolic block or a deficiency where symptoms are primarily due to the defective synthesis of compounds distal to the block or from the defective transportation of an essential molecule through membranes.

Subgroup 1A: Accumulation of small molecules (diffusible water-soluble)

- Causes acute or progressive "intoxication".
- Symptoms and signs result primarily from accumulation of the "intoxicating" compound and can be reversed as soon as it is removed.
- Does not interfere with fetal development.
- Presents after a symptom-free interval (days to weeks).
- "Metabolic crisis" induced by food and catabolism.
- Most disorders are treatable.
- Most disorders have metabolic marker(s) & are detectable by:
  - First line tests: blood glucose, blood ammonia, blood acid-base status, blood lactate, urine/ blood ketones.
  - Second line tests: analysis of plasma amino acids (AA), urine organic acids (OA) and dried blood spot (DBS) acylcarnitines ± plasma total homocysteine.
  - Almost all disorders can be diagnosed by molecular genetic testing. Bloods can be taken first with DNA extracted and stored with consent in the event of an ill patient who may succumb before diagnostic results are out.

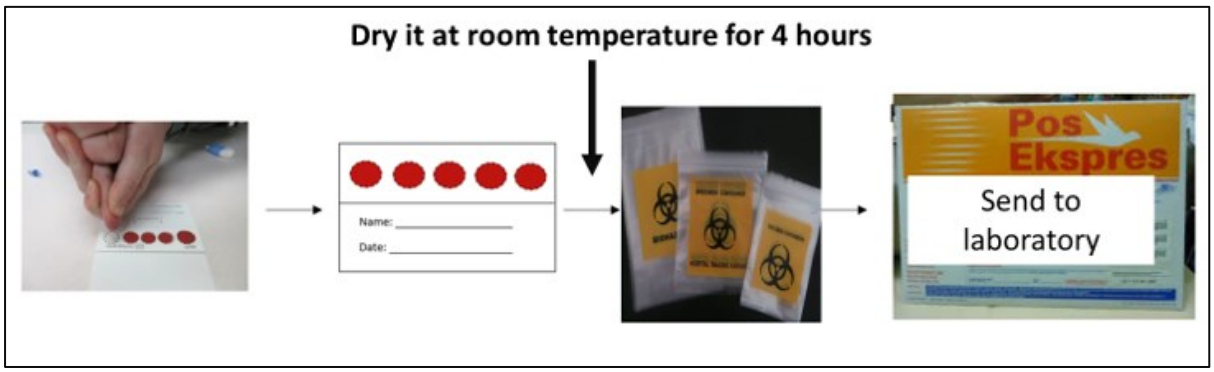
Interpretation of initial first line investigation results

- Serves as a guide only: consult the metabolic team with abnormal results.
- Table below is most relevant for neonates and small infants.
- Must also take into account the clinical context, including the period of fasting prior, hydration status, stage of illness and what fluids or other management that have been started.

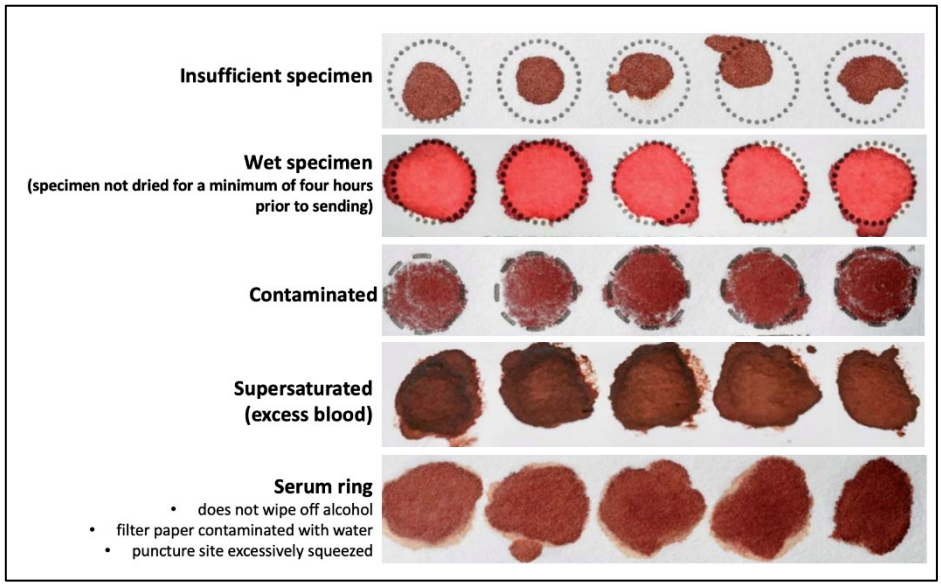
	Glucose	Lactate	Metabolic acidosis	Ammonia	Anion Gap[#]	Urine ketones
Maple Syrup Urine Disease	Low or Normal	Normal	Variably present	Normal	May be increased	Positive
Organic Acidurias	Low or Normal	May be high	Very acidotic	May be high	Usually increased	Positive
Fatty Acid Oxidation Disorders	Low or Normal	May be high	Variably present	May be high	May be increased	Negative or low
Urea Cycle Disorders	Normal	Normal	Early, respiratory alkalosis Late, metabolic acidosis May be normal	High	Normal	Negative

[#]Anion gap = $[Na^+] – [Cl^- + HCO_3^-]$ (Normal 7 – 16 mmol/L)

Sterile blood collection tube: purple top (K2 EDTA tube) for ammonia and grey top for lactate.
Send immediately to the laboratory on ice.



Collection of dried blood spot specimen according to the recommended procedure



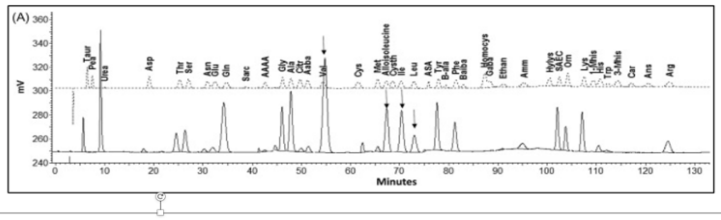
Dried blood spot specimens deemed unsuitable

Amino acid analysis, urine organic acid analysis and acylcarnitine analysis are fundamental to the diagnosis and management of many IEMs in particular small molecule disorders

Amino acids quantitative analysis

Instrument: Amino acids analyser Method: ion exchange chromatogrphy

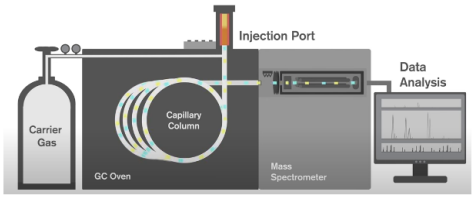
Chromatogram



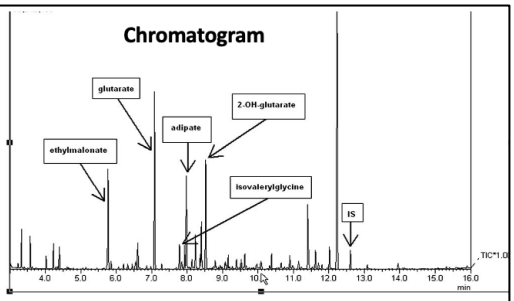
Amino acids analysis is used in the diagnosis & monitoring of aminoacidopathies such as PKU, MSUD and UCD where analysis of a single or very few amino acids present in high concentration is sufficient. Plasma is the preferred sample for amino acid analysis for the diagnosis of most disorders. Plasma should be separated as soon as possible and frozen if analysis is delayed. If serum is used, the sample should be immediately centrifuged after clotting. Urine amino acid analysis is usually less informative than analysis of plasma samples due to the wide reference intervals for urinary excretions of most amino acids. Urine amino acid analysis should be reserved for assessment of renal tubular transport abnormalities and disorders of amino acid transport. CSF amino acid analysis is essential in the diagnosis of neurotransmitter disorders such as NKH, serine deficiency disorders, and pyridoxal-phosphate-dependent epilepsy.

Urine organic acid analysis (qualitative, quantitative)

Instrument: Gas chromatogrphy mass spectrometry



Chromatogram

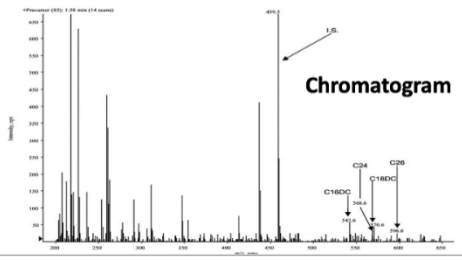


Urine organic acid analysis is an excellent diagnostic test for the organic acidemias involving propionic, methylmalonic, isovaleric acid and glutaric acids. It also detects succinylacetone which is a hallmark of tyrosinemia type I, and abnormal compounds related to fatty acid oxidation defects. Most laboratories provide a qualitative interpretation of the urine organic acids profile. Quantitative analysis is performed when small concentrations of critical metabolites may lead to a diagnosis. Urine sample collected during episodes of acute decompensation is most informative. If urine cannot be shipped to the laboratory immediately, it must be frozen at −20° C.

Acylcarnitines analysis (quantitative)

Instrument: Tandem mass spectrometry

Chromatogram



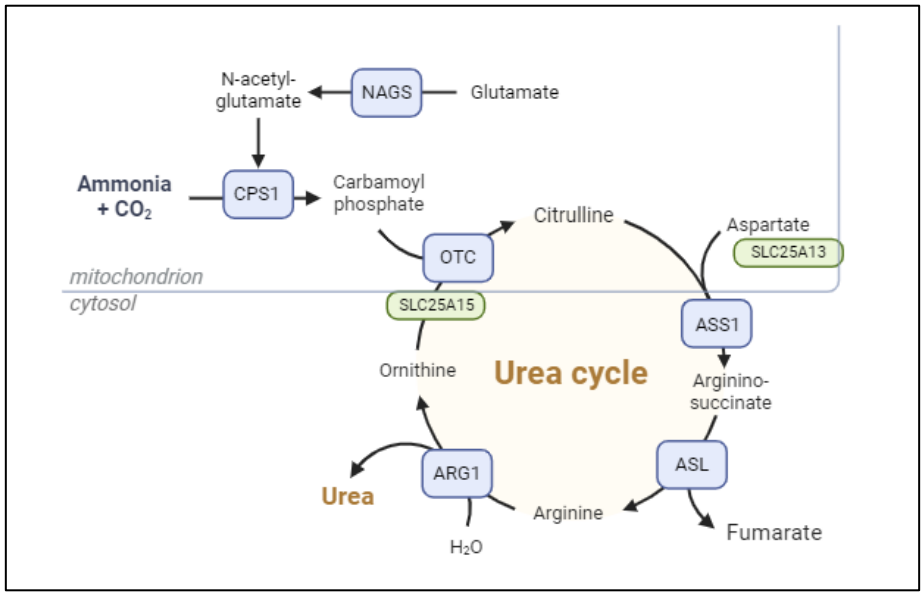
Tandem mass spectrometry is a major technological advance in the screening for inborn errors of metabolism. It has the advantage of sensitive and simultaneous multiple disease screening with minimal sample requirement using dried blood spot specimen. Quantitative acylcarnitine analysis is now widely available as a noninvasive initial investigation in patients suspected to have underlying disorders of fatty acids metabolism. It is also able to detect aminoacidopathies, and organic acidemias; and is widely used in expanded newborn screening program worldwide.

Main disorders:

(i) Urea Cycle Disorders (UCD)

Most common cause of severe hyperammonemia. There are >100 diagnosed patients in Malaysia. Argininosuccinate synthase deficiency and argininosuccinate lyase deficiency are the two most common types of UCD in Malaysia [Chen 2010]. Arginase deficiency patients are mostly from East Coast states due to a common pathogenic variant resulting from a complex re-arrangement of the *ARG1* gene [Mohseni 2014].

Biochemistry and Genetics: Urea Cycle



Ammonia is a byproduct of the metabolism of protein, mainly from muscle and food. The urea cycle is the main pathway for ammonia detoxification. The complete cycle is found only in periportal hepatocytes and involves mitochondrial as well as cytosolic enzymes and transporters. A deficiency in any of these enzymes or transporters due to its respective gene defects causes a urea cycle disorder (a.k.a. primary hyperammonemia). See table below.

Enzyme / transporter	Gene	Inheritance	Disorder
N-acetylglutamate synthase	*NAGS*	AR	NAGS deficiency
Carbamoyl-phosphate synthetase 1	*CPS1*	AR	CPS1 deficiency
Ornithine transcarbamylase	*OTC*	X-linked	OTC deficiency
Argininosuccinate synthetase	*ASS1*	AR	ASS deficiency = Citrullinemia type 1
Argininosuccinate lyase	*ASL*	AR	ASL deficiency = Argininosuccinic aciduria
Arginase 1	*ARG1*	AR	Arginase deficiency
Mitochondrial ornithine transporter ORNT1	*SLC25A15*	AR	Hyperornithinemia hyperammonemia-homocitrullinuria (HHH) syndrome

AR: autosomal recessive.
SLC25A13 gene encodes the citrin transporter protein. Its deficiency causes citrin deficiency (see page 20)

Clinical: Severe neonatal onset form: Rapidly progressive encephalopathy in the first days of life after a short symptom-free interval. Respiratory alkalosis is common early, but metabolic acidosis may mask it later if systemic shock develops. The initial presentation is often described as "sepsis-like" but unlike neonatal sepsis, blood pressure is usually in the (high) normal range or even increased in the early stages of hyperammonemic encephalopathy. In some patients signs of acute liver failure, including coagulopathy, may be found.

Late onset form: Failure to thrive, feeding problems, vomiting, chronic/unexplained neurological symptoms, episodic encephalopathy with lethargy, ataxia, seizures, behavioural/ psychological problems, protein aversion.

Arginase deficiency differs from other UCD because patients rarely present in the neonatal period but may manifest as progressive spastic paraplegia and developmental delay between 2 - 4 years old, sometimes with episodic hyperammonemia.

Diagnosis: ↑↑ NH_4^+, Plasma amino acid – abnormal profile, ↑urine orotic acid (identifies OTC deficiency and differentiates from NAGS/CPS1 deficiency).

Molecular genetic testing is recommended for definitive confirmation.

Blood NH_4^+ values	**Neonates:**	**Healthy**	**<110 µmol/L**
		Sick	Up to 180 µmol/L
		Suspect IEM	>200 µmol/L
	After the neonatal period:	Healthy	<50 µmol/L
		Suspect IEM	>100 µmol/L

Acute treatment: Emergency management of acute hyperammonemia: Rapid and efficient management is of utmost importance – short time-span from first symptoms to irreversible brain damage and death.

The prognosis is considered very poor in patients with any of the following characteristics: 1) Coma >3 days; 2) significantly elevated intracranial pressure; 3) Blood NH_4^+ concentration in plasma >1000 µmol/L.

Step 1: Basic life support

- Ensure basic life function (ventilator and circulatory support).

Step 2: Stop the offending precursor nutrients

- Stop protein intake (for a maximum 48 hours).

Step 3: Promotion of anabolism

- Give hypercaloric management.
- Intravenous infusion 10% (or higher) glucose with appropriate electrolytes (Na^+, K^+) at the rate according to patient's age, at least:
    - 10 mg/kg/min in a neonate
    - 8 mg/kg/min in infants
    - 6 mg/kg/min in all others
- Administration of iv lipids (1-2g/kg/d) or oral protein-free medical formula will provide additional energy and help promote anabolism.

- In a neonate, aim to achieve 120 – 140 kcal/kg/day. In older infants or children, aim to achieve 120 – 140% caloric requirement for age and sex.
- Adjust volume according to individual demands and hydration status. Check glucose; add insulin if necessary.
- Effective antiemetic (Granisetron or Ondansetron) to control vomiting.

Step 4: Detoxification

- Pharmacotherapy: Remove NH_4^+ using iv nitrogen scavengers (sodium benzoate and sodium phenylbutyrate) and replenish urea cycle intermediates (arginine, citrulline (CPS1 deficiency, OTC deficiency)).

Drug*	Loading dose over 1.5 – 2 hrs (bolus)	Followed by maintenance dose over 24 hrs
iv L-Arginine**	250 mg/kg	250 mg/kg
iv sodium benzoate	250 mg/kg	250 mg/kg
iv Sodium phenylbutyrate	250 mg/kg	250 mg/kg
***Should be diluted in glucose 5% to a total volume of 25 – 50 ml and administered as a bypass to the regular infusion. **400 mg/kg in ASL deficiency. Avoid Arginine in arginase deficiency.**		

- In undiagnosed patients in whom hyperammonemia could be caused by either UCD (a.k.a. primary hyperammonemia) or organic acidurias (a.k.a. secondary hyperammonemia), consider adding carglumic acid (see section on organic acidurias).
- Check blood NH_4^+ after 2 hrs. Thereafter, it must be determined at least every 3 - 4 hours until the acute situation is successfully managed.
- Reintroduction of protein/essential amino acids (oral or parenteral) must not be delayed for more than 48 hours.
- If at any time during the crisis the blood NH_4^+ escalates to >500 µmol/L or if the patient is encephalopathic (seizures, severely reduced consciousness or coma), continuous renal replacement therapy should be started as soon as possible.
- The method will depend on the experience of the local hospital but hemodiafiltration or haemodialysis have been proven most efficient while peritoneal dialysis with currently used solutions may be considered only if a more effective dialytic technique cannot be applied or for bridging to this more effective technique. Do not perform exchange transfusion.
- Management to induce anabolism and infusion of nitrogen scavengers must be continued during and after completion of renal replacement therapy to prevent rebound hyperammonemia.
- When blood NH_4^+ is persistently >1,000 µmol/L and prolonged coma, evaluate whether to aim at all out treatment or to start palliative care.

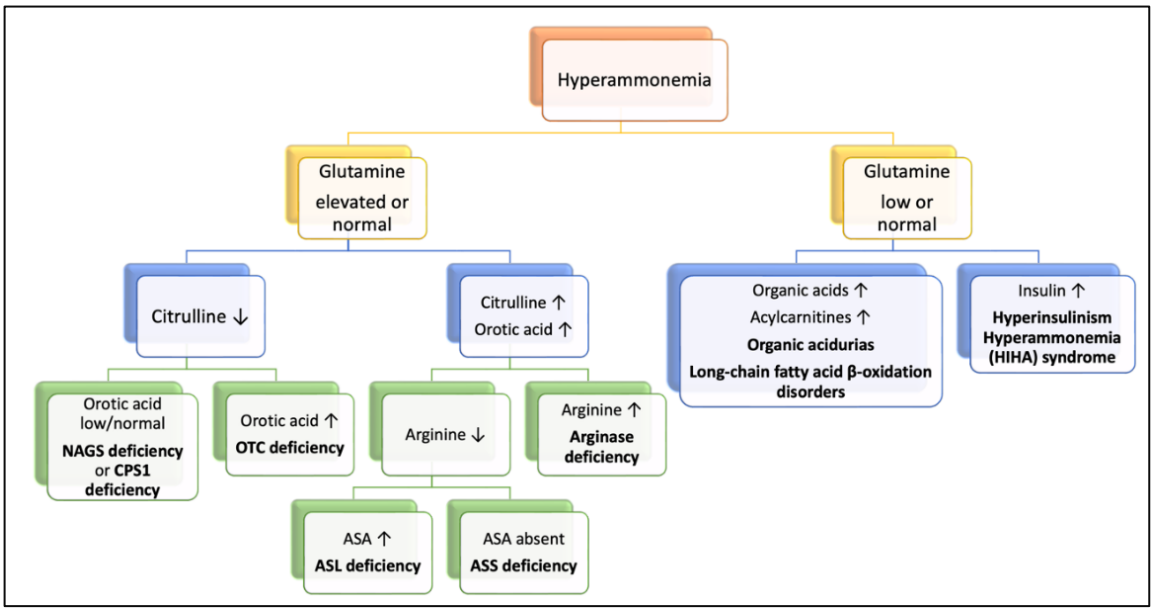
Step 5: Other supportive treatment

- Introduce supporting therapies, such as:
    - Treating infections
    - Managing seizures

Long-term treatment:

- Low-protein diet (titrate according to individual protein tolerance/safe level) – combination of low but high biological value natural protein and medical formula containing essential amino acids (30-50% of the protein requirement).
- Ensure sufficient intake of essential nutrients & calories to promote normal growth.
- May require nasogastric tube feeding or gastrostomy.
- Oral sodium benzoate 250 - 500 mg/kg/day.
- Oral sodium or glycerol phenylbutyrate 250 - 500 mg/kg/day.
- CPS1 or OTC deficiency: Oral arginine or citrulline (preferred) 100-200 mg/kg/day. ASS or ASL deficiency: Oral arginine 200 - 400 mg/kg/day.
- Carglumic acid in individuals with NAGS deficiency and partially responsive CPS1 deficiency.
- Give vitamins and trace elements.
- Consider lactulose.
- Consider early liver transplantation, particularly in individuals with severe neonatal onset OTC and CPS1 deficiency.
- Monitor growth and laboratory values regularly (Target blood NH_4^+ <80 µmol/L, glutamine <1,000 µmol/L, glycine 100 - 150 µmol/L, arginine 80-150 µmol/L, essential amino acids should all be in the normal range).
- Acute decompensation: Dietary indiscretion causes NH4+ to increase but only rarely results in acute decompensation and encephalopathy. In contrast, infections and injuries trigger a large endogenous mobilization of muscle protein and can precipitate metabolic crisis and hospitalisation.
- Treatment of intercurrent illness (with poor feeding, vomiting, diarrhea, fever): In order to prevent metabolic decompensation, it is imperative to prepare & educate the parents/caretakers to an individualized home-based sick day management plan. To interrupt the catabolism in its early stage:
    - Stop protein intake (not more than 48 hours).
    - Give sufficient fluid (water, juice, electrolyte solution) and extra calories (carbohydrates as glucose polymer).
    - If tolerated, special medical formula should be continued.
    - Reintroduce protein after 48 hours and increase stepwise until the amount of maintenance treatment is reached. E.g. one day ½ of the normal amount of protein, next day ¾, then full amount. (Prolongation of inadequately low protein intake increases the risk of protein catabolism).
    - Immediate hospital admission and check blood ammonia if the clinical condition deteriorates, oral intake is poor at home or the disease course is prolonged.
        - Administer caloric management that promotes anabolism even if blood ammonia is normal, until patient recovers.

Biochemical diagnostic pathway for the Urea Cycle Disorders



NAGS: N-acetylglutamate synthase; CPS1: Carbamylphosphate synthase I; OTC: Ornithine transcarbamylase; ASS: Argininosuccinate synthase; ASL: Argininosuccinate lyase; ASA: Argininosuccinic acid

(ii) Maple Syrup Urine Disease (MSUD)

More than 100 patients have been diagnosed in Malaysia. Most patients have the classical neonatal-onset MSUD. The *BCKDHB* gene was the most commonly affected (45%), *DBT* (39%) and *BCKDHA* (16%) [Ernie 2018]. *DBT* variant c.1196C > G (p.S399C) was the recurring variant among patients from the East Coast states. East Malaysia recorded almost no patient.

Clinical: (1) Severe neonatal onset form: progressive encephalopathy starting on 3rd-5th days of life: irritability, poor feeding, lethargy, intermittent apnea, opisthotonus, and stereotyped movements such as "fencing" and "bicycling" are followed by coma and central respiratory failure. No abnormalities in routine laboratory tests except ketonuria. Hypoglycemia is rare. Odor of urine may be highly characteristic (maple syrup-like). Acute cerebral oedema is a well-recognized complication (fully reversible if treated early), may progress to cerebral herniation.

(2) Attenuated forms (intermittent, intermediate): developmental delay, fluctuating/progressive neurological disease, recurrent ataxia, spastic diplegia, chronic vomiting with failure to thrive.

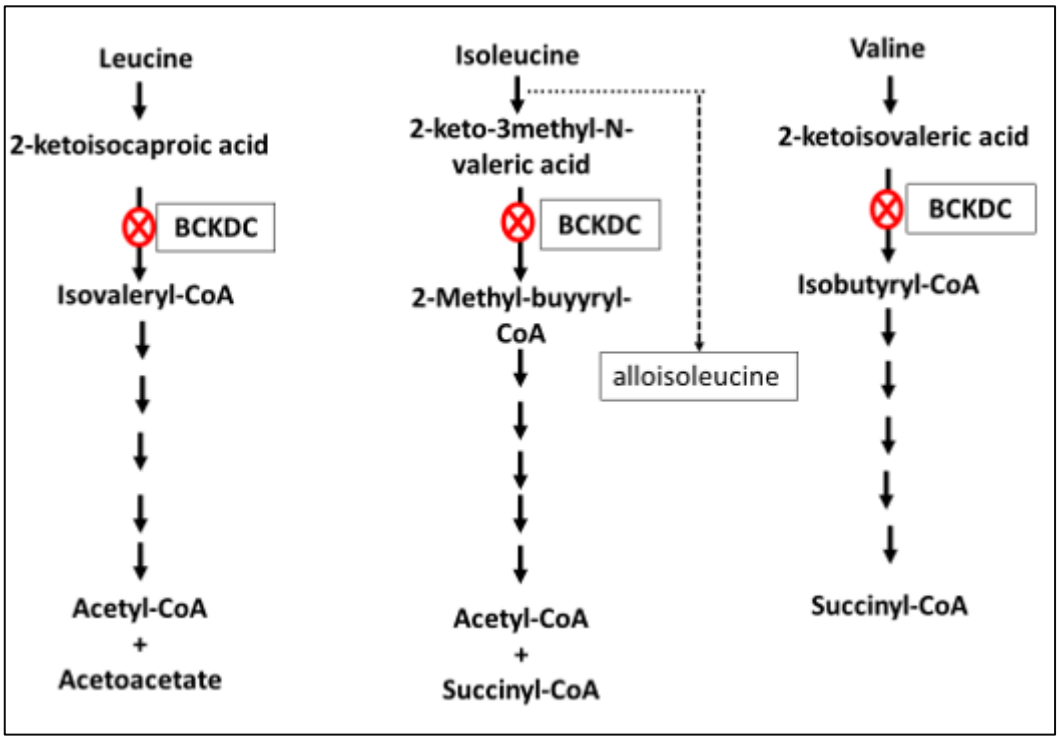
(3) Other children/adult: neurologic signs of intoxication vary and can include cognitive impairment, hyperactivity, sleep disturbances, hallucinations, mood swings, focal dystonia, choreoathetosis, and ataxia.

Diagnosis: Plasma AA: elevation of branched-chain amino acids (BCAAs) -↑↑↑Leucine (Leu, most neurotoxic), ↑Isoleucine (Ile), ↑Valine (Val). Presence of alloisoleucine is diagnostic.

Urine OA: ↑ branched-chain keto- and hydroxyacids, e. g. 2-OH-isovaleric acid, 2-ketoisocaproic acid.

Molecular genetic testing is recommended for definitive confirmation: *BCKDHA, BCKDHB, DBT* and *DLD* genes.

The branched-chain ketoacid dehydrogenase complex (BCKDC) is defective in MSUD. BCKDC has four subunit components (E1a, E1b, E2, and E3). Biallelic pathogenic variants in one of the four genes can result in decreased activity of the enzyme complex.



Acute Treatment:

Step 1: Basic life support

- Ensure basic life functions (ventilator and circulatory support).

Step 2: Stop the offending precursor nutrients

- Stop BCAAs/ natural protein intake (for 24 to 72 hours).

Step 3: Promotion of anabolism

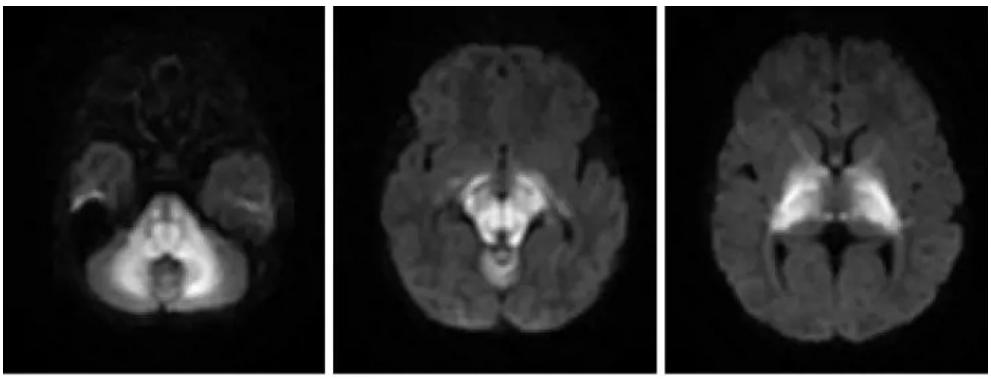
- Step (i) – Hypercaloric/anabolic nutritional support that contains iv 10% (or higher) glucose infusion, BCAA-free formula (enteral feeding via tube feeding + perfusor) and iv lipid (1-2 g/kg/d) ± iv insulin (if persistent hyperglycemia or glucosuria) to enhance protein anabolism. Effective antiemetic (Granisetron or Ondansetron) to control vomiting. Serial monitoring of blood BCAAs levels is essential.
- Step (ii) - Plasma concentrations of Val and Ile will normalize before Leu concentrations normalize. If Val and Ile fall below normal, then it will become rate limiting for protein synthesis → avoid secondary deficiency of Ile and Val by early oral supplementation (each 20 – 80 mg/ kg/ day)
- Step (iii) – Reintroduce Leu to the diet when plasma Leu drops to ≤ 400 µmol/L by adding natural protein e.g. breastmilk or infant formula.

Step 4: Detoxification

- Promoting protein anabolism is key to reducing toxic levels of BCAAs. Reduction in leucine concentration at 750 µmol/L or more per 24 hrs is normally achievable with hypercaloric nutritional therapy.
- However, in patients with very high concentrations of BCAAs or if nutritional therapy is insufficient, consider continuous renal replacement therapy (to reduce risk of cerebral herniation).

Step 5: Other supportive treatment

- Introduce supporting therapies, such as:
  - Treating infections
  - Managing seizures
  - Managing cerebral edema



Axial diffusion-weighted magnetic resonance images in Neonatal-onset MSUD shows high signal mainly involves the globus pallidus, thalamus, internal capsule, brainstem, and cerebellar white matter; these represent the typical myelinated areas in normal full-term neonates at birth.

Long-term treatment:

- Dietary restriction of BCAAs (especially leucine) by restricting natural protein (number of protein or Leu exchange is titrated to patient's Leu tolerance) and use of BCAA-free medical formulas and low protein foods, and careful supplementation of isoleucine and valine (regular monitoring of plasma BCAAs); sufficient intake of essential nutrients & calories to promote normal growth.
- Consider liver transplant in individuals with severe clinical phenotype (poor metabolic control, frequent metabolic decompensations).
- **Acute decompensation:** Dietary indiscretion causes plasma BCAAs to increase but only rarely results in acute decompensation and encephalopathy. In contrast, infections and injuries trigger a large endogenous mobilization of muscle protein and can precipitate metabolic crisis and hospitalization.
- Early intervention of any intercurrent illness to prevent metabolic decompensation is a very important aspect of long-term care. Parents/ caretakers should be educated to initiate sick day protocol upon the first signs of an illness: Increasing BCAA-free medical formula intake to 120% of the usual intake, decreasing leucine intake by 50%–100%, and providing small but frequent feedings throughout a 24-hour period. Minor illnesses can be managed at home. Immediate hospital admission if poor oral intake, the clinical condition deteriorates, or the disease course is prolonged or in serious cases.

(iii) Classical organic acidurias: isovaleric aciduria (IVA), propionic aciduria (PA), isolated methylmalonic aciduria due to methylmalonyl-CoA mutase or Cbla or Cblb deficiency (MMA)

These are the most common organic acidurias in Malaysia with combined number of patients >100 in Malaysia. Iban from Sarawak has the most cases of Methylmalonyl-CoA mutase deficiency invariably due to a homozygous missense pathogenic variant in *MUT* gene, c.982C>T (p.L328P) [unpublished data from Clinical Genetics Department HKL].

Clinical: Severe neonatal onset form: Metabolic encephalopathy "intoxication type"- lethargy, feeding problems, vomiting, dehydration, truncal hypotonia or limb hypertonia, coma, multi-organ failure progressing to death, unusual sweaty feet odour in IVA.

Late onset form: Recurrent episodes of metabolic crisis (ketoacidotic coma, lethargy, ataxia, focal neurological signs); chronic progression of organ dysfunction (brain, heart, kidney, pancreas, optic nerve).

Laboratory/ Diagnosis: Ketonuria, persistent metabolic acidosis, ↑anion gap, ↑lactate, ↑NH_4^+, hypoglycemia or hyperglycemia, neutropenia, thrombocytopenia, pancytopenia, ↑AST/ALT.

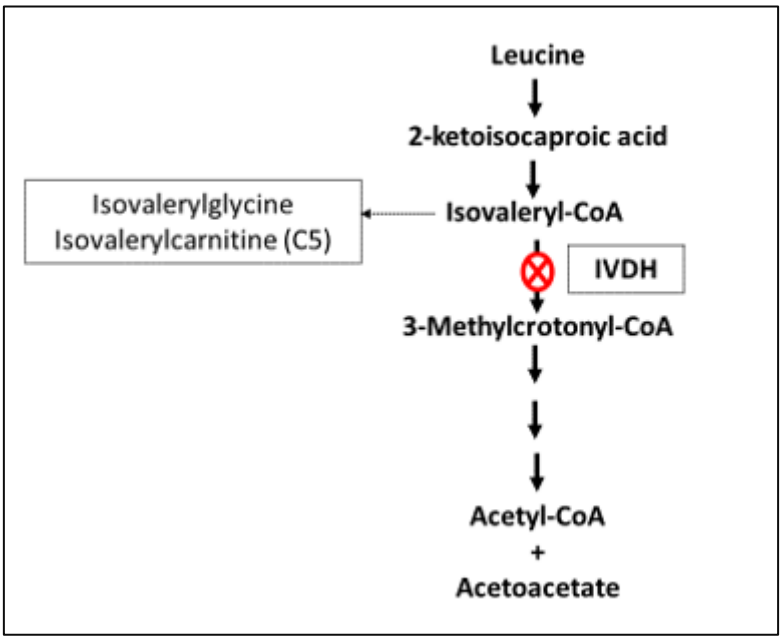
Urine OA: specific metabolite in IVA - ↑3-hydroxyisovaleric acid, isovalerylglycine; PA - ↑propionic acid, propionylcarnitine, 3-hydroxypropionate and methylcitrate; MMA - ↑methylmalonic acid.

DBS acylcarnitines; IVA - ↑C5, PA & MMA: ↑C3.

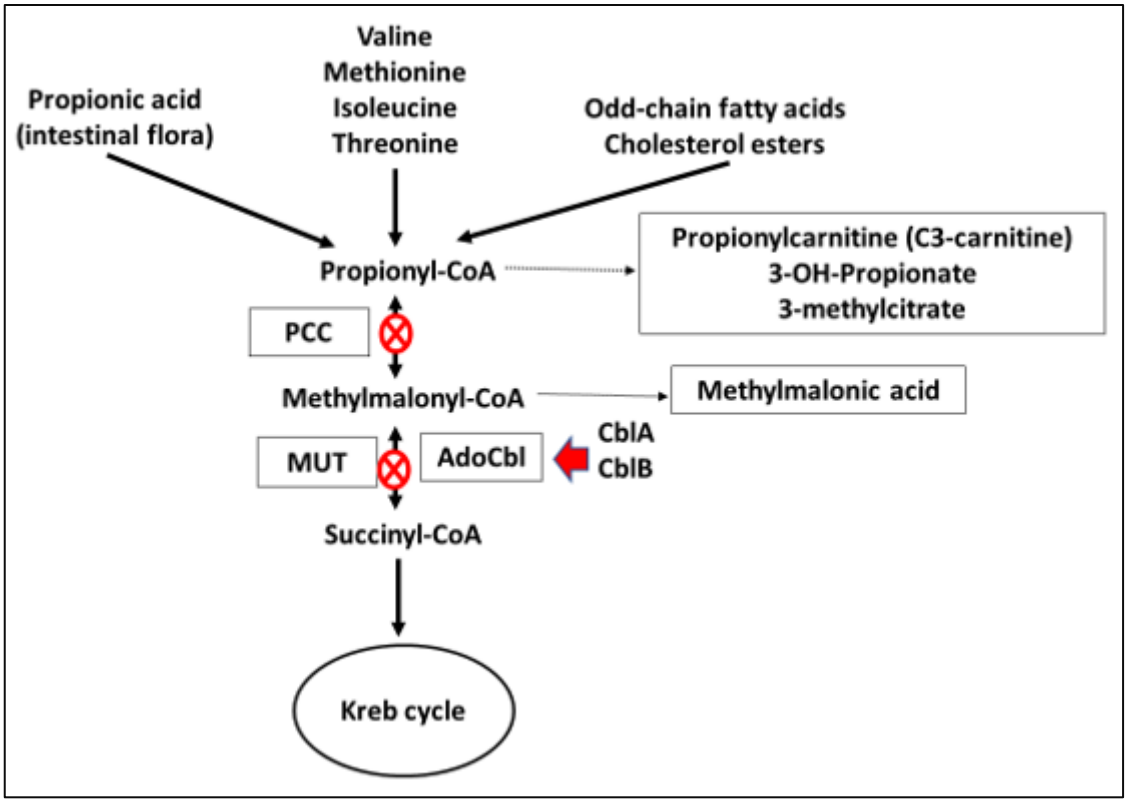
Plasma AA: ↑Glycine.

Molecular genetic test is recommended for definitive confirmation: *PCCA* and *PCCB* genes for PA*; MMUT, MMAA* and *MMAB* genes for MMA*; IVD* gene for IVA.

In isovaleric aciduria, isovaleryl-CoA dehydrogenase (IVDH) is defective.



Propionyl-CoA carboxylase (PCC) is defective in propionic aciduria. Isolated methylmalonic aciduria is due to methylmalonyl-CoA mutase (MUT), CblA or CblB deficiencies.



Acute Treatment:

Step 1: Basic life support

- Ensure basic life function (ventilator and circulatory support).

Step 2: Stop the offending precursor nutrients

- Transiently stop natural protein intake (24 – 48 hours).

Step 3: Promotion of anabolism

- Induce anabolism with iv 10% (or higher) glucose infusion and protein-free formula (enteral feeding via tube feeding + perfusor, iv lipids (1-2 g/kg/d) ± effective antiemetic (Granisetron or Ondansetron).

Step 4: Detoxification & correction of metabolic acidosis

- Correct metabolic acidosis with iv sodium bicarbonate.

Using sodium bicarbonate to treat acidosis
HCO_3^- deficit **ΔHCO_3^- = normal HCO_3^- (24mmol/L) - actual HCO_3^-** **HCO_3^- deficit is corrected for volume of distribution** **HCO_3^- deficit = ΔHCO_3^- x weight (Kg) x 0.4, or in infant use 0.5 for the correction factor** **Treatment:** **Replace ½ of the HCO_3^- deficit in the first 1 – 3 hours** **Replace ½ over the next 24 hours**

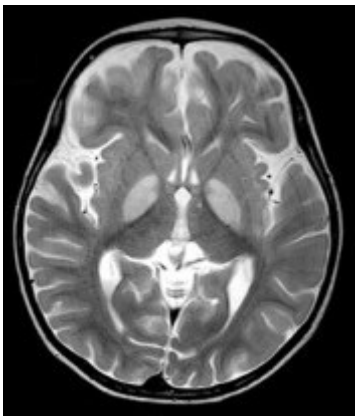
- Stimulate NH_4^+detoxification by oral Carglumic acid (loading dose of 50–100 mg/kg followed by 200 mg/kg/day in 3 divided doses).
- Enhance removal of toxic metabolites and prevent carnitine depletion by iv/oral L-carnitine (100–200 mg/kg/day).
- Consider continuous renal replacement therapy if metabolic acidosis and hyperammonemia remain intractable.

Step 5: Other supportive treatment

- Introduce supporting therapies, such as:
  - Treating infections
  - Managing seizures

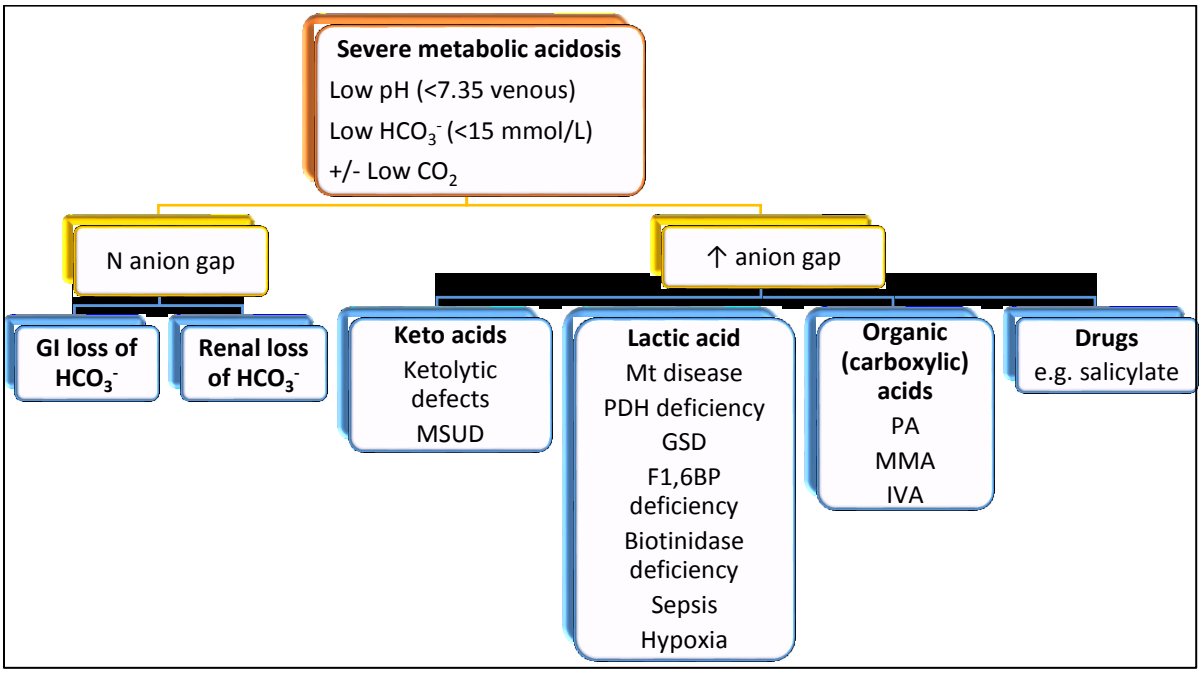
Long-term treatment:

- Diet: Low natural protein diet (0.5–1.5 g/kg/day) aiming to reduce toxic precursor AAs while maintaining sufficient intake of essential nutrients & calories; supplementation of precursor-free special formula.
- L-carnitine (50–100 mg/kg/day).
- Intermittent oral metronidazole (PA and MMA; 10–20 mg/kg/day for 10 consecutive days each month).
- Consider maintenance dose of carglumic acid in patient with frequent acute hyperammonemic episodes
- Every patient with MMA should be tested for responsiveness to vitamin B12 (when in stable condition) – IM vitamin B12 for 5 days with serial urine MMA quantification. >50% reduction in urine mean MMA is indicative of responsiveness.
- Monitor complications: Pancreatitis, basal ganglia infarct (PA, MMA), chronic kidney disease (MMA), cardiomyopathy and prolonged QTc (PA), optic atrophy (PA).
- Consider liver transplant in individuals with severe clinical phenotype.
- Early intervention of any intercurrent illness to prevent metabolic decompensation. Sick day protocol upon the first signs of an illness, increasing to 120% caloric intake using medical formula and glucose polymer (e.g. Carborie).



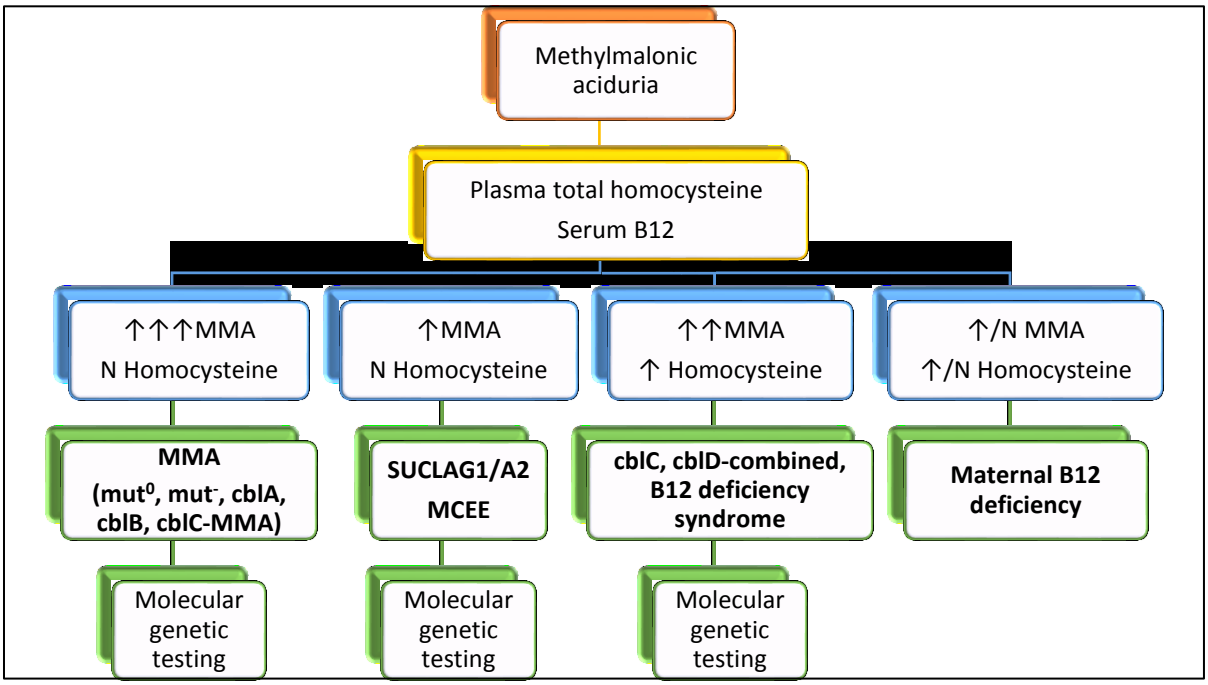
MRI brain (axial, T2). High signal in the bilateral globus pallidus in MMA.

Algorithm to aid understanding of the aetiology of metabolic acidosis based on the anion gap



PDH: Pyruvate dehydrogenase; GSD: Glycogen storage disorders; F1,6BP: Fructose-1,6-bisphosphatase; Mt mitochondrial

An algorithm for the differential diagnoses of elevated urine methylmalonic acid



B12 deficiency syndromes include intrinsic factor deficiency, Imerslund-Gräsbeck syndrome, and others causes of abnormal gastrointestinal B12 absorption. Cbl: cobalamin; MMA: methylmalonic acid; Mut: Methylmalonyl-CoA mutase Complete (mut^0 complete deficiency of the enzyme, mut^- partial deficiency of the enzyme), MCEE: Methylmalonyl-coenzyme A epimerase.

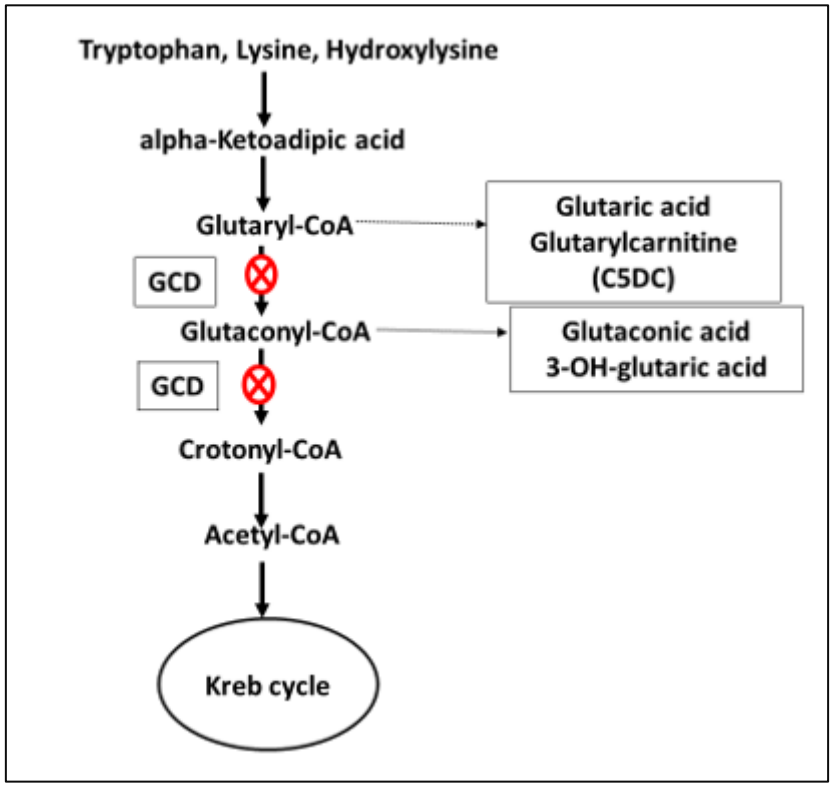
(iv) Cerebral organic aciduria: Glutaric aciduria type I (GA1)

Delayed diagnosis is common in the absence of universal expanded newborn screening program in Malaysia. There are about 20 patients diagnosed symptomatically in Malaysia, often due to private pathogenic variants of the *GCDH* gene [Abdul Wahab 2016].

Clinical: Macrocephaly (early sign), temporal brain hypoplasia, acute or insidious onset of striatal damage at the age of 6 – 36 months, complex movement disorder with predominant dystonia and truncal hypotonia. Metabolic/ lactic acidosis, hyperammonemia and hypoglycemia are inconsistent/absent.

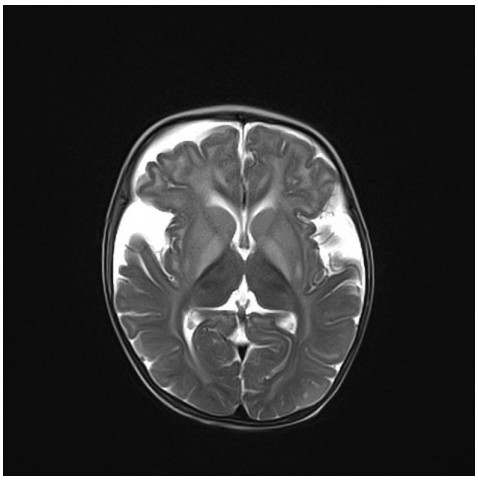
Diagnosis: Urine OA: ↑glutaric acid, 3-OH-glutaric acid (diagnostic).
DBS acylcarnitines: ↑C_5DC; molecular genetic testing is recommended for definitive confirmation (*GCDH* gene), and also recommended in low-excretors where glutaric acids are not detected in the urine.

Glutaryl-CoA dehydrogenase (GCD) is defective in Glutaric aciduria type 1



Treatment:

- Low-lysine diet while maintaining sufficient intake of essential nutrients & calories, often combined with the use of lysine-free, tryptophan-reduced medical formula (beware of tryptophan deficiency).
- Carnitine supplementation (100 mg/kg/day).
- Emergency treatment during catabolic episodes to protect against encephalopathic crises/acute striatal injury. Vomiting and diarrhoea is particularly dangerous – even in the absence of fever.
- ↓ or omit natural protein for 24 – 48 hrs.
- Frequent high carbohydrate feeds every 3 – 4 hourly (added glucose polymer).
- If tolerated, special medical formula should be continued, providing 120% caloric intake supplemented with glucose polymer.
- ↑ L-carnitine supplementation up to 200 mg/kg/day.
- If feeds are not tolerated, admit hospital for high-dose (10% or more) iv glucose infusion and give medical formula via tube feeding + perfusor ± effective antiemetic (Granisetron or Ondansetron). iv lipid (1-2g/kg/day) provide additional energy and help promote anabolism.
- With increasing age, and in particular after age 6 years, the risk of acute neurological insult appears to be much reduced.



MRI brain (T2, axial). Enlarged Sylvian fissures with "bat-wing" configuration in Glutaric aciduria type I. Subdural haemorrhage may occur. Basal ganglia injury frequently occurs following acute crisis.

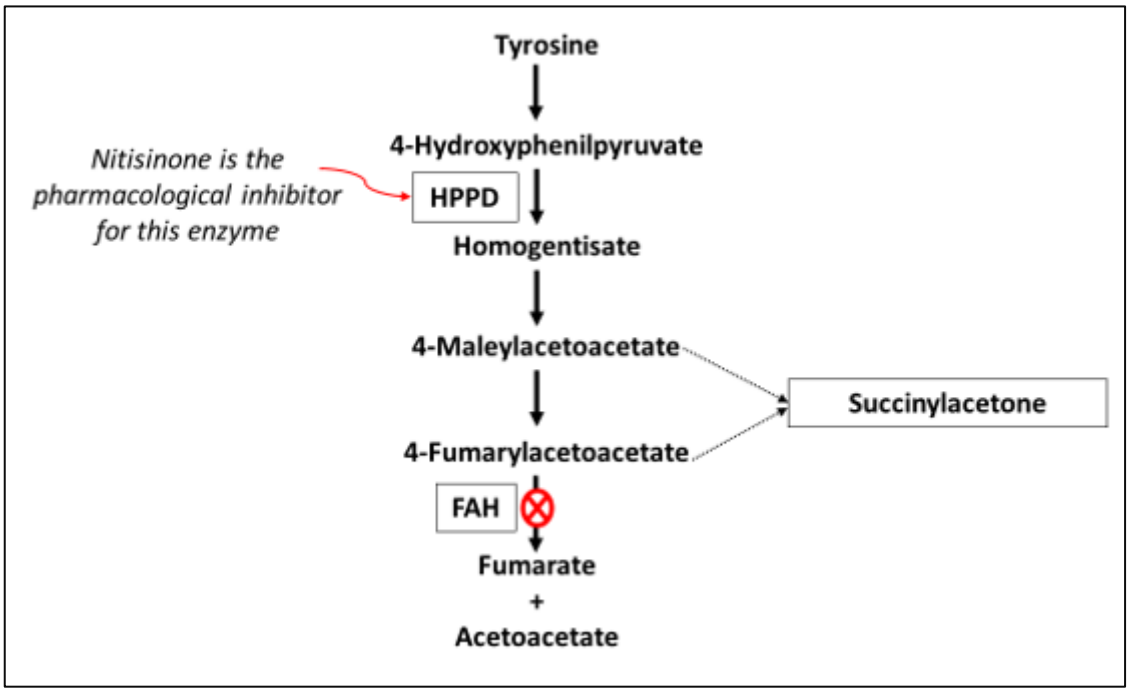
(v) Tyrosinemia type 1

More than 10 patients had been diagnosed in Malaysia [unpublished data from Clinical Genetics Department HKL].

Clinical: Acute: (neonate/infant): Severe liver failure, vomiting, bleeding, septicaemia, hypoglycemia, renal tubulopathy (renal Fanconi syndrome). Chronic: Hepatomegaly, cirrhosis, growth retardation, rickets, renal tubulopathy, neuropathy, neurological crises (due to porphyrins).

Diagnosis: Urine OA - ↑succinylacetone (diagnostic); plasma AA - ↑Tyr, ↑Met, ↑α-fetoprotein; Urine porphyrins - ↑δ-aminolevulinic acid. Molecular genetic test is recommended: *FAH* gene.

Defects in Fumarylacetoacetate hydrolase (FAH) are responsible for tyrosinemia type I.



HPPD: 4-hydroxyphenylpyruvate dioxygenase

Treatment: Nitisinone (NTBC) 1-2 mg/kg/day (inhibitor of 4-OH-phenylpyruvate dioxygenase, blocks the accumulation of toxic metabolites; beware of ↑Tyr); Phe- + Tyr-restricted diet

Prognosis: Good with nitisinone; liver transplant no longer needed in most patients.

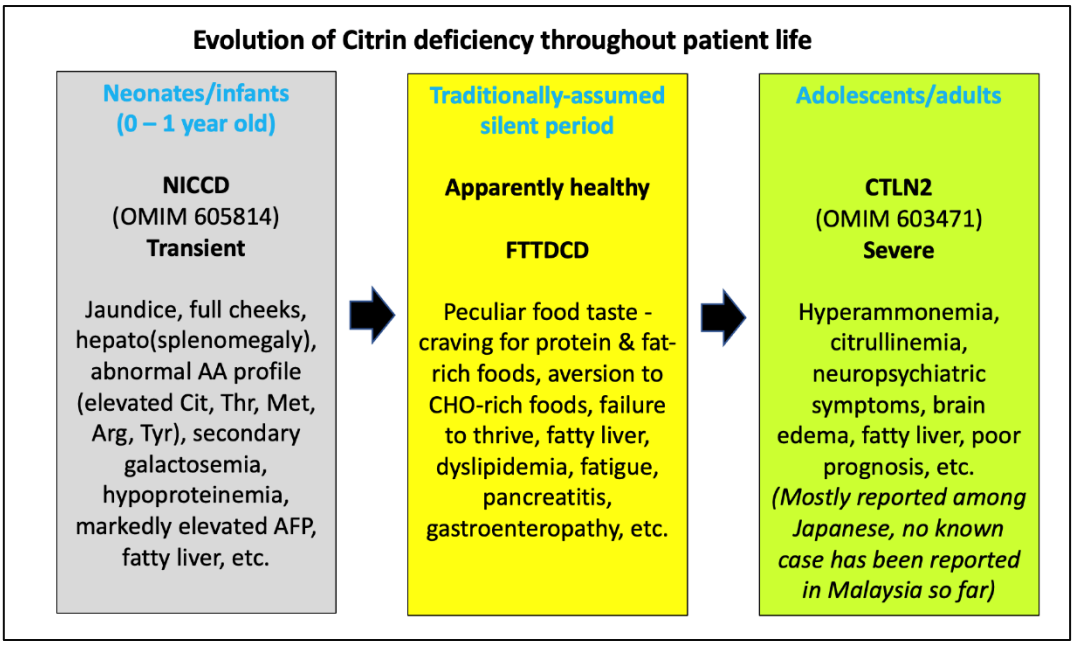
Complications: Hepatocellular carcinoma (watch AFP), renal failure.

(vi) Others

DISORDER	CLINICAL	DIAGNOSIS	TREATMENT
Classical galactosemia (not as common as Western countries in Malaysia)	Progressive symptoms after the start of milk feeds, prolonged jaundice, liver dysfunction, Gram negative organisms sepsis, bilateral cataract → death from hepatic and renal failure.	↑ Galactose, ↑Galactose-1-Phosphate, ↓Galactose-1-phosphate uridylyltransferase (GALT). Molecular genetic test: *GALT* gene.	Lactose-free, galactose-restricted diet.
Hereditary fructose intolerance	Following dietary exposure to fructose, sucrose, or sorbitol: nausea, vomiting, abdominal pain, renal tubulopathy, hepatomegaly, liver dysfunction, failure to thrive. Metabolic disturbances: hypoglycemia, lactic acidemia, hypophosphatemia, hyperuricemia, hypermagnesemia, hyperalaninemia	Molecular genetic test: *ALDOB* Fructose challenge not recommended	Strict fructose-restricted diet, vitamins supplement
Citrin deficiency (a.k.a. Citrullinemia Type II) (common in Malaysia [Ngu 2010, Chew 2010])	Neonatal/infantile period: prolonged direct hyperbilirubinemia, cholestatic jaundice, full cheeks, hepato-(spleno)megaly, secondary galactosemia, cataract. Childhood period: failure to thrive, craving for protein & fat-rich foods, aversion to CHO-rich foods, fatty liver, dyslipidemia. Adult: liver cirrhosis, hyperammonemia crisis.	Plasma AA: ↑citrulline, ↑Threonine, ↑Methionine, ↑Tyrosine (Abnormalities are transient during neonatal/infantile period). Molecular genetic test recommended for patient and all asymptomatic siblings: *SLC25A13* gene.	Neonatal/infantile: lactose/galactose-free formula (during cholestasis#), MCT oil, lipid soluble vitamins. Older ages: Low CHO diet, MCT oil (monitor growth), protein and lipid rich diet. Avoid high-carbohydrate meals and alcohol. # Use normal infant formula once the cholestasis has resolved.

Classical homocystinuria (~15 patients in Malaysia [A Habib 2024])	Progressive myopia, lens dislocation, Marfan-like appearance, cerebral venous sinus thrombosis /other thromboembolism, stroke, epilepsy, intellectual disability.	↑↑ Plasma total homocysteine (tHcy) (>150 µmol/L). Molecular genetic test (*CBS* gene).	Pyridoxine 50 -100 mg/day for 4 weeks. If no response: methionine-restricted diet, betaine (to keep tHcy <50 µmol/L).
Phenylketonuria (common in Western countries, rare in Malaysia)	Untreated: severe intellectual disability, epilepsy, hypopigmentation.	Plasma AA: ↑↑phenylalanine (Phe), ↓ tyrosine (DD: BH_4 deficiency) Molecular genetic test (*PAH* gene)	BH4 responsiveness test. If no response: Phe-restricted diet (to keep Phe 120 – 360 µmol/L).
Glycine encephalopathy (a.k.a. nonketotic hyperglycinemia) (a significant number of patients are Penans from Sarawak [N Azimah 2014])	Neonatal intractable seizures, hypotonia, lethargy, hiccups, apnea, EEG: burst suppression (mimics hypoxic ischemic encephalopathy). Attenuated form: developmental delay, epilepsy.	CSF: Plasma glycine ratio >0.08 (normal <0.02). Molecular genetic test for confirmation (*GLDC* and *AMT* genes).	Evaluate whether to aim at all out treatment or palliative care. To treat: Dextromethorphan 5 – 15 mg/kg/day, benzodiazepines, sodium benzoate (to keep plasma glycine <300 µmol/L).
Sulfite oxidase deficiency & Molybdenum cofactor deficiency (MoCoD) (~10 patients diagnosed in Malaysia [Ngu 2009])	Infantile epileptic encephalopathy, MRI brain – cystic encephalomalacia.	Fresh urine sulphite test: positive; ↑ urine sulphocysteine, ↓serum uric acid in MoCoD. Molecular genetic test: *SUOX, MOCS1, MOCS2* genes.	Substitution of cPMP in MoCoD type I. Symptomatic/ palliative care for others.
Cobalamin C defect (~10 patients diagnosed in Malaysia [A Habib 2024])	Neonatal/ infantile feeding difficulties, lethargy, neurological deterioration, encephalopathy, seizures, abnormal movement, pancytopenia. Attenuated form: developmental delay, behavioural issues, epilepsy.	Plasma AA: ↓methionine, Urine OA: ↑MMA, Plasma tHcy ↑. Molecular genetic test: *MMACHC* gene.	IM hydroxocobalamin 1 mg 3 – 5 times/week, betaine, folate, methionine supplement.
Biotinidase deficiency (BTD) & holocarboxylase synthase (HLCS) deficiency (~10 patients diagnosed in Malaysia [Mardhiah 2019])	Skin rashes, hair loss, progressive neurological symptoms, metabolic acidosis, immune deficiency.	↑ lactate, urine OA: 3-OH-isovaleric acid, DBS acylcarnitines: ↑C5OH, DBS biotinidase enzyme activity: ↓in BTD, normal in HLCS deficiency. Molecular genetic test: *BTD* and *HLCS* genes.	Biotin 5-10mg/day (BTD), 10-20mg/day (HLCS deficiency).

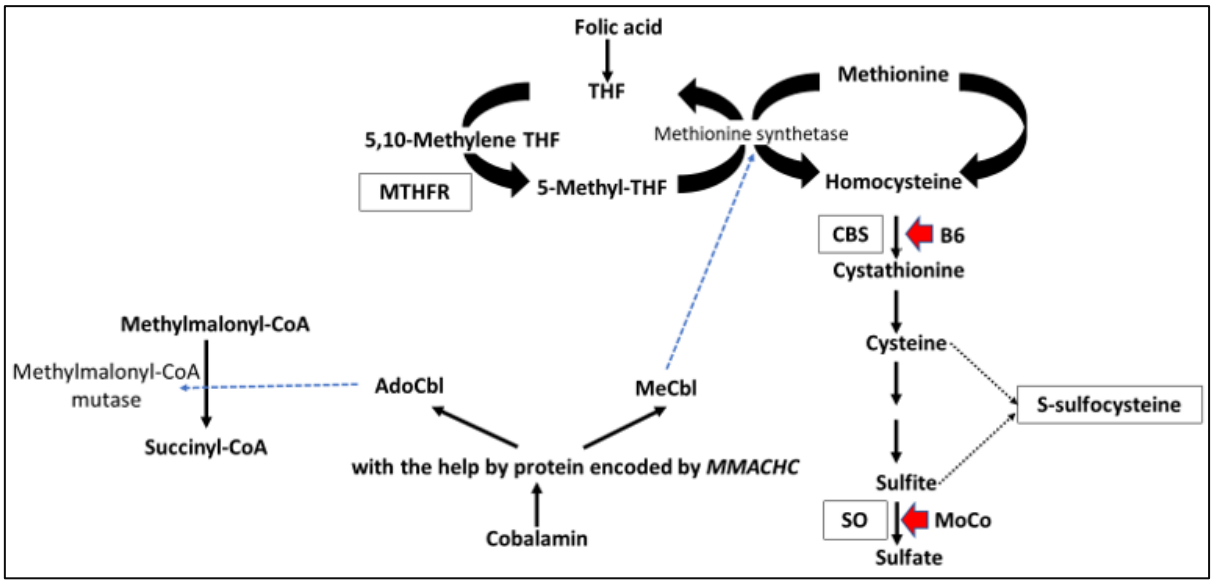
Antiquitin deficiency (pyridoxine-responsive seizures)	Neonatal onset epileptic seizures.	↑ Piperidine-6-carboxylate (P6C), ↑ pipecolic acid, ↑ α-aminoadipic semialdehyde (urine, CSF, plasma). Molecular genetic test: *ALDH7A1* gene.	Pyridoxine 5-15 mg/kg/day. Consider folinic acid, diet lysine restriction and arginine supplement.
Methylenetetra-hydrofolate reductase (MTHFR) deficiency	Progressive neurological deterioration, apnea.	Plasma AA: ↓methionine, plasma tHcy ↑. Molecular genetic test: *MTHFR* gene.	IM hydroxocobalamin 1mg/day, betaine, folinic acid, methionine supplement.
Cystinuria	Recurrent kidney stones, risk of chronic kidney disease.	Urine AA: ↑↑ Cys, ↑ Arg, Lys, Orn. Plasma AA: normal Molecular genetic test: *SLC3A1*, *SLC7A9*	High fluid intake, alkalinization of the urine, in selected cases try penicillamine 1-2 g/day and consider captopril. Surgical management may be needed
Hypoxanthine: guanine phosphoribosyl-transferase (HPRT) deficiency (X-linked disorder)	Lesch-Nyhan syndrome (complete deficiency): motor retardation, dystonia, spasticity, choreoathetosis, epilepsy, self-mutilation, uric stones (radiolucent) → renal failure, gout. Kelley-Seegmiller syndrome (partial deficiency): gout, renal stones, mild neurological symptoms but never self-mutilation.	↑↑uric acid Urine purine analysis: ↑ hypoxanthine Molecular genetic test: *HPRT* gene	Symptomatic, plentiful fluids, allopurinol (high dose), S-adenosylmethionine may reduce self-mutilation [Chen 2014].
Acute intermittent porphyria (hydroxy-methylbilane synthase deficiency)	Acute episodes of abdominal pain, nausea, vomiting, constipation, tachycardia, hypertension, muscle weakness due to peripheral neuropathy, respiratory muscle paralysis, seizures, mental changes, and hyponatremia; lasting days to weeks. Urine may be reddish brown or red (not a constant finding). Various triggers: stress, drugs, menstruation, fasting, etc. Long term complications: liver cancer, renal failure.	Urine porphobilinogen (PBG) ≥10 times the upper limit of normal (quantitative analysis is a must) Molecular genetic test: *HMBS* gene	IV human hemin is the most effective treatment for severe acute attack. Mild attack: IV fluids that contain 10% glucose with added sodium and potassium (hypotonic solutions should be avoided because of the risk of hyponatremia). Supportive treatment: pain relief, control of hypertension, prevention of nausea and vomiting, prompt treatment of seizures, and maintenance of fluid and electrolyte balance.



AA = amino acids; AFP = alpha-fetoprotein; Arg = arginine; Cit = citrulline; CTLN2 = adult-onset type II citrullinemia; FTTDCD = failure to thrive and dyslipidemia caused by citrin deficiency; Met = methionine; NICCD = neonatal intrahepatic cholestasis caused by citrin deficiency; Thr = threonine; Tyr = tyrosine

Pathway of sulfur-containing amino acid metabolism

Classical homocystinuria is due to defective cystathionine beta synthase (CBS). B6 (pyridoxine) is cofactor for CBS. Sulfite oxidase (SO) deficiency is isolated or due to Molybdenum cofactor (MoCo) deficiency. Methylenetetrahydrofolate reductase (MTFHR) deficiency impaired the re-methylation of homocysteine to methionine. AdoCbl and MeCbl are the active forms of cobalamin, functioning as cofactors for methylmalonyl-CoA mutase and methionine synthetase respectively. Formation of AdoCbl and MeCbl require proteins encoded by a number of genes including *MMACHC*. Cobalamin C disorder is due to defective *MMACHC*.



Subgroup 1b: Deficiency of small molecules

- Symptoms result primarily from the defective synthesis or transportation of an essential molecule (e.g. non-essential amino acids, neurotransmitters, cofactors, etc.).
- Defects often cause neurodevelopment disruption, with congenital presentation.
- Share many characteristics with disorders in the complex molecules.
- Most but not all are irreversible.
- A few are or should be treatable by supplementing the missing product distal to the block.
- Metabolic markers are not always present.

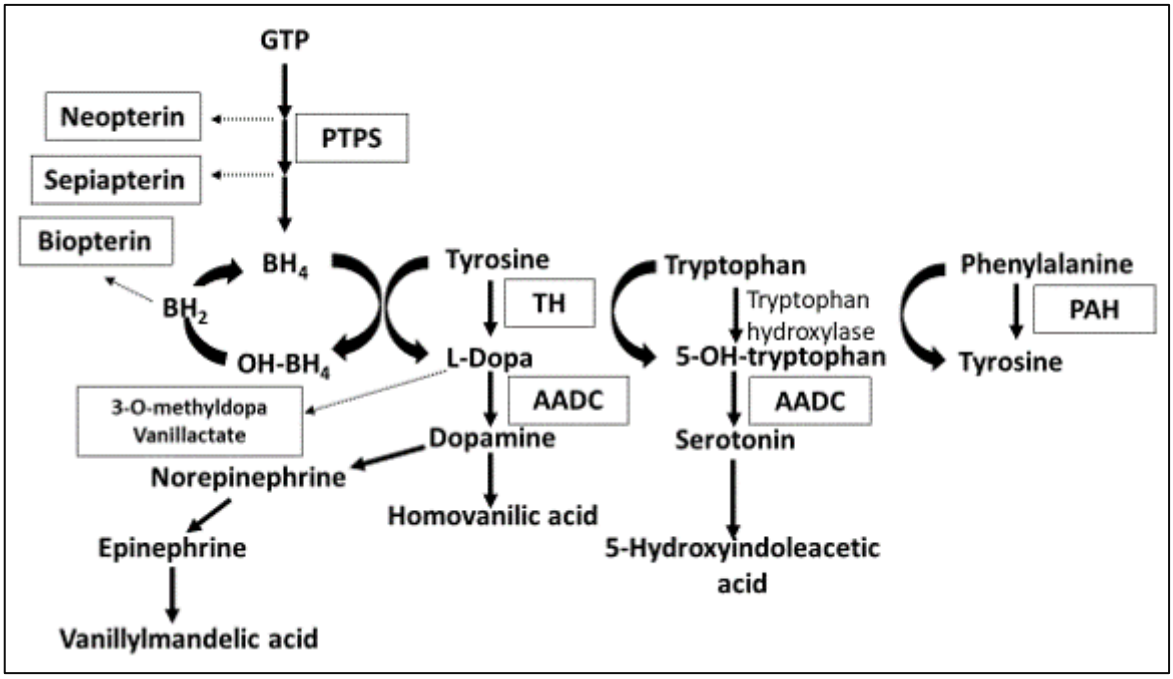
Main disorders:

DISORDER	CLINICAL	DIAGNOSIS	TREATMENT
AA synthesis defects **- Serine** **- Glutamine** **- Asparagine**	Early onset epileptic encephalopathy, brain malformations, microcephaly, severe intellectual disability.	Plasma/CSF amino acids. Molecular genetic test.	Not or poorly treatable except serine deficiency (serine supplement).
Neurotransmitters (NT) synthesis disorders: **- Tyrosine hydroxylase (TH) deficiency#** **- Aromatic L-amino acid decarboxylase (AADC) deficiency##** **- BH_4 deficiency (a.k.a. atypical phenylketonuria)###**	TH deficiency: severe dopamine deficiency with two phenotypes: (1) a severe neonatal onset complex encephalopathy with oculogyric crises, (2) an infantile onset, progressive, hypokinetic-rigid syndrome with dystonia.	CSF NT analysis Molecular genetic test.	L-Dopa 1 -10 mg/kg/day (start at low dose, gradually titrate to optimal dose as patients often have hypersensitivity to L-Dopa).
	AADC deficiency: severe combine dopamine and serotonin deficiencies. Infantile onset, progressive hypokinesia, truncal muscular hypotonia, limb rigidity, oculogyric crises, feeding difficulties, insomnia, temperature instability.	CSF NT analysis. Molecular genetic test.	Pyridoxal phosphate trial, dopamine agonists (response is often poor). Consider intracerebral gene therapy.
	BH_4 deficiency: severe combine dopamine and serotonin + ↑ plasma phenylalanine. Infantile onset, developmental delay, progressive hypokinesia, truncal muscular hypotonia, limb rigidity, oculogyric crises, feeding difficulties, insomnia, temperature instability.	Plasma AA: ↑ Phe CSF NT analysis. Molecular genetic test.	BH_4 5 mg/kg/day, L-Dopa 1 -10 mg/kg/day, 5(OH)tryptophan 1 – 5 mg/kg/day.

[#] ~ 15 patients diagnosed in Malaysia [Dhashene 2023]
[##] "High" incidence among Malaysian Chinese due a common 'Chinese' *AADC* variant c.[714+4A>T] [Himmelreich 2023]
[###] Most common cause of BH_4 deficiency in Malaysia is PTPS deficiency. It is more common among Malaysian Chinese due a few common 'Chinese' *PTS* variants [Chiu 2012].

Pathway of BH_4, dopamine and serotonin synthesis

6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency results in BH_4 deficiency. Tyrosine hydroxylase (TH) deficiency causes dopamine insufficiency. Aromatic L-amino acid decarboxylase (AADC) deficiency causes impaired synthesis of both dopamine and serotonin. BH_4 is also the cofactor for phenylalanine hydroxylase (PAH), its deficiency results in classical phenylketonuria.



Chapter 3

GROUP 2: DISORDERS INVOLVING ENERGY METABOLISM

These consist of IEMs with symptoms due, at least in part to a deficiency in energy production or utilization within the liver, myocardium, muscle, brain, and other tissues.

In general, cytoplasmic energy defects are generally less severe and more treatable than mitochondrial energy defects.

Diagnosis is by functional tests measuring glucose, lactate, ketones and other biomarkers (AA, organic acids, acylcarnitines) in blood, CSF and urine and confirmed by enzyme assays and/or molecular genetic testing.

Main disorders:

(i) Glycogen storage disorders (GSD)

Three main clinical presentations:

- Liver: hypoglycemia, hepatomegaly, growth retardation (GSD Ia, Ib, VI, IX, Oa)
- Muscle: exercise intolerance, muscle cramps, cardiomyopathy (GSD 0b, II, V, VII)
- Mixed/generalized: cardiomyopathy, liver/muscle involvement (GSD III, IV, IX)

Diagnosis is confirmed by molecular genetic testing (preferred) with/ without biopsy (histology) or enzyme studies (rarely done nowadays).

Inheritance: autosomal recessive except certain subtypes of GSD IX.

- **GSD Ia and GSD Ib**

  GSD 1a is due to Glucose-6-phosphatase deficiency. GSD Ib is due to defects in transporting Glucose-6-P into the endoplasmic reticulum for the action of Glucose-6-phosphatase. These two are the most severe type of hepatic GSD. GSD Ia is the most common type of GSD in Malaysia (>50 patients) [Abdul Wahab 2022].

  Clinical: First manifestation usually at the age of 3-6 months: recurrent hypoglycemia 3-4 hrs after meals, truncal obesity, hepatomegaly, doll-like face, failure to thrive, short stature. Bleeding tendencies such as epistaxis and easy bruising due to dysfunctional von Willebrand factor and platelet aggregation (despite normal platelet count). GSD Ib variant: same as above, plus neutropenia, leukocyte dysfunction, bacterial infections, diarrhea, inflammatory bowel disease (IBD).

  Diagnosis: ↓ Glucose, metabolic acidosis, ↑ lactate, lipemia, ↑ triglycerides, ↑ uric acid. Elevation of 3-methylglutaconic acid has been reported. Molecular genetic testing (single gene testing for *G6PC* and *SLC37A4* genes, or a multigene panel for GSD) is preferred over invasive liver biopsy.

  Treatment: Avoid hypoglycemia through frequent carbohydrate intake: Frequent meals (every 2 to 3 hrs in infants, 4-hourly from childhood); slowly resorbed carbohydrates (uncooked corn-starch 1 – 2.5 gm/kg/feed), limited fructose and lactose/galactose (vegetables, fruits); lactose-free milk + calcium; multivitamins, vitamin D. Nights: 4-hourly uncooked corn-starch; or continuous feeds (infants 12 hrs, adults 8 - 10 hrs) via gastrostomy starting as soon as possible after the last daytime meal. Simple sugar is not encouraged in GSD due to risk of rebound hypoglycemia. Glucose

polymers e.g. carborie is preferred if clinically necessary. Overtreatment with carbohydrate may be associated with insulin insensitivity.

Allopurinol for hyperuricaemia and ACE inhibitors for proteinuria. Perioperative glucose infusion for surgeries to prevent hypoglycemia. Management of platelet dysfunction includes antifibrinolytics (tranexamic acid), deamino-8-D-arginine vasopressin (DDAVP) and platelet transfusion during bleeding episodes or when undergoing invasive procedures.

GSD Ib: If neutropenia/ infections, add Filgrastim (G-CSF). Consider Empagliflozin (SGLT2 inhibitor).

Monitor: Use non-invasive continual blood glucose monitoring device (keep pre-prandial blood glucose > 3.5 - 4.0 mmol/L); monitor renal profile and urine for microalbuminuria, liver function, calcium, phosphate, blood gas, uric acid, lipid profile (triglyceride concentration <6.0 mmol/l); body mass index between 0.0 and + 2.0 SDS; half-yearly ultrasound scan of the liver, and blood pressure checks, 3-yearly echo and bone density scan from age 10 yrs.

Complications: After the first decade if poor metabolic control: delayed puberty, liver adenomas/carcinomas, anemia, gout, osteoporosis, proteinuria, renal failure. Combined oral contraceptive pills (high-dose estrogen) should be avoided, especially if liver adenoma detected.

- **GSD III (debrancher enzyme deficiency)**

Clinical: Clinical signs and hypoglycemia (ketotic) are usually milder than GSD I. Elevated serum creatine kinase is often observed. In adolescence and adulthood, liver disease becomes less prominent but patients develop progressive skeletal myopathy and cardiomyopathy.

Diagnosis: *AGL* gene analysis.

Treatment: Frequent meals and uncooked corn starch to prevent hypoglycemia (especially during infancy), protein intake of 3 g/kg/day or 25% of total energy is recommended to prevent breakdown of endogenous muscle protein in times of high glucose requirement and preserves skeletal and cardiac muscles. High-fat diet may reduce cardiomyopathy.

Complications: Hepatic cirrhosis and hepatic adenomas have been reported.

- **GSD IV (phosphorylase deficiency), GSD IX (phosphorylase kinase deficiency)**
  - They are clinically indistinguishable from each other

Clinical: Variable clinical severity affecting primarily the liver: hepatomegaly, elevated serum transaminases, ketotic hypoglycemia, hyperlipidemia, and poor growth

Diagnosis: GSD VI is caused by deficient activity of hepatic glycogen phosphorylase (encoded by the *PYGL* gene).

GSD IX is caused by deficient activity of phosphorylase kinase, the enzyme subunits of which are encoded by various genes: α (*PHKA1* in muscle, *PHKA2* in liver), β (*PHKB*), ɣ (*PHKG1*, *PHKG2*), and δ (*CALM1*, *CALM2*, *CALM3*).

Complications: Short stature, delayed puberty, and cirrhosis have been reported.

Treatment: Symptomatic patients: high protein diet, frequent meals and uncooked corn starch therapy (may be required at bedtime to prevent overnight hypoglycemia), avoidance of long

periods of fasting. Patients with very mild or no metabolic derangements may not need nutritional intervention.

- **Fanconi-Bickel syndrome**

- Deficiency in glucose transporter 2 (GLUT-2) results in hepatorenal glycogen accumulation leading to severe renal tubular dysfunction and impaired glucose and galactose metabolism.
- **Clinical:** Infantile onset - failure to thrive, hepatosplenomegaly, polyuria, normo/hypokalemic metabolic acidosis, hypophosphatemia and rickets (due to proximal tubule dysfunction) fasting hypoglycemia and post-feeding hyperglycemia. Puberty delay. Generalized osteoporosis.
- **Diagnosis:** Molecular genetic test – *SLC2A2* gene
- **Treatment:** Frequent feeds, uncooked corn starch, plenty of fluids, electrolyte replacement, vitamin D supplement.

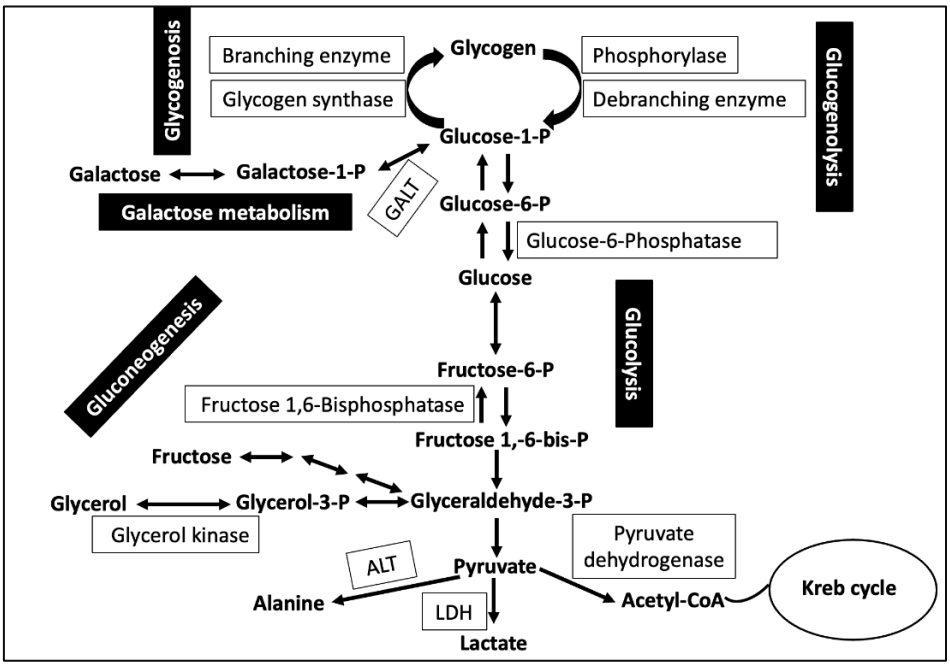
(ii) Disorders of gluconeogenesis

The typical feature is recurrent hypoglycaemia with lactic acidosis ± ketosis ± fluctuating hepatomegaly.

DISORDER	CLINICAL	DIAGNOSIS	TREATMENT
Fructose-1,6-bisphosphate deficiency[#]	Acute crisis (often neonatal) with hypoglycemia, hepatomegaly, metabolic acidosis, hyperventilation, ketosis, ↑lactate, coma, seizures, brain damage.	↑Lactate, ↑ketones, Urine OA): 2-oxoglutaric acid, glycerol, glycerol-3-phosphate. Molecular genetic test: *FBP* gene.	Usually responds rapidly to treatment with intravenous/oral glucose ± sodium bicarbonate. Avoidance of fructose/ sucrose/ sorbitol. Nocturnal feeds/ uncooked cornstarch. Frequent intake of glucose when unwell (sick day protocol). **Table sugar is sucrose and therefore not suitable when unwell.**
Glycerokinase (GK) deficiency	Isolated GK deficiency: Mostly male (X-linked). Recurrent vomiting, ↑ ketones, hypoglycaemia. Complex GK deficiency (Contiguous gene syndrome due to Xp21 deletion) – congenital adrenal hypoplasia ± Duchenne muscular dystrophy, sometimes OTC deficiency.	Urine OA: ↑ glycerol. Pseudo-Hypertriglyceridemia. Molecular genetic test: *GK* gene.	Treatment of associated conditions; fat-restricted diet.

[#] A number of patients have been reported in Malaysia [Moey 2018]

Carbohydrate metabolism



GALT: Galactose-1-phosphate uridylyltransferase, LDH: Lactate dehydrogenase, ALT: Alanine transaminase

(iii) Disorders of fatty acid beta oxidation (FAOD)

A group of about 20 defects in fatty acid transport and mitochondrial β-oxidation that are inherited as autosomal recessive disorders. Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency which is common in western countries is not common in Malaysia [unpublished data from Clinical Genetics Department HKL]. A few long-chain FAOD have been reported [A Habib 2021].

Pregnancies of mothers heterozygous for FAOD have been associated with development of severe pre-eclampsia, acute fatty liver of pregnancy and HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) in mothers and intrauterine growth retardation in infants.

The typical feature is hypoketotic hypoglycemic coma, which may be accompanied by signs of liver dysfunction. The first manifestation is frequently in late infancy, precipitated by fasting or infection with vomiting.

Clinical: Severe deficiencies of the carnitine shuttle and long-chain fatty acid oxidation cause severe neonatal lactic acidosis, hyperammonemia (esp. in Carnitine-acylcarnitine translocate (CACT) deficiency), hepatopathy, cardiac arrhythmias and cardiomyopathy. The condition is often lethal.

Milder deficiency variants of long-chain fatty acid oxidation and the carnitine shuttle may manifest in adolescence or early adulthood as chronic muscle weakness, pain, or recurrent rhabdomyolysis (sometimes precipitated by exercise or infection), or cause acute or chronic cardiomyopathy.

Other manifestations include retinopathy, often starting in infancy, and peripheral neuropathy.

Diagnosis: DBS acylcarnitines analysis is usually diagnostic but may be normal when well. It is important to repeat when unwell. Urine organic acid and serum total/ free carnitine may be helpful. Molecular genetic test for confirmation

Treatment: Avoid fasting, early intervention in intercurrent illness e.g. gastroenteritis, etc.

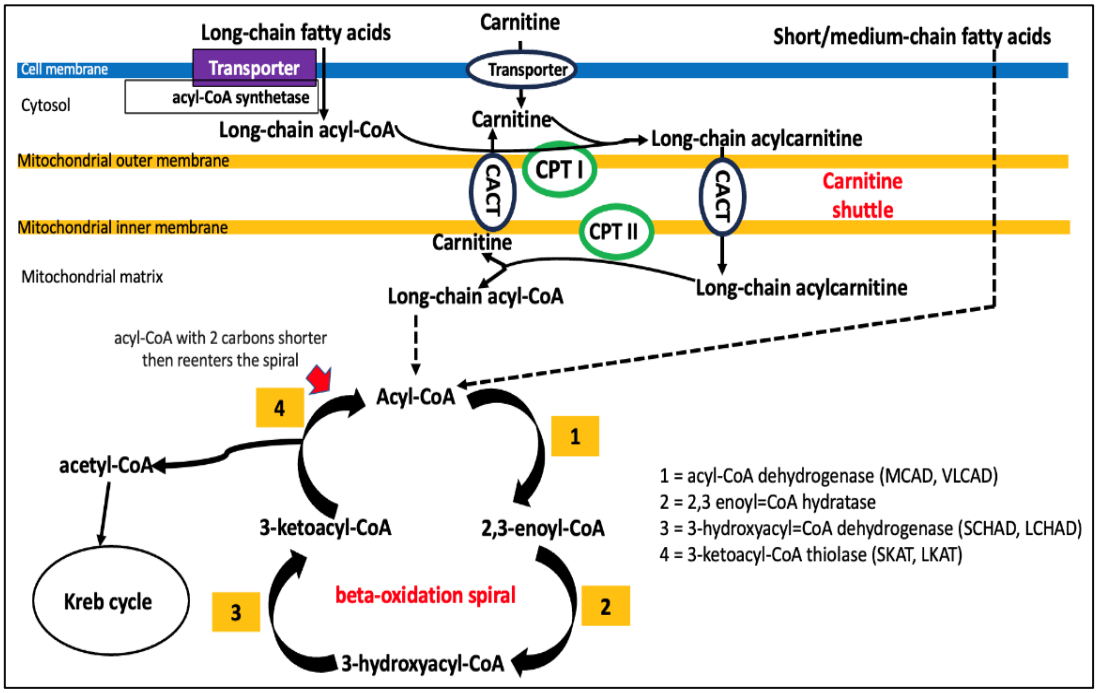
- Acute: High dose iv glucose (7 - 10 mg/kg/min). **Do not give iv lipids**.
- **In proven disorders of long-chain fatty acid oxidation and the carnitine shuttle:**
  - Acute: Dialysis (rarely needed); Low-fat formula + medium-chain triglyceride (MCT) oil, or MCT-rich medical formula via nasogastric tube or gastrostomy as slow perfusor as needed.
  - Long-term: Restrict dietary long-chain fatty acids; Low-fat formula + MCT oil, or MCT-rich medical formula; frequent meals, continuous nocturnal feeding; supplementation of essential long-chain fatty acids (alpha-linoleic, linoleic); consider triheptanoin.
  - Do not give MCT in medium chain FAODs e.g. MCAD deficiency.

- **Multiple acyl-CoA dehydrogenase deficiency (a.k.a. Glutaric aciduria type 2)**
  - Deficient electron transfer from the FAD-dependent dehydrogenases to the respiratory chain; does not only affect fatty acid oxidation but also dehydrogenases involved in the metabolism of amino acids (e.g. Val, Leu, Ile, Trp, Lys).
  - Clinical: Severe form: neonatal acidosis, hypotonia, hypoglycemia, hyperammonemia, hepatomegaly; sweaty feet odor; facial dysmorphism, congenital malformations (renal cysts, hypospadias, etc.); usually fatal in the first weeks of life.
  - Attenuated forms: episodic hypoglycemia, liver dysfunction; cardiomyopathy; progressive encephalopathy, epilepsy; myopathy. Sometimes riboflavin responsive
  - Diagnosis: DBS acylcarnitines. Urine OA: ↑ lactic, ↑glutaric, ↑ethylmalonic, ↑ dicarboxylic acids. Molecular genetic test:
  - Treatment: Avoidance of fasting; frequent meals, low-fat diet; trial of riboflavin 100 - 150 mg/day.

- **Carnitine transporter deficiency (a.k.a. Systemic primary carnitine deficiency)**
  - Carnitine is required for the transfer of long-chain fatty acids from the cytoplasm to the mitochondrial matrix for beta-oxidation. Carnitine is transported inside the cells by transporter OCTN2 transporter present in the heart, muscle, and kidney. In kidneys, absent of OCTN function results in reduced reabsorption of carnitine.

  - Clinical:
    - Hypoketotic hypoglycemic, hepatomegaly, elevated transaminases, and hyperammonemia.
    - Skeletal myopathy and/or elevated CK.
    - Cardiomyopathy, arrhythmias.
    - Unexplained fatigability in adults.
  - Diagnosis: ↓ plasma free carnitine levels (< 5 µmol/L, normal 20–50 µmol/L), *SLC22A5* gene analysis.
  - Treatment: Lifelong treatment with a high dose of oral L-carnitine (100 to 200 mg/kg daily dose in 3 divided doses).

Fatty acid metabolism



CPT1: Carnitine palmitoyltransferase I, CPT2: carnitine palmitoyltransferase II, CACT: carnitine acylcarnitine transporter, VLCAD: Very long-chain acyl-CoA dehydrogenase, MCAD: medium-chain acyl-CoA dehydrogenase, SCAD: short-chain acyl-CoA dehydrogenase, LCHAD: Long-chain 3-hydroxyl-CoA dehydrogenase, LCKAT: Long-chain 3-ketoacyl-CoA thiolase, SCHAD: Short-chain 3-hydroxyl-CoA dehydrogenase, SKAT: Short-chain 3-ketoacyl-CoA thiolase. VLCAD, MCAD and SCAD are responsible for metabolism of acyl-CoAs of chain lengths C_{12-18}, C_{6-10} and C_{4-6}, respectively.

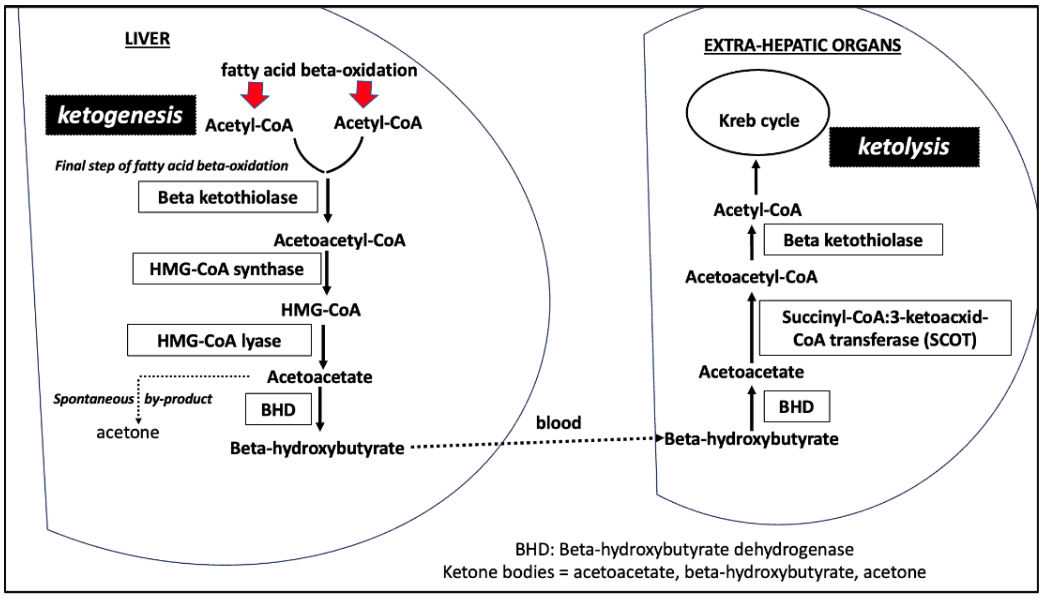
Disorders of ketone body metabolism

DISORDER	CLINICAL	DIAGNOSIS	TREATMENT
HMG-CoA synthase deficiency#	Acute hypoketotic hypoglycaemia, relative short fasting tolerance.	Urine OA: dicarboxylic aciduria without ketosis, ↑ 4-hydroxy-6-methyl-2-pyrone (only in acute sample). Molecular genetic test: *HMGCS2* gene.	Avoidance of fasting.
HMG-CoA lyase deficiency#*	Acute hypoketotic hypoglycaemia, metabolic acidosis, liver disease, Reye-like crisis.	Urine OA: ↑ 3-hydroxy-3-methylglutaric acid, ↑3-methyglutaconic acid. Molecular genetic test: *HMGCL* gene.	Acute: as for organic aciduria – high dose iv glucose, carnitine. Do not use iv lipids. Long term: low fat diet (25% of daily energy requirement), mild protein restriction, carnitine.

Succinyl-CoA:3-ketoacxid-CoA transferase (SCOT) deficiency##	Recurrent severe ketoacidosis, hyperketotic hypoglycemia	Fed state: persistent ketonuria; Fasting state: excessive ketonuria. Molecular genetic test for confirmation: *OXCT1* gene.	Acute: high dose iv glucose (7 - 10 mg/kg/min). Long term: avoidance of fasting, adequate caloric intake
Methylacetoacetyl-CoA thiolase deficiency (a.k.a. β-ketothiolase deficiency) ##	Recurrent severe ketoacidosis, hyperketotic hypoglycaemia ± ↑ NH_4^+ ± ↑ lactate	Urine OA: specific metabolites (e.g. ↑2-methyl-3-hydroxybutyrate, ↑tiglylglycine, ↑2-methylacetoacetate). Molecular genetic test: *ACAT1* gene.	

#Ketogenesis disorders; ##Ketolytic disorders; *also affects Leucine catabolism

Ketone Body Metabolism



(iv) Pyruvate dehydrogenase complex deficiency

Clinical: Neonatal encephalopathy, lactic acidosis, brain malformations (e.g. corpus callosum agenesis), dysmorphism; progressive encephalopathy in infancy (incl. Leigh or Leigh-like syndrome, focal brainstem lesions), apnea, episodic weakness, seizures; intermittent acute peripheral neuropathy, dystonia: childhood-onset intermittent episodes of weakness and ataxia. Most frequent *PDHA1* variant (X-linked) – males are more severely affected than females.

Diagnosis: ↑Lactate, pyruvate, alanine in body fluids, n- ↓lactate/pyruvate ratio. Molecular genetic test: *PDHA1* gene (x-linked, males more often and more severe than females, most common), less frequent *PDHX*, *DLD*, *PDHB*, *DLAT*, *PDP1* genes.

Treatment: Trial of thiamine (150 - 1000 mg/day), ketogenic diet.

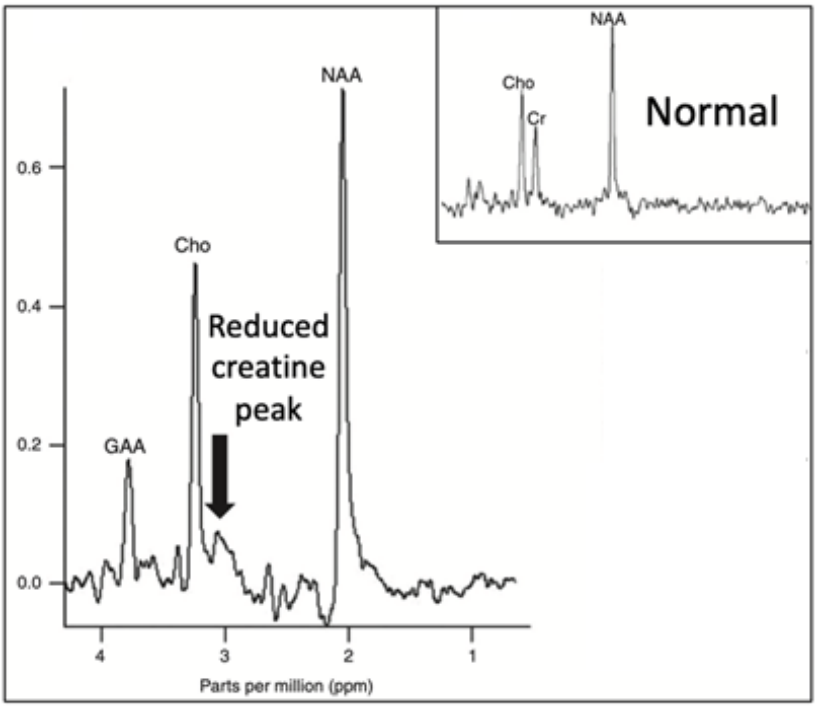
Prognosis: Often poor in early onset patients

(v) Disorders of creatine biosynthesis or transport

Clinical: Intellectual disability, speech impairment and epilepsy due to cerebral creatine deficiency.

Diagnosis: Low creatine concentrations in the brain can be recognized by MR spectroscopy; abnormal concentrations of creatine/creatinine and its precursor guanidinoacetate are usually found in serum and urine. Molecular genetic test: *GAMT* and *AGAT* genes (biosynthesis), *SLC6A8* gene (transporter, x-linked).

Treatment: Creatine 400 mg/kg/day.



MR Spectroscopy (MRS) of the brain in creatine deficiency. Creatine peak is reduced at 3.0 parts per million (ppm).

(vi) Glucose transporter protein deficiency (GLUT1 deficiency)

Clinical: Severe forms: epileptic encephalopathy of infancy or early childhood, (secondary) microcephaly, psychomotor retardation. Milder variants: (exercise induced) fluctuating movement disorders (ataxia, spasticity, dystonia, chorea), childhood/juvenile/adult-onset absence epilepsy.

Diagnosis: CSF analysis (following a 4 - 6 hrs fast): ↓ Glucose < 2.7 mmol/l, CSF/blood Glucose ratio <0.45 (normal 0.65 ± 0.1), n-↓ lactate/alanine. Molecular genetic test: *SLC2A1* gene.

Treatment: Ketogenic diet; avoidance of drugs that inhibit GLUT1 (e.g. barbiturates, chloral hydrate, diazepam, tricyclic antidepressants, ethanol, methylxanthines/green tea).

Prognosis: Satisfactory with early treatment.

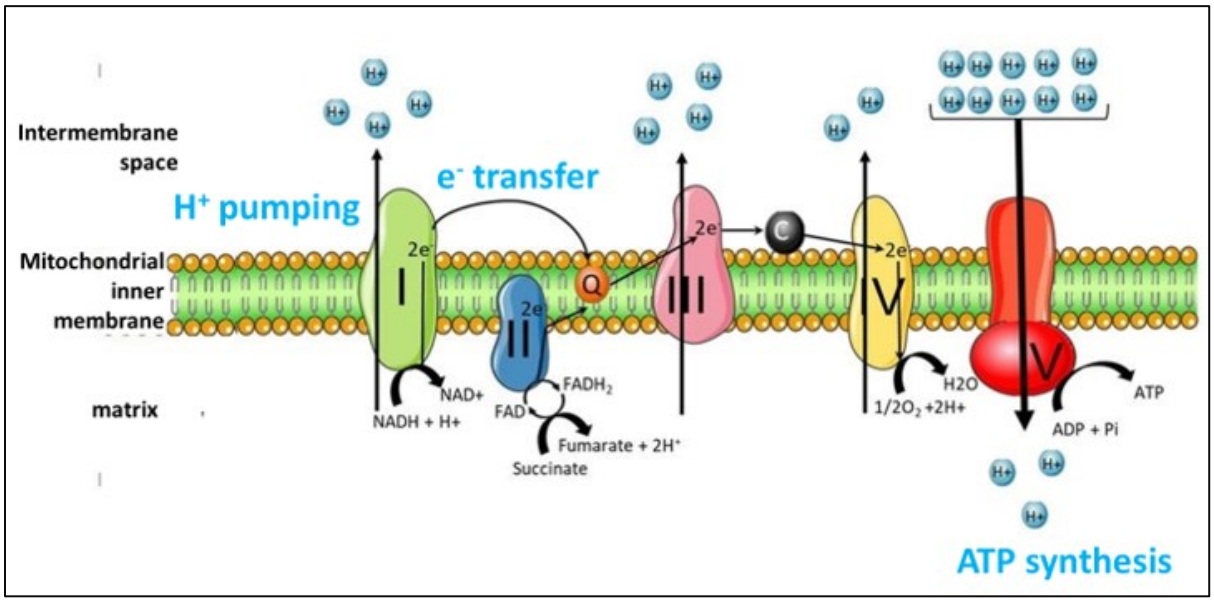
(vii) Primary mitochondrial disorders (PMDs)

As a group, PMDs is the most common type IEM in Malaysia. Few hundred patients have been diagnosed in Malaysia. The most common disease phenotypes are Leigh syndrome and MELAS [unpublished data from Clinical Genetics Department HKL]

- PMDs consist of a heterogeneous group of inborn errors of oxidative phosphorylation/ mitochondrial energy production caused by pathogenic variants in several hundred different nuclear and mitochondrial DNA genes.
- Mutations in nuclear genes coding for respiratory enzyme complexes structural subunits and assembly factors or proteins needed for mitochondrial DNA (mtDNA) maintenance & replication or gene expression predominate in younger age group.
- MtDNA mutations, often inherited in variable heteroplasmy (a situation where mutant and wild-type mtDNA coexist within the same cell) levels from mother, are more frequently associated with specific clinical syndrome and may present at any age.
- Heteroplasmy level may vary between cells, tissues, organs or different individuals carrying the same mtDNA mutation.
- A higher heteroplasmy (60% -80%) may lead to clinical symptoms while a low level of heteroplasmy may be clinically silent. E.g. for m.8993T>G and m.8993T>C pathogenic variants:

Heteroplasmy level	Clinical features
≤ 60%	Asymptomatic
~70% - 90%	NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa) syndrome
≥ 90%	Leigh syndrome

Mitochondrial respiratory enzyme complexes



Genetic origin of mitochondrial respiratory chain			
Complex	**Subunits**	**Nuclear encoded**	**mtDNA encoded**
I	44	37	7
II	4	4	0
III	11	10	1
IV	14	11	3
V	19	17	2

The range of possible symptoms/signs in PMDs are very diverse; could arise from isolated organ (e.g. LHON), or multiple organs (more often)

- **Central Nervous system:** Seizures, acute encephalopathy, developmental delay, neuroregression, migraine, ataxia, stroke (-like) episodes, malformations
- **Peripheral nervous system:** Neuropathy
- **Eye:** Optic neuropathy, retinopathy, ophthalmoplegia, ptosis
- **Ear:** Sensorineural hearing loss
- **Heart:** Cardiomyopathy, arrhythmia
- **Muscle:** Fatigue, exercise intolerance, myopathy
- **Gastrointestinal system:** Pseudo-obstruction, delayed gastric emptying
- **Liver:** Hepatopathy
- **Endocrine system:** Diabetes, hypothyroidism, hypoparathyroidism, hypogonadism.
- **Kidney:** Renal tubulopathy, glomerulopathy
- **Blood:** Sideroblastic anemia, pancytopenia, neutropenia

- It may manifest at any age: antenatal, neonatal, infancy, childhood, or adulthood.
- Intrauterine development may be affected, resulting in IUGR and brain malformations.
- Young children frequently present with encephalomyopathy while myopathies predominate in older children.
- Variable disease course: progressive, relatively static for long periods of time, or fluctuate with acute illness.

Diagnosis:

Biochemical Screening Tests in Suspected Mitochondrial Disease	
Test	**Remarks**
Blood lactate	Measure on several occasions, elevated levels are a helpful clue, but normal levels do not exclude mitochondrial disease. Beware of artefactual elevation (struggling child, excessive squeezing); lactate may also be elevated in hypoxia, after seizure, and with certain drugs.
CSF lactate	May be elevated, particularly when there is CNS involvement, but normal levels do not exclude mitochondrial disease.
Plasma amino acids	Elevated alanine may suggest a chronic/persistent elevation in blood lactate (May be the only clue in some patients with normal blood lactate)
Urine organic acids	Urine organic acid analysis may show Kreb's cycle intermediates such as fumarate. In addition, it can show elevated lactic acid, ethylmalonic acid, 3-methylglutaconic acid, MMA.

Congenital lactate acidosis in newborns

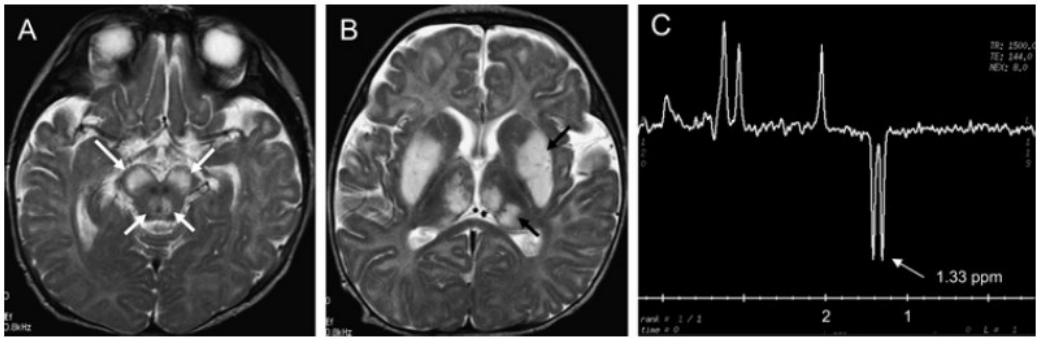
Typical characteristics of elevated lactate by cause in newborns

	Clinical Characteristics	Lactate	Lactate/ Pyruvate Ratio	Other biochemical Features
Spurious	Well appearing; Difficult phlebotomy; delayed sample processing.	<4 mmol/L	High	None
Secondary to hypoxia	Hypotension, poor tissue perfusion; history of hypoxia or tissue injury.	<5 mmol/L in most cases	High	None
Secondary to organic acidurias or Fatty acids oxidation defects	Sudden decompensation at 2-3 days after birth in previously well patient.	5 – 10 mmol/L in most cases	High	May have hyperammonemia. Urine OA: specific metabolites suggestive of organic aciduria, ketonuria (organic acidurias), or dicarboxylic aciduria (fatty acid oxidation defect). Acylcarnitine profile: Fatty acid oxidation defect.
Primary due to pyruvate metabolic defect	Illness apparent around 24 hours after birth; structural brain anomalies; fetal alcohol-like facial features.	10 – 20 mmol/L	Normal	Plasma amino acids: Very high alanine; citrulline is high in pyruvate carboxylase deficiency.
Primary due to PMDs (a.k.a Primary lactic acidosis) – generally poor prognosis	Hypertrophic cardiomyopathy; structural anomalies; lens clouding or cataracts; intrauterine growth restriction.	10 – 20 mmol/L	High	Plasma amino acids: high alanine, proline, and often glutamine. Urine organic acids: variable elevations of 3-methylglutaconic acid, fumarate, ethylmalonic acid, dicarboxylic aciduria.

Neuroradiological studies

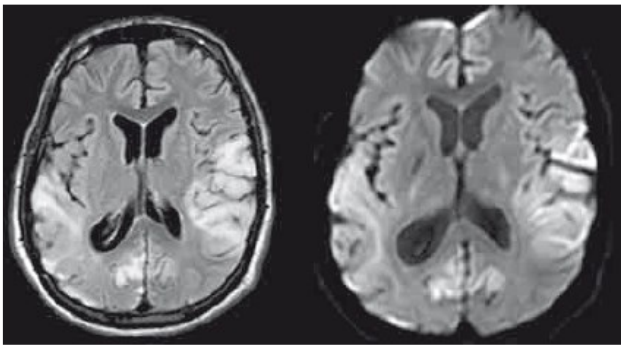
There are certain neuroradiological findings that are sensitive and quite suggestive in diagnosis of mitochondrial disease. MR spectroscopy may reveal lactate accumulation. CT brain may reveal symmetrical calcification, e.g. in basal ganglia. Four recognized MRI brain patterns are as follows:

- Leigh syndrome pattern



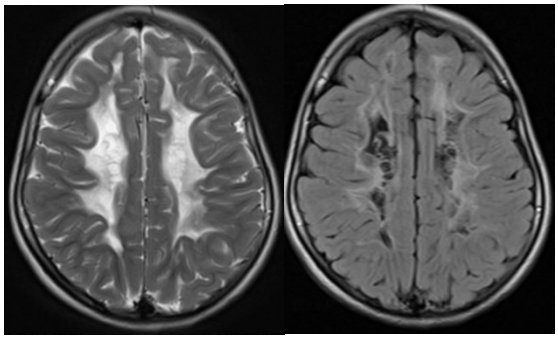
Characteristic brain MRI pattern of Leigh syndrome. (A) Axial T2 weighted images show important bilateral hyperintensities in the brainstem (white arrows). (B) Axial T2 weighted images show hyperintensities in the basal ganglia and thalami (black arrows). (C) Magnetic resonance spectroscopy shows a lactate peak at 1.33 parts per million (ppm) (white arrow).

- Stroke-like pattern



Fluid-attenuated inversion recovery (left) and diffusion-weighted (right) MR brain images of a patient with MELAS showing the patchy abnormalities in multiple regions of the cortical tissue (not confined to a vascular territory). Acute changes may fluctuate, migrate, or even disappear completely during the acute to subacute phase.

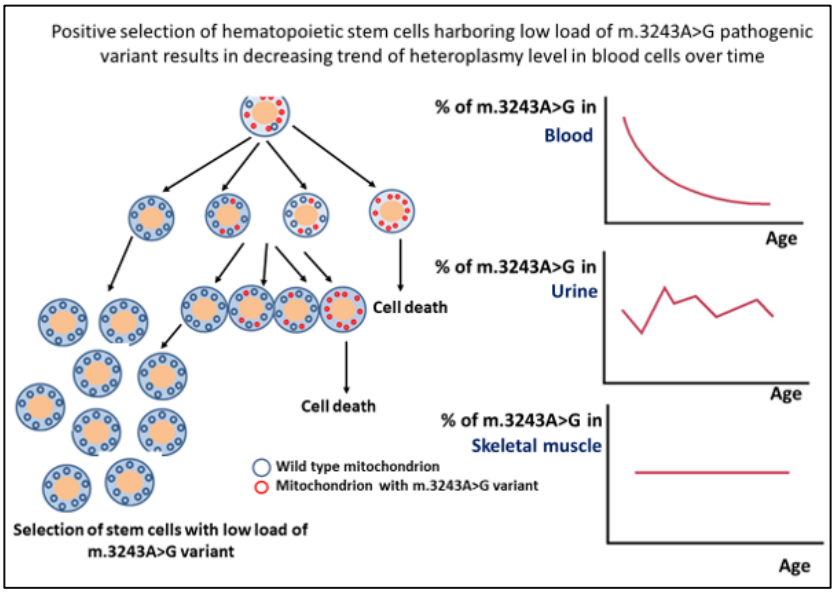
- Leukodystrophy (frequently diffuse, patchy, or cystic/cavitating white matter lesions).



Axial T2 (left) and FLAIR (right) shows vacuolization of white matter

- Cerebral or cerebellar atrophy (non-specific) or pontocerebellar hypoplasia (e.g. PCH6).

Molecular genetic testing has rapidly evolved to become the confirmatory test of choice for the definitive diagnosis of mitochondrial disease.

Molecular Genetic Diagnostic Approaches for Mitochondrial Disease	
Molecular Genetic Test	**Remarks**
Targeted mtDNA genetic testing for point mutation and/or single large-scale deletion	When the clinical presentation is typical, targeted genetic testing allows confirmation of a clinical diagnosis. For example, testing for common mtDNA mutations for MELAS; single large-scale deletion test for Kearns-Sayre syndrome. A negative blood result does not rule out the diagnosis because of tissue-dependent and age-dependent variation in heteroplasmy level (blood heteroplasmy level decreases significantly over time). It should be repeated on other tissues (that are clinically more severely affected and/or heteroplasmy level shows very little age-dependent variation) e.g. uroepithelial cells (urine sample), muscle, buccal mucosa cells, etc.  Wellcome Centre Mitochondria Research at Newcastle University, UK has developed an online tool to produce corrected blood and urine m.3243A>G heteroplasmy levels. **https://newcastle-mito-apps.shinyapps.io/m3243ag_heteroplasmy_tool/**
Targeted nuclear gene sequencing	Example: Sequencing of *POLG1* when clinical presentation (childhood onset progressive encephalopathy, seizures, liver failure) is typical.
Targeted nuclear gene panel	When there are findings indicating a particular condition that is genetically heterogeneous. For example, mtDNA depletion panel when mtDNA depletion syndrome is suspected.

Whole exome/genome sequencing that includes mitochondrial genome sequencing	This is the most comprehensive approach. It not only allows diagnosis of a mitochondrial disorder but also other disorders that are in the differential diagnosis. However, there is a likelihood of finding more variants of unknown significance.

Treatment:

- A multidisciplinary team is required to provide supportive care.
- General measures:
  - Ensure adequate intake of calories, fluids and electrolytes; avoid fasting
  - Avoid/treat condition with high energy consumption:
    - Treat fever efficiently.
    - Treat seizures/epilepsy efficiently (avoid valproate)
- Metabolic acidosis is common in PMDs. Types of acidosis: ketoacidosis (due to impaired β-hydroxybutyrate oxidation), lactic acidosis and renal tubular acidosis. Symptoms and signs with acidosis: mental state changes, hyperpnea, bradycardia, hypotension, arrhythmia, anorexia, vomiting, failure to thrive, delayed myelination, etc.
- Acute treatment:
  - Correct metabolic acidosis with iv Sodium Bicarbonate.
  - Monitor for hypernatremia and respiratory failure.
  - Minimize dextrose to maintain euglycemia (High dextrose can cause increased elevation in lactate).
- Long term treatment: Consider ketogenic diet.

- Contraindicated drugs:
  - Sodium valproate -Absolute contraindication in PMD caused by *POLG* mutations. In other PMDs, may be considered in exceptional circumstances).
  - Aminoglycosides – For short term, emergency use – the benefits outweigh the risks. For long term elective use – must exclude mtDNA mutations (m.1555A>G, m.1494C>T in MT-RNA1) that are associated with aminoglycoside-induced sensorineural hearing loss.
  - Lactated Ringers (LR) - If Lactate < 5 mmol/L, LR is not contraindicated. If LR is deemed the most appropriate resuscitation fluid, benefit may outweigh risk for individual patients.

- General anesthesia and surgery
  - In general, most PMD patients tolerate anesthetics well
  - Most risk is attributable to the severity and extent of organ involvement such as cardiomyopathy, respiratory muscle weakness, bulbar dysfunction or severe lactic acidosis. Perform baseline comorbidity evaluation.
  - Fasting should be minimized and caloric and fluid intake maintained.
  - Small intravenous boluses of propofol (avoid prolonged use in the maintenance of anaesthesia), benzodiazepines, or ketamine.

- Stroke-like episode (SLE)
  - A subacute, evolving brain syndrome.
  - Diagnosis of a SLE requires the combination of clinical assessment, MRI and EEG. (and other differential diagnoses excluded)

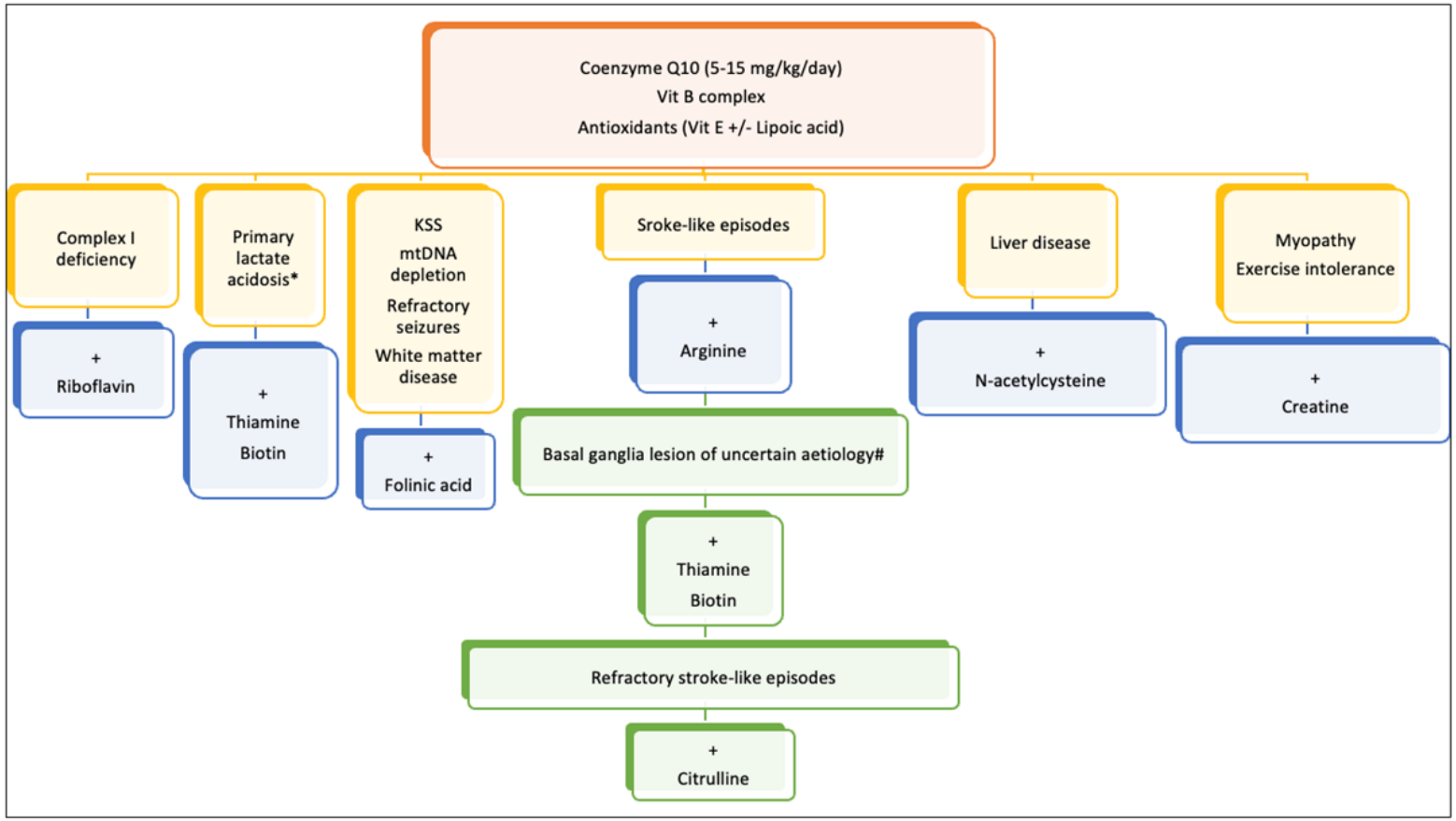
- Clinical: headache, nausea and vomiting, complex visual symptoms including visual filed defects, encephalopathy, focal onset seizures (with or without associated focal neurological deficits) including epilepsia partialis continua, new-onset neuropsychiatric symptoms (excessive anxiety, aggressiveness, agitation or psychosis - SLE involve frontal, temporal or limbic lobe)
- MRI brain: cortical and subcortical signal abnormalities not confined to vascular territories.
- EEG: focal epileptic discharges.

  - Genetic aetiology: m.3243A>G (most common), *POLG,* other rarer mtDNA mutations
  - Possible pathophysiology mechanisms of SLE: i. mitochondrial angiopathy (impaired vasodilation due to reduced nitric oxide production); ii. mitochondrial cytopathy (causes cytotoxic edema due to ATP depletion, disrupted calcium homeostasis, etc. →neuronal hyperexcitibility → driver of SLE is seizure activity → neuronal death if untreated)
  - Treatment: **To treat the seizures/cytotoxic edema** - iv levetiracetam is recommended, but phenytoin (with cardiac monitoring) or phenobarbitone (with respiratory monitoring) can be used. Avoid valproate. **To treat angiopathy** – iv arginine infusion 250 - 500 mg/kg over 60-90 minutes given within 3 hours of symptom onset is recommended. May be given daily for up to 5 days. May stop sooner if neurologic symptoms resolve.
  - Chronic or prophylactic treatment is aimed to decrease the frequency of SLE: oral arginine 100-300mg/kg/day.

- Although randomized, controlled trial data are lacking, supporting preclinical evidence and favourable risk-benefit ratios justify empiric trial of vitamins and cofactors to support mitochondrial function in PMDs with further modification based on tolerability concerns or additional patient-specific genetic and/or clinical phenotypes.

- PMDs that are potentially treatable using specific therapies:
  - Disorders of Coenzyme Q_{10} biosynthesis – high dose of CoQ_{10} (30mg/kg/day).
  - LHON – Idebenone 900mg/day (it has been shown to be effective in stabilizing and restoring vision in patients treated within 1 year of onset of vision loss, ideally as soon as possible from onset, maintains for at least 1 year or until a plateau in term of improvement is reached).
  - Thymidine kinase 2 deficiency - Nucleoside (deoxycytidine and deoxythymidine) treatment.

Medications used for mitochondrial disease



Dose: Vitamin E 1-2 IU/day, Lipoic acid 50-600mg/day, Biotin 10-20mg/day, Riboflavin 50-400mg/day, Thiamine 50-300mg/day, Arginine 150-300mg/kg/day, Citrulline 150-300mg/kg/day, Folinic acid 1.5-5 mg/kg/day, N-acetylcysteine 10mg/kg/day, Creatine 100mg/kg/day

#While investigating for *SLC19A3*-related Biotin-thiamine-responsive basal ganglia disease; *While investigating for biotinidase deficiency & PDH deficiency

Common classical mitochondrial syndromes				
Disease (onset)	**Diagnostic criteria**	**Symptoms of Acute Decompensation**	**Common (Chronic) Symptoms**	**Genetic**
Leigh Syndrome (infancy or early childhood)	Both A and B: A. Neurodevelopmental delay and stepwise regression B. Bilateral lesions of the basal ganglia, midline brainstem or both by brain CT, brain MRI (T2/FLAIR hyperintense lesions)	- Rapid progression to respiratory failure - Dysphagia/drooling - Profound (lactic) acidosis - Cardiomyopathy - Metabolic stroke: Increased weakness, seizures - Hepatopathy - Neuroregression	- Global Developmental Delay - Hypotonia/hypertonia - Movement disorder - Dysconjugate gaze - Elevated lactate in blood, CSF - Abnormal MRI brain - Bilateral basal ganglia and/or brainstem lesions - Eating & swallowing difficulties - FTT - Metabolic acidosis	-mtDNA point mutations: m.8993T>G/C, other mtDNA mutations (maternally inherited) -Nuclear gene mutations: *SURF1*, etc. (>100 genes, mostly autosomal recessive, some are X-linked)
MELAS Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like Episodes (4 – 60 yrs, mean at 14 yrs for paediatric cases)	**All of A – C:** **A. Stroke-like episodes (sudden-onset focal neurological deficit with brain MRI or CT showing a cerebral lesion that does not conform to a large vessel territory and typically affects cortex and adjacent white matter)** **B. Encephalomyopathy** **C. Lactate acidosis**	**Stroke-like episodes:** - **Focal neurological deficits** - **Abnormal MRI brain with acute DWI+** - **Medically actionable with IV arginine** **Seizures:** - **EPC (epilepsia partialis continua) or** **Status epilepticus**	- **Migraine** - **Vision loss** - **Hemiplegia** - **Seizures** - **Short stature, FTT** - **Deafness** - **Diabetes** - **Hyperglycemia, hypoglycemia** - **Avoid over treating** - **DM results from insulin deficiency and resistance** - **Elevated lactate w/wo metabolic acidosis**	**mtDNA point mutations: 80% m.3243A>G, 20% other mtDNA mutations (maternally inherited)**

Common classical mitochondrial syndromes (cont.)				
Disease (onset)	**Diagnostic criteria**	**Symptoms of Acute Decompensation**	**Common (Chronic) Symptoms**	**Genetic**
Myoclonic Epilepsy with Ragged-Red Fibers (MERRF) (5 – 15 yrs)	All of A – C: A. Myoclonic seizures or myoclonus plus seizures B. Ataxia C. Mitochondrial myopathy (RRF, raised CK)	- Encephalomyopathy - Myoclonic epilepsy	- Ataxia - Neuropathy - Progressive dementia	mtDNA point mutations: 80% m.8344A>G, 20% other mtDNA mutations (maternally inherited)
Alpers Syndrome (infancy or early childhood)	All of A – C: A. Psychomotor regression B. Intractable seizures (confirmed by EEG and lack of response to at least two anti-seizure medications) C. Hepatopathy (Liver disease/dysfunction)	Seizures: - Refractory status epilepticus - Epilepsia partialis continua (EPC) - Continuous focal seizure or myoclonus Fulminant liver failure: - Triggered by sodium valproic - Provoked by fever, infection	- Developmental regression - Fluctuating LFTs - Elevated CSF protein - Cerebral folate deficiency - Brain MRI may show global atrophy or stroke like lesions	Nuclear genes: mostly *POLG* mutations (autosomal recessive)
Hepatocerebral syndrome (most common phenotype of Mitochondria DNA depletion syndrome in paediatric ages) (infancy or early childhood)	**All of A-C A. Progressive or persistent liver dysfunction B. Encephalopathy C. Any one of i. Cognitive impairment ii. Increased skeletal muscle tone (spasticity) iii. Hyperactive reflexes iv. Diffuse, patchy, or cystic white matter lesions evident on brain MRI**	- **Neonatal hypoglycaemia** - **Liver dysfunction/failure (may mimic neonatal hemochromatosis)** - **Encephalopathy** - **Infection-associated deterioration**	- **Global developmental delay** - **Epilepsy** - **Myoclonus**	**Nuclear genes including *DGUOK*, *TWNK*, etc.**

Common classical mitochondrial syndromes (cont.)				
Disease (onset)	**Diagnostic criteria**	**Symptoms of Acute Decompensation**	**Common (Chronic) Symptoms**	**Genetic**
Kearns-Sayre Syndrome (KSS) due a single large scale mtDNA deletion ***(< 20 yrs)***	All of A – D: A. Ptosis, Progressive External Ophthalmoplegia (PEO) or both B. Pigmentary retinopathy C. Cardiac conduction block D. Skeletal muscle involvement	- Hypocalcemia/ hypoparathyroidism: - Tetany, seizures, rickets - Diabetes mellitus - Pancreatitis/pancreatic insufficiency - Stroke-like episodes	- CPEO (chronic progressive external ophthalmoplegia) - SNHL (sensorineural hearing loss) - Elevated CSF protein - Cerebral folate deficiency - Ataxia - FTT, short stature - Renal failure - Hepatopathy - Brain MRI Leigh syndrome or white matter changes - Cardiac conduction defects (some have pacemaker)	Single large scale mtDNA deletion (sporadic)
Chronic progressive external ophthalmoplegia (CPEO) (early adulthood, occasionally childhood)	A ± B A. Ptosis, Progressive External Ophthalmoplegia (PEO) B. Limb myopathy, exercise intolerance, and dysphagia		- Ptosis (bilateral) - Limited peripheral vision owing to restricted eye movement: Additional features may include proximal limb weakness, pharyngeal	50%: Single large scale mtDNA deletion; 50%: nuclear genes including *POLG*
Leber hereditary optic neuropathy (LHON) (12 – 30 yrs)	**Both A and B:** **A. Acute-onset central vision loss in one or both eyes** **B. Family history compatible with maternal inheritance and not autosomal dominant**	- **Visual loss**	- **Subacute painless visual loss; sequentially affect both eyes** - **Males > females 4:1**	**mtDNA mutations: m.11778G>A, m.3460G>A, m.14484T>C (usually homoplasmic, maternal inherited, variable penetrance)**

Chapter 4

GROUP 3: COMPLEX MOLECULE DISORDERS

- This expanding group encompasses diseases that disturb the metabolism of complex molecules that are neither water-soluble nor diffusible: sphingolipids (SPL), triglycerides (TG) phospholipids (PL), complex long chain fatty acids (LCFA), cholesterol and bile acids, glycosaminoglycans (GAGs), oligosaccharides (OLS), glycoproteins, glycolipids and nucleic acids.
- Metabolism of complex molecules take place in organelles (mitochondria, lysosomes, peroxisomes, endoplasmic reticulum and Golgi apparatus) and most pathways involve several organelles and require transporters.
- Clinical symptoms are permanent, very often progressive, independent of intercurrent events, and unrelated to food intake. Most disorders do not present with acute crises.
- There are two subgroups in complex molecule disorders, defined by whether the phenotype primarily results from an accumulation or a deficiency.

Subgroup 3A: Accumulation of complex molecules

- Catabolism or transport defects lead typically to storage of a visible compound like in classical lysosomal storage diseases (LSD).
- In general, there are no antenatal manifestations although in some severe forms, this is possible such as hydrops or malformations.
- Neurological presentations include progressive neurodegeneration with or without obvious visceral storage signs.
- Diagnosis is mostly based on urine screening (mucopolysaccharides, oligosaccharides, etc.), leukocyte enzyme analysis and/or molecular genetic testing.

Main disorders:

(i) Disorders of glycosaminoglycan degradation/Mucopolysaccharidoses (MPS)

- More than 100 patients have been diagnosed in Malaysia. All MPS are autosomal recessive except Hunter disease (MPS II), which is X-linked. In Malaysia, MPS II is the commonest type of MPS [Ngu 2017].
- **Clinical:** Affected children often appear normal at birth but subsequently develop chronic progressive disease symptoms and signs. Affected organ systems vary between diseases and include the skeletal system and connective tissue (dysostosis multiplex, growth failure, joint contractures, facial dysmorphism, hernias, etc.), the nervous system (progressive neurological abnormalities, developmental regression, etc.), sensory organs (corneal clouding, deafness), internal organs (hepatosplenomegaly, cardiomyopathy, etc.), and others.

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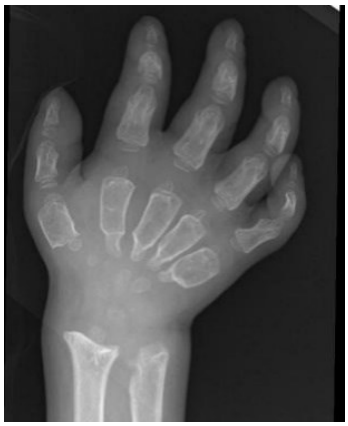
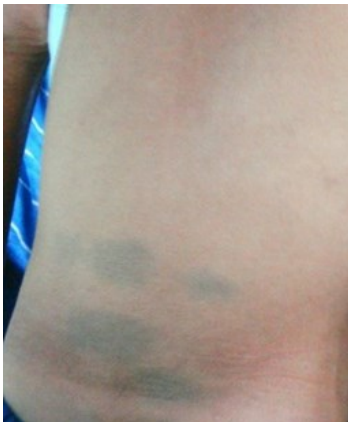
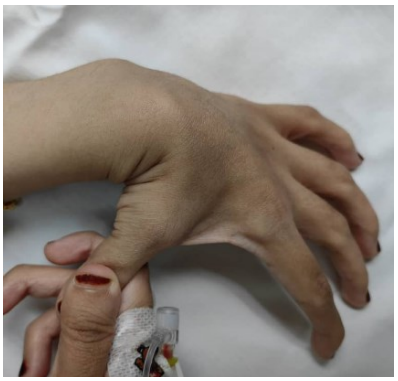


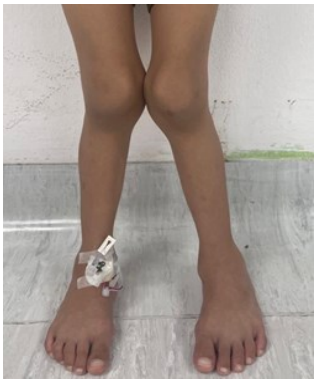
Tapering of the proximal ends of metacarpals and distal phalanges



Extensive and persistent Mongolian spots are often found in patients with MPS in Malaysia (and other East Asian countries) especially those with neurological involvement



Hypermobile joints in MPS IVA resulting in ulnar deviation of both wrists. Other types of MPS cause progressive joint contractures.



Genu valgum (knock knees) in MPS IVA

MPS IVA (Morquio syndrome A) is mainly a skeletal dysplasia [Leong 2019]. Growth modulation surgery is recommended in patients with MPS IVA who have signs of genu valgum and should be performed as early as possible during the period of growth.

- **Diagnosis:** Primarily by the analysis of glycosaminoglycans (GAG) in urine; confirmation by leukocyte enzyme analysis and molecular genetic testing.
- **Treatment:** Enzyme replacement therapy (ERT) for non-cerebral manifestations in MPS I, II, IVA, VI, and VII. Brain-penetrating ERT for MPS II may be considered. Hematopoietic stem cell transplant (HSCT) is the treatment of choice for severe MPS I to prevent cognitive decline (must be performed before the age of 2 years). It can be used in MPS VI, but risks compared to ERT should be balanced. HSCT is controversial in MPS II. Many patients will require surgical management including hernia repair, ear ventilation tube insertion, tonsillectomy and/or adenoidectomy, cardiac valve replacement, carpal tunnel decompression, corneal transplant, spinal surgery and orthopedic surgery.

- **Monitoring:** Multisystemic monitoring is required including yearly audiology, eye assessment, sleep study, echocardiogram and ECG. Baseline MRI cervical spine, followed by 1 to 2 yearly imaging to monitor for cervical cord compression.
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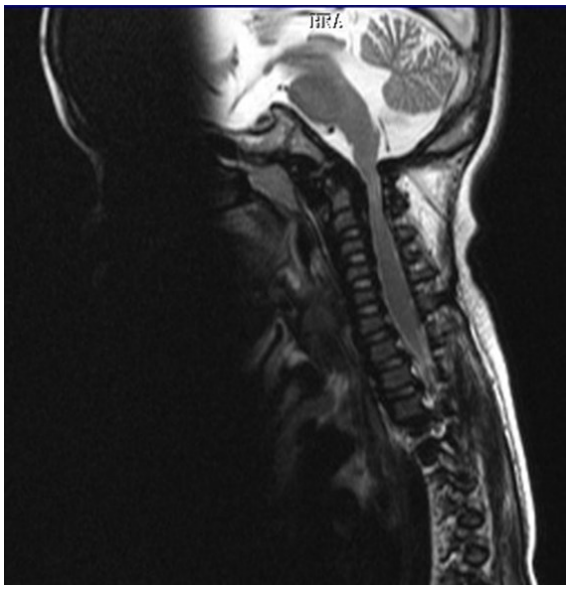


Cervical cord compression in a patient with MPS VI

- **Considerations for anaesthesia:** Due to the risk of upper airway obstruction, preoperative sedative premedication should be used with caution in patients with MPS. Assessment of the upper and lower airway anatomy, cardiac function (including an ECG and echocardiogram), and potential cervical spine instability and compression, should be performed and functions as a baseline evaluation prior to any procedure that requires sedation or anaesthesia.

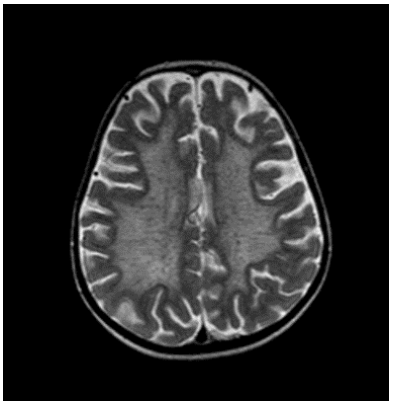
(ii) Pompe disease

More than 30 patients have been diagnosed in Malaysia. Majority are infantile-onset patients. All are CRIM negative [Chan 2023].

- **Clinical:** Infantile-onset: Hypertrophic cardiomyopathy, skeletal myopathy, hypotonia, respiratory failure, large tongue, failure to thrive, typical ECG (P waves, massive QRS complexes and shortened PR intervals), fatal in the first year if untreated.
- Late-onset: Slowly progressive limb-girdle muscle weakness (hip flexors are most affected) results in gait abnormalities, difficulties in climbing stairs and reaching overhead; diaphragmatic muscle weakness results in respiratory insufficiency without cardiac involvement; trunk muscle (paravertebral) and abdominal muscle weakness; hyperCKemia, scoliosis, rigid spine, and myotonic discharges on electromyography (EMG). Patients may notice the first signs of respiratory muscle dysfunction when lying down, including: orthopnoea, sleep disordered breathing, morning headache, dyspnoea at rest and after exercise.
- **Diagnosis:** Enzyme assay (α-glucosidase) in dried blood spots, molecular genetic test of the *GAA* gene.
- **Treatment:** ERT, multidisciplinary supportive care including intensive physiotherapy.
- Some infantile-onset patients are susceptible to CNS pathologies such as white matter hyperintense foci in the brain detected by neuroimaging and developmental assessments.

(iii) Disorders of sphingolipid degradation/sphingolipidoses

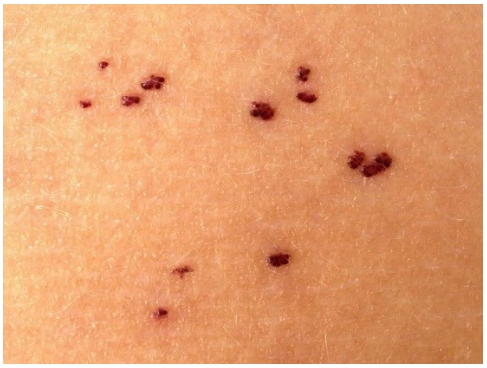
- There are many types of sphingolipidoses: Gaucher disease, acid sphingomyelinase deficiency, GM_1 gangliosidosis, GM_2 gangliosidosis, Fabry disease, Krabbe disease, metachromatic leukodystrophy, etc. More than 100 patients have been diagnosed in Malaysia [Leong 2016].
- **Clinical:** Sphingolipids are of special importance in the nervous tissue and reticuloendothelial system. Most sphingolipidoses present with prominent neurological symptoms: developmental delay/regression, epilepsy, ataxia, spasticity. Hepatosplenomegaly and cherry-red macula spot are not uncommon. Dysmorphism (e.g. coarse facies) ± skeletal deformities (dysostosis multiplex) are present in GM_1 gangliosidosis. Skin angiokeratoma is a feature of Fabry disease (X-linked inheritance). Radiologically distinct leukodystrophies are metachromatic leukodystrophy and Krabbe disease. Histological features include foam cells in the bone marrow or vacuolated lymphocytes. Due to the overlapping clinical features, it is challenging to diagnose the specific type of sphingolipidoses on clinical grounds alone.
- **Diagnosis:** ↑plasma chitotriosidase (in some), enzyme analyses in leukocytes or dried blood spots, and molecular genetic testing.
- **Treatment:** Specific therapies are available for some conditions, e.g. ERT for non-neuronopathic types of Gaucher disease and acid sphingomyelinase deficiency. Fabry disease – There are 2 options: IV ERT and oral migalastat. The latter option is only for adults with a *GLA* gene variant that is amenable (responsive) to it. To find out whether a specific *GLA* mutation has been classified as amenable, visit https://www.galafoldamenabilitytable.com/hcp



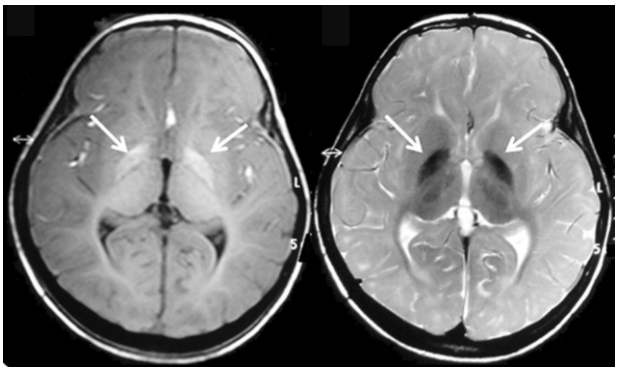
Classical MRI brain findings in metachromatic leukodystrophy. Axial T2 image: symmetric confluent areas with high signal intensity in the periventricular white matter with tigroid and leopard skin patterns

(iv) Oligosaccharidoses (deficiency breakdown of sugar side chains of glycoproteins) e.g. fucosidosis, sialidosis, alpha-mannosidosis, etc.

- Most patients with fucosidosis in Malaysia are Ibans from Sarawak (unpublished data from Clinical Genetics Department HKL).
- **Clinical:** Progressive neurological symptoms, epilepsy, developmental regression, coarse facies, dysostosis multiplex (mild), angiokeratoma.
- **Diagnosis:** Urine oligosaccharides analysis, enzyme analyses in leukocytes or dried blood spots, and molecular genetic testing.
- **Treatment:** Symptomatic.



Angiokeratoma

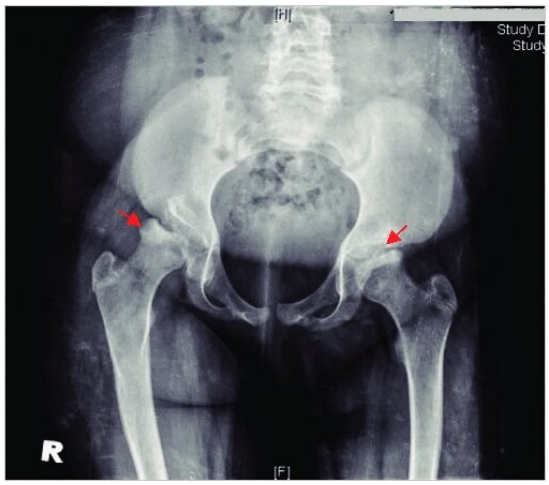


Classical MRI brain findings in fucosidosis. T1 axial (left), T2 axial (right). Bilateral globus pallidi (arrows) show hyperintensity on T1 and marked hypointensity on T2 images. Hypomyelination is evident by diffuse white matter hyperintensity on T2 with normal appearance on T1 images.

(v) Mucolipidoses (ML)

About 20 patients with mucolipidoses have been diagnosed in Malaysia [Ngu 2016].

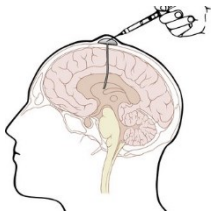
- **Clinical:** Combined clinical features of MPS and sphingolipidoses.
- ML II (I-cell disease) – Hurler-like but earlier, neonatal onset coarse facies, severe dysostosis multiplex.
- ML III – Childhood onset, mild-to-moderate dysostosis multiplex, joint stiffness, coarse facies, etc.
- **Diagnosis:** ↑GAG, ↑multiple lysosomal enzymes in the plasma (due to failure in the transport of soluble lysosomal enzymes from the Golgi apparatus into the lysosome), molecular genetic testing.
- **Treatment:** Symptomatic.



Pelvic X-ray of a patient with ML III: avascular necrosis of femoral heads (red arrows). Bilateral hip joint disease may initially be misdiagnosed as Legg-Calve-Perthes disease [Ngu 2016].

(vi) Neuronal ceroid lipofuscinoses (CLN)

- **Clinical:** Epilepsy, cognitive and developmental regression, loss of vision (retinal disease), specific EEG changes with photostimulation (CLN1).
- **Diagnosis:** DBS enzyme assays are available for CLN 1 and CLN2. Molecular genetic testing for other types.
- **Treatment:** Intracerebroventricular ERT for CLN2. Symptomatic treatment for other types of CLN.



Intracerebroventricular ERT is administered via Ommaya reservoir

(vii) Infantile nephropathic cystinosis

Nephropathic cystinosis is caused by defective transport of the amino acid cystine out of lysosomes.
Clinical: Renal Fanconi syndrome, poor growth, hypophosphatemic/calcipenic rickets, polyuria, polydipsia, dehydration, vomiting, metabolic acidosis, corneal crystals, photophobia. If left untreated, patients develop end-stage kidney failure by late childhood.
Diagnosis: Elevated cystine in leukocytes, molecular genetic testing: *CTNS* gene.
Treatment: Symptomatic treatment for renal Fanconi syndrome, oral cysteamine, cysteamine eye drops.
Prognosis: Satisfactory if treated early. However, if treated late extra-renal complications occur – myopathy, endocrine abnormalities, blindness (if not treated with cysteamine eye drops)

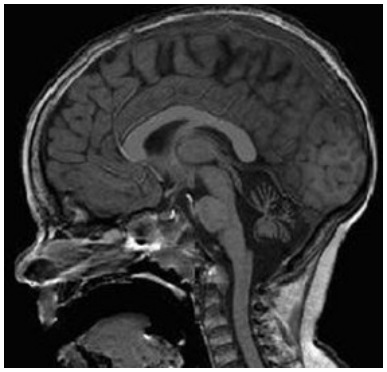
Subgroup 3B: Deficiency of complex molecules

- Defects of synthesis, recycling, intracellular transportation/trafficking of complex molecules.
- No storage material.
- May interfere with fetal development.
- Most are irreversible disorders.
- Many have multisystemic presentations. Almost all present as chronic or progressive diseases independent of food and intercurrent events. Most involve the nervous system.
- Only few have metabolic markers (peroxisome and cholesterol disorders).
- For all the others the diagnosis is mostly made by molecular genetic testing.
- In general, there are no specific treatments with rare exceptions.

Main disorders:

(i) Congenital disorders of glycosylation (CDG)

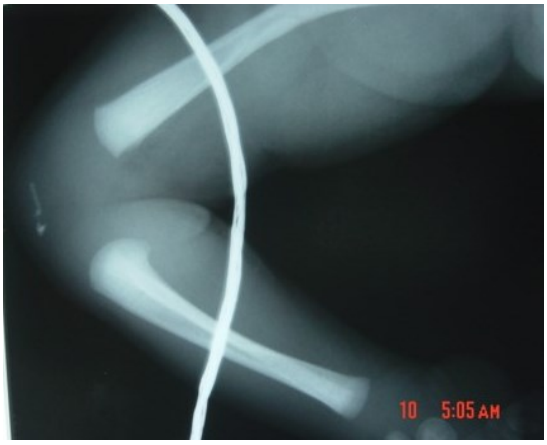
- It is a large group of rare genetic disorders (~ 160 disorders) that affect the addition of sugar building blocks, called glycans, to proteins in cells throughout the body.
- **Clinical:** CDGs should be considered in any unexplained clinical condition particularly in multiorgan disease with neurological involvement but also in non-specific developmental disability. Many CDGs interfere with neurodevelopment in the fetal period.
- **Diagnosis:** Serum transferrin isoelectric focusing can be used for the screening of N-linked glycosylation defects. No biomarkers are available for the other types of CDGs, for which diagnosis would require molecular genetic testing.
- The most common CDG is **Phosphomannomutase deficiency (PMM2-CDG or CDG1a):**
  - **Clinical:**
    Infancy: Hypotonia, failure to thrive, dysmorphism (inverted nipples & unusual fat pads); multisystem disease (pericardial effusion, liver disease, coagulation defects, endocrine abnormalities), severe cases are fatal in infancy.
    Late infancy to childhood: Global developmental delay, severe cerebellar atrophy, ataxia, seizures, stroke-like episodes, retinitis pigmentosa, skeletal deformities, endocrine abnormalities (e.g. hypothyroidism, hypogonadism).
  - **Diagnosis:** Serum transferrin isoelectric focusing- Type I pattern. Enzyme assay and molecular genetic testing.
  - **Treatment:** Symptomatic.



Severe diffuse cerebellar atrophy in PMM2-CDG

(ii) Peroxisomal disorders

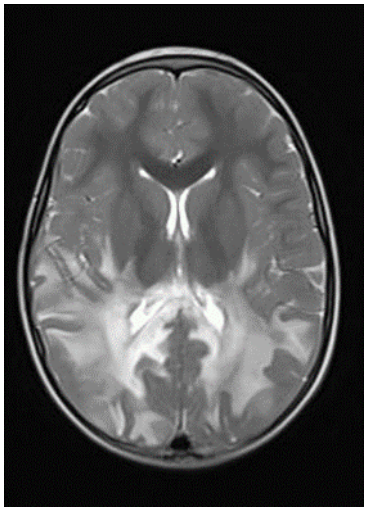
- A group of IEMs affecting either peroxisome biogenesis or a specific single enzyme involving the catabolism of very long chain and branched chain fatty acids (phytanic acid), or complex molecule synthesis like bile acids or plasmalogens.
- **Clinical:** Many present at birth with multiple malformations e.g. Zellweger syndrome. Others present later between the first and second decade of life with neurodegenerative disorders (Refsum disease, X-linked adrenoleukodystrophy, etc.)
- **Diagnosis:** ↑plasma VLCFA is found in majority of peroxisomal disorders. Many other biomarkers. Molecular genetic testing for definitive confirmation.
- **Treatment:** Mostly symptomatic.
- **Zellweger syndrome:** Neonatal presentation with severe hypotonia, seizures, liver dysfunction (severe jaundice, cholestasis), dysmorphism, skeletal abnormalities, sensorineural deafness, retinopathy, cataracts, failure to thrive; X-ray: Stippling of the epiphyses; MRI brain: pachypolymicrogyria; usually fatal within a few months.



Calcific stippling in Zellweger syndrome

- **X-linked adrenoleukodystrophy (ALD):** Most common peroxisomal disorder.

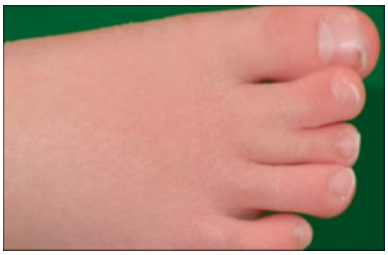
  Clinical: Childhood cerebral form: Behavioral changes (e.g. ADHD), visual/hearing impairment, intellectual regression, ataxia, adrenal insufficiency, MRI brain - leukodystrophy predominantly involving the occipital lobe. Decerebration within 2 - 4 years.
  Adrenomyeloneuropathy (Adolescent/young adult): Progressive spastic paraparesis, sphincter problems, adrenal insufficiency. Isolated Addison disease (childhood-adult): Very rare.
  Diagnosis: ↑plasma VLCFA. Molecular genetic testing: *ABCD1* gene.
  Treatment: Early hematopoietic stem cell transplantation. "Lorenzo's oil" is not effective.



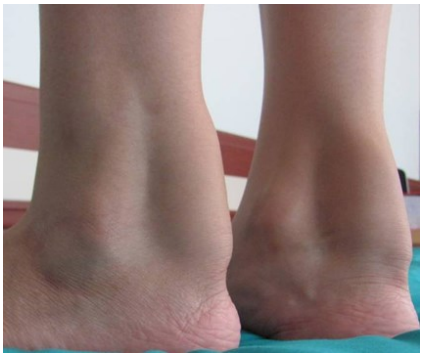
Classical MRI brain findings in X-linked adrenoleukodystrophy. Axial T2 image: High signal intensity in the parieto-occipital lobes

(iii) Others

- **Phospholipids (PL), glycosphingolipids (GSL) and fatty acids (FA) (long, very long, ultra long chain FA) synthesis and remodelling defects:** A spectrum of progressive neurodegenerative symptoms, myopathy and cardiomyopathy (e.g. Barth syndrome), orthopedic signs (bone and chondrodysplasia), syndromic ichthyosis, and retinal dystrophy.
- **Cholesterol and bile acid synthesis defects** present either with syndromes with multiple malformations or birth defects such as in Smith-Lemli-Opitz (SLO) syndrome, neonatal cholestasis, or with late-onset neurodegenerative disorders such as cerebrotendinous xanthomatosis (treatable by chenodeoxycholic acid).
- **Glycosaminoglycans (GAG) synthesis disorders** should be suspected in patients with a combination of characteristic clinical features in more than one connective tissue: bone and cartilage (short long bones with or without scoliosis), ligaments (joint laxity/dislocations) and the subepithelium (skin, sclerae). Some disorders present as distinct clinical syndromes with bone dysplasias.



Syndactyly of second and third digits of the foot in Smith-Lemli-Opitz syndrome.



Tendon xanthomas in cerebrotendinous xanthomatosis

Chapter 5

LIST OF MEDICATIONS AND NUTRITIONAL PRODUCTS USED IN THE TREATMENT OF INBORN ERRORS OF METABOLISM AT THE CLINICAL GENETICS DEPARTMENT, HOSPITAL KUALA LUMPUR

Emergency medications for hyperammonemia

Drugs	Dose	Indication
L-arginine (Intravenous)	Loading dose (short infusion over 90 to 120 min): 250mg/kg >10 yrs. 5.5g/m^2 Maintenance dose: 250 - 500mg/kg/day >10 yrs. 5.5g/m^2/day	Hyperammonemia due to urea cycle disorders except arginase deficiency
Carglumic acid (Oral)	Up to 100 mg stat, then 100 – 250 mg/kg/day	Hyperammonemia due to NAGS deficiency, organic acidurias and FAOD.
Sodium benzoate (Intravenous)	Loading dose (short infusion over 90 to 120 min): 250mg/kg >10 yrs. 5.5g/m^2 Maintenance dose: 250 - 500mg/kg/day >10 yrs. 5.5g/m^2/day	Hyperammonemia due to urea cycle disorders except arginase deficiency
Sodium phenylbutyrate (Intravenous)	Loading dose (short infusion over 90 to 120 min): 250mg/kg >10 yrs. 5.5g/m^2 Maintenance dose: 250 - 500mg/kg/day >10 yrs. 5.5g/m^2/day	Hyperammonemia due to urea cycle disorders

Other emergency medications

Drugs	Dose	Indication
L-arginine (Intravenous)	Slow infusion 250 - 500 mg/kg over 60 to 90 minutes given within 3 hours of symptom onset is recommended. May be given daily for up to 5 days	MELAS
Nitisinone (Oral)	1 - 2mg/kg/day	Tyrosinemia type I
Panhematin (Hemin) (Intravenous)	1 - 4 mg/kg/day over at least 30 minutes for 3 to 14 days based on clinical signs	Acute porphyria

Vitamins, cofactors and related medications

Drugs	Dose	Indication
Betaine	100mg/kg/day	Hyperhomocysteinemia due to cystathionine beta-synthase deficiency and cobalamin C disorder
Biotin	10 - 20 mg/day	Biotinidase deficiency, holocarboxylase synthase deficiency, biotin-thiamine-responsive basal ganglia disease
L-carnitine	50 - 200mg/kg/day	Systemic primary carnitine deficiency, organic acidurias
Chenodeoxycholic acid	10 - 20 mg/kg/day Adult: 750 mg/day	Cerebrotendinous xanthomatosis
Creatine	400mg/kg/day	Cerebral creatine deficiency
Coenzyme Q_{10}	5 - 15 mg/kg/day	Primary CoQ_{10} deficiency. May consider a therapeutic trial for other primary mitochondrial diseases
Cysteamine (Eye drops)	One drop in each eye 4 times a day	Cystinosis
Cysteamine (Oral)	1.30 g/m^2/day >12 years: 2g/day	Cystinosis
Folinic acid	3 – 5 mg/kg/day in 3 doses	Cerebral folate deficiency
Hydroxocobalamin (B12)	IM 1 mg/day, trial for 5 days; then 1 - 10 mg daily or every other day	B12-responsive methylmalonic academia, cobalamin C disorder
Idebenone	900mg/day	Leber hereditary optic neuropathy
Ornithine	100 - 800mg/kg/day	Cerebral creatine deficiency
Pyridoxine (B6)	100 mg then 30 mg/kg/day in 3 divided doses for 3 days; Maintenance 5 - 15 mg/kg/day	Antiquitin deficiency. B6-responsive cystathionine beta-synthase deficiency
Pyridoxal phosphate	30 - 50 mg/kg/day in 3 to 6 divided doses, trial for 3 days	Pyridoxal phosphate-responsive seizures. May consider a therapeutic trial in aromatic L-amino acid decarboxylase deficiency
Riboflavin (B2)	100 - 400 mg/day	Multiple acyl-CoA dehydrogenase deficiency. May consider a therapeutic trial for primary mitochondrial diseases especially in complex I deficiency.
Sapropterin	5mg/kg/day	BH_4 deficiency, BH_4 responsive phenylketonuria
S-adenosylmethionine	400 - 1000mg/day	Lesch-Nyhan syndrome
Thiamine (B1)	150 - 300 mg/day	Pyruvate dehydrogenase deficiency. Thiamine pyrophosphokinase (TPK) deficiency
Vitamin A	100 - 400 IU/kg/day	Abetalipoproteinemia
Vitamin E	100 - 300 IU/kg/day	Abetalipoproteinemia

Long-term maintenance medications for hyperammonemia

Drugs	Dose	Indication
Arginine	Oral 250-500mg/kg/day	Urea cycle disorders except arginase deficiency
Citrulline	Oral 250-500mg/kg/day	Urea cycle disorders except arginase deficiency
Sodium benzoate	Oral 250-500mg/kg/day	All Urea cycle disorders
Sodium phenylbutyrate	Oral 250-500mg/kg/day	All Urea cycle disorders

Enzyme replacement therapy

Drugs	Dose	Indication
Alglucosidase (iv)	20 - 40mg/kg 2 weekly	Pompe disease
Avalglucosidase (iv)	20 - 40mg/kg 2 weekly	Pompe disease
Cerliponase alfa (Intracerebroventricular)	300 mg 2 weekly	CLN2
Elosulfase alfa (iv)	2mg/kg weekly	MPS IVA
Galsulfase (iv)	1mg/kg weekly	MPS VI
Idursulfase (iv)	0.5mg/kg weekly	MPS II
Idursulfase beta (iv)	0.5mg/kg weekly	MPS II
Imiglucerase (iv)	40 - 60 units/kg 2 weekly	Gaucher disease
Laronidase (iv)	0.58 mg/kg weekly	MPS I
Vestronidase alfa-vjbk (iv)	4mg/kg 2 weekly	MPS VII

A range of nutritional therapy products to treat inborn errors of metabolism including special foods and milk formula for special medical purposes, single amino acid supplements (e.g. valine, isoleucine, methionine powder) and food supplements are also available through collaboration with the Dietetic Department of Hospital Kuala Lumpur.

Milk formula for special medical purposes and food supplements available in Hospital Kuala Lumpur

Chapter 6

INVESTIGATING CHILDREN SUSPECTED OF HAVING A CONDITION WITH GENETIC BASIS INCLUDING INBORN ERRORS OF METABOLISM

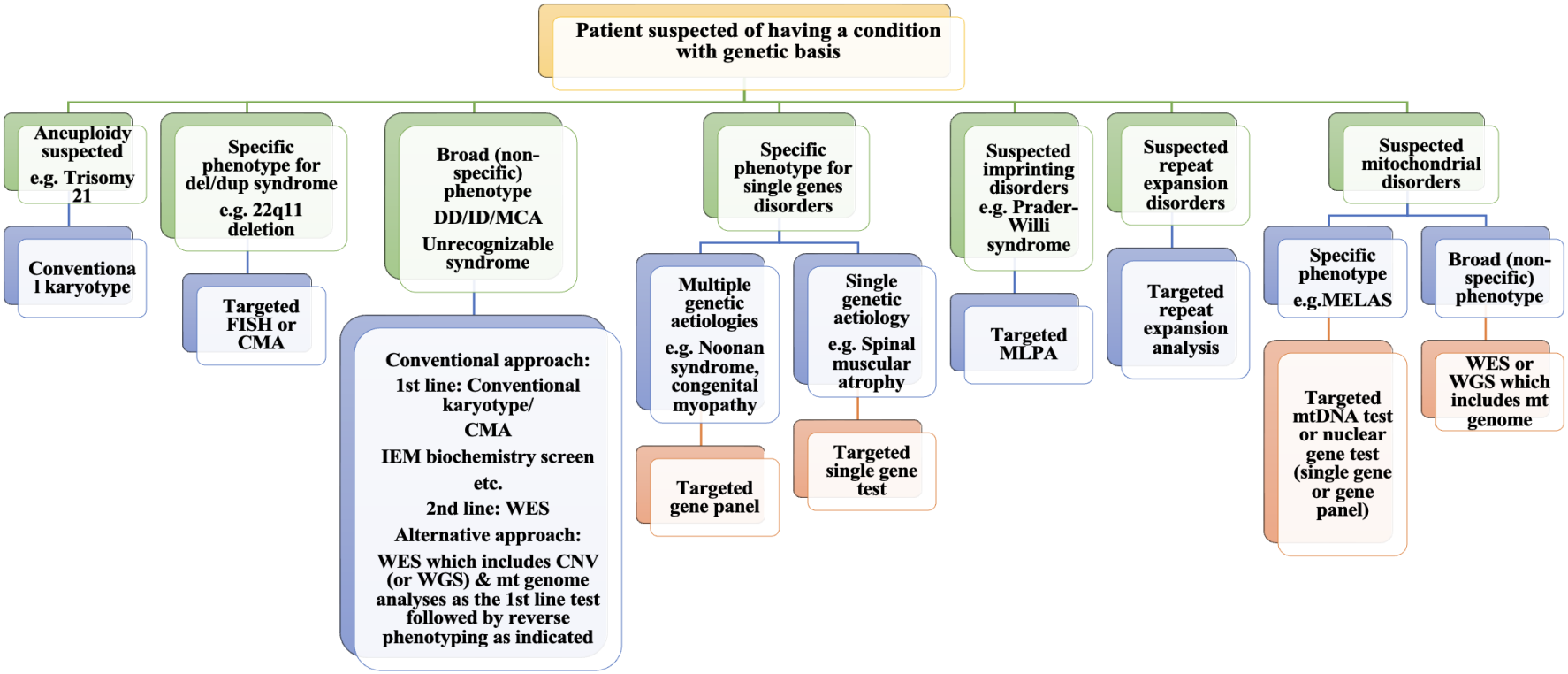
- Our understanding of the genetic aetiologies of many paediatric disorders has grown substantially in recent years. IEMs often need to be considered concurrently with other genetic conditions.

- Genetic diseases could be due to
  - **Chromosomal abnormalities** which include numerical abnormalities (aneuploidy) and structural abnormalities (deletion, duplication, insertion, inversion, and translocation). Numerical abnormalities and large structural abnormalities are detectable by conventional karyotyping. Detection of small structural abnormalities such as microdeletion and microduplication (a.k.a. copy number variations (CNV)) require molecular tools and techniques, examples include:
    - Fluorescence *in situ* hybridization (FISH)
    - Multiplex Ligation-dependent Probe Amplification (MLPA)
    - Chromosomal Microarray Analysis (CMA), either
      - Single-nucleotide polymorphism (SNP) array or
      - Array comparative genomic hybridization (aCGH)
    - Using software tools to infer CNV from data generated from Next generation sequencing (NGS)-based tests [targeted gene panels, whole exome sequencing (WES), and whole genome sequencing (WGS)].
  - **Pathogenic DNA sequence variants in single genes**, detectable by DNA sequencing.
  - **Unstable nucleotide repeat expansion**, e.g. congenital central hypoventilation syndrome, myotonic dystrophy.
  - **Epigenetic modifications (such as genomic imprinting)**, e.g. Angelman syndrome, Prader-Willi syndrome.
  - **Mitochondrial genome abnormalities**.

- Choosing an appropriate genetic test is a multi-factorial consideration: clinical phenotype, availability of the tests, diagnostic urgency, financial resources, etc. Consult the Clinical Genetics/Metabolic team if indicated.

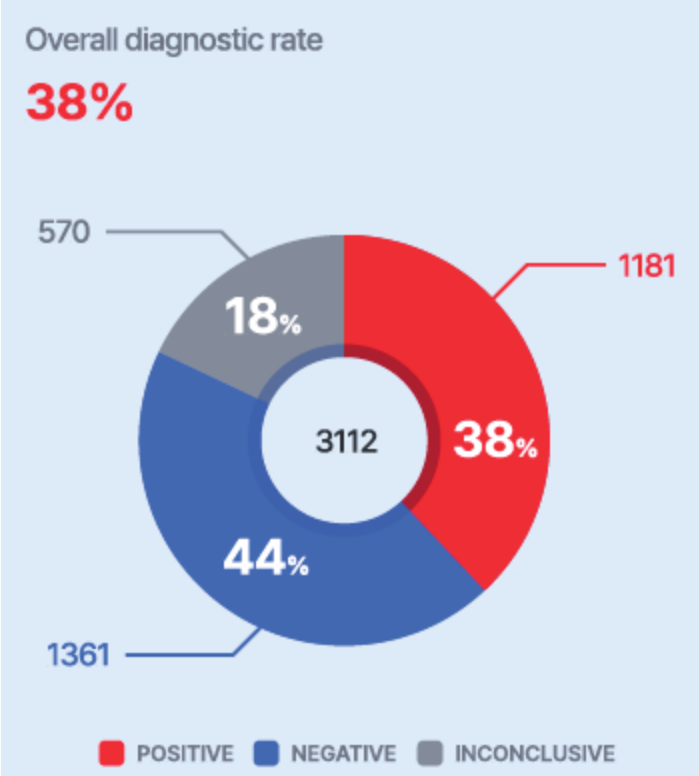
- "Genetic first" approach with early genome-wide analyses such as whole exome sequencing (WES) or whole genome sequencing (WGS) may be easier, more cost-effective and potentially more rapid in arriving at a definitive diagnosis than a combination of several biochemical (IEMs) screening tests that have limited sensitivity and specificity.

General considerations in determining the appropriate genetic test



DD: developmental delay; ID: Intellectual disability, MCA: Multiple congenital anomalies

Our real world experience of using whole exome sequencing for investigation of patients with unsolved diagnosis at the Clinical Genetics Department, Hospital Kuala Lumpur (2020 – 2023)



Up to now, with regular re-classification (of variants of uncertain significance) and reanalysis, at least an additional 2% of the patients have a definitive diagnosis. Reanalysis involves revisiting and reinterpreting the sequencing raw data in light of new scientific discoveries, updated databases or if a patient has new symptoms or signs.

Differential genetic diagnoses of some common paediatric conditions

Clinical phenotype	Differential diagnoses (First think of treatable disorders)	Tests to be considered (modify according to specific clinical phenotype)

Acute neurological deterioration "Intoxication" symptoms	• Urea cycle disorders
	• Maple syrup urine disease
	• Organic acidurias (Propionic aciduria, methylmalonic aciduria, Isovaleric aciduria, etc.)
	• Multiple carboxylase (biotin) deficiency
	• Carbonic anhydrase VA deficiency
		• First line tests:
		  • blood: glucose, NH_4^+, acid-base status, lactate
		  • Urine/blood ketones
		• Second line tests: plasma amino acids, urine organic acids and dried blood spot (DBS) acylcarnitines ± plasma total homocysteine

Epileptic encephalopathy	• Antiquitin deficiency (vitamin B6-responsive seizures) • Early-onset vitamin B6-dependent epilepsy due to *PLPBP* deficiency	• First line tests: Paired plasma and CSF amino acids, urine organic acids, DBS acylcarnitines, serum uric acid, fresh urine sulphite, urine sulphocysteine, urine purine &

	• Pyridox(am)ine -5'-phosphate oxidase deficiency (Pyridoxal phosphate responsive seizures) • Folinic acid responsive seizures • Multiple carboxylase (biotin) deficiency • GLUT1 deficiency • Serine deficiency • Creatine deficiency • Glycine encephalopathy • Sulfite oxidase deficiency • Molybdenum cofactor deficiency • Adenylosuccinate lyase deficiency • Congenital disorders of glycosylation • Several channelopathies (e.g. *SCN1A*, *SCN2A*, *KCNQ2*, etc. mutations) • Synaptopathies • Brain malformations (e.g. lissencephaly, etc.)	pyrimidine, urine/plasma/CSF Piperideine-6-carboxylate (P6C), urine pipecolic acid, urine α-aminoadipic semialdehyde, biotinidase assay, DBS/urine/plasma creatine & guanidinoacetate, serum transferrin isoelectric focusing, neuroimaging + MR spectroscopy • Second line tests: Consider molecular genetic test (epilepsy gene panel or whole exome sequencing)
Severe hypotonia mimicking neuromuscular disorders	• Fatty acid oxidation disorders • Carnitine transport defects • Biogenic amine (neurotransmitters) deciencies • Riboflavin transport defects • Primary CoenzymeQ_{10} defects • Mitochondrial myopathy • Peroxisome biogenesis defects • Congenital disorders of glycosylation (N- glycosylation defects/O glycosylation defects /IEM of the dolichols) • Prader-Willi syndrome	• First line tests: DBS acylcarnitines, plasma total & free carnitines, blood lactate, serum uric acid, serum CK, serum prolactin, CSF neurotransmitters, CSF/urine pterins, urine organic acid (vanillactate), serum transferrin isoelectric focusing, PWS/AS MS-MLPA. • Second line tests: Consider molecular genetic test (neurometabolic gene panel or whole exome sequencing)
Liver failure/dysfunction	• Galactosemia • Hereditary fructose intolerance • Tyrosinemia Type I • Urea cycle disorders • Bile acid synthesis defects • Long chain fatty acids β-oxidation disorders • Wilson disease • α-1 antitrypsin deficiency • Transaldolase deficiency • CDG1b • mtDNA depletion syndrome • GSD Type IV • Peroxisomal biogenesis disorders	• First line tests: DBS Galactose-1-Phosphate & Galactose-1-phosphate uridylyltransferase, plasma amino acids, urine organic acids, urine succinylacetone, urine bile acids analysis, dried blood spot acylcarnitines, plasma VLCFA, blood lactate, serum γ-GT, urine polyol, serum copper, serum caeruloplasmin, quantitation & phenotyping of α-1 antitrypsin • Second line tests: Consider molecular genetic test (liver

	• Hereditary haemochromatosis	disease gene panel or whole exome sequencing)
Cholestatic liver disease	<u>High γ-GT:</u> • Biliary atresia • Alagille syndrome • Galactosemia • Hereditary fructose intolerance • Tyrosinemia Type I • Citrin deficiency • Progressive familial intrahepatic cholestasis Type III • α-1 antitrypsin deficiency • mtDNA depletion syndrome • Peroxisomal biogenesis disorders • Niemann-Pick Disease Type C • HFN1B defect <u>Low/normal γ-GT:</u> • Progressive familial intrahepatic cholestasis Type I and II • Bile acid synthesis defects • Smith-Lemli-Opitz syndrome • TJP2 defect • Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome • MYO5B defect	• First line tests: DBS Galactose-1-Phosphate & Galactose-1-phosphate uridylyltransferase, plasma amino acids, urine organic acids, urine succinylacetone, urine bile acids analysis, plasma VLCFA, blood lactate • Second line tests: Consider molecular genetic test (*JAG1* gene ± *NOTCH2* gene analysis for Alagille syndrome, *SLC25A13* gene analysis for Citrin deficiency, liver disease/jaundice gene panel or whole exome sequencing)
Cardiomyopathy	• Primary carnitine deficiency • Fatty acid oxidation disorders • Infantile-onset Pompe disease • Primary mitochondrial disorders • Barth syndrome • Rasopathies	• First line tests: DBS acylcarnitines, blood lactate, urine organic acids, plasma total & free carnitines, DBS α-glucosidase enzyme assay. • Second line tests: Consider molecular genetic test (cardiomyopathy gene panel or whole exome sequencing)
Persistent/recurrent hypoglycemia	• Glycogenosis defects • Gluconeogenesis defects • Congenital hyperinsulinism • Fatty acid oxidation disorders • Carbonic anhydrase VA deficiency	• First line tests: Blood lactate, blood NH_4^+, lipid profile, serum uric acid, serum CK, DBS acylcarnitines, urine organic acids, serum insulin. • Second line tests: Consider molecular genetic test (hypoglycemia gene panel or whole exome sequencing)
Predominant dysmorphism +/- malformations	• Peroxisomal defects • Cholesterol defects	• First line tests: Detailed dysmophology analysis, Plasma VLCFA, total cholesterol, serum

	• Congenital disorders of glycosylation (N- glycosylation defects/O glycosylation defects/GPI anchor synthesis defects) • Menkes disease • Lysosomal storage disorders • Chromosomal abnormalities (Copy number variations (CNV)) • Genetic syndromes	transferrin isoelectric focusing, serum copper, ceruloplasmin, urine glycosaminoglycans (GAGs), urine oligosaccharides, karyotype. • Second line tests: Consider lysosomal enzyme assay, molecular genetic test (chromosomal microarray or whole exome sequencing which include CNV detection or WGS)
Unexplained global developmental delay/intellectual disabilities	• Chromosomal abnormalities (Copy number variations (CNV)) • Syndromic single gene disorders (e.g., Fragile X, Rubinstein-Taybi) • Nonsyndromic single gene disorders • Metabolic disorders (e.g., phenylketonuria, Smith-Lemli-Opitz syndrome) • Acquired/non-genetic causes	<u>Conventional approach:</u> • 1st line: Detailed dysmophology analysis, neuroimaging, audiology, ophthalmology assessement, karyotype, Fragile X gene test, T4/TSH, Creatine kinase, blood NH_4^+, blood lactate, plasma amino acids, DBS acylcarnitine, plasma total homocysteine, urine organic acid, DBS/urine/plasma creatine & guanidinoacetate, serum transferrin isoelectric focusing, Urine glycoaminoglycans, urine purine & pyrimidine, serum copper & ceruloplasmin, biotinidase assay • 2nd line: Chromosomal microarray, WES <u>Alternative approach:</u> • WES + CNV analysis (or WGS) + mtDNA genome sequencing as the 1st line test. Perform reverse phenotyping as indicated.

Post-mortem investigations

- If a child dies of an unknown, possible genetic disease including an IEM, it is essential to collect representative post-mortem samples and discuss their analysis with the Clinical Genetics/Metabolic team. Without a diagnosis, genetic counselling of the parents and reliable risk assessment for future children is not possible.
- Samples/investigations to collect:
- <u>Mandatory</u>
  - Save a blood sample for potential genetic testing.

- Option 1: Save 5mls whole blood in EDTA bottle (purple top). Send it immediately (at room temperature) to genetic laboratory for DNA extraction and storage. If the transport is not immediately available, keep the blood in a refrigerator (4°C, maximum storage duration: not more than 4 weeks). For longer storage (months to years), blood sample should be stored in a freezer at -20°C to -80°C and remain there until they can be shipped to the laboratory.
- Option 2: Keep and store a dried blood spot (DBS) specimen (spotted onto an appropriate filter paper card).
- Basic IEM tests: plasma amino acid, urine organic acid, DBS acylcarnitines. (If possible, collect the blood and urine samples prior to expected death. Autolysis during the process of dying causes intracellular fluid to mix with extracellular fluid. This may lead to misleading changes of metabolites concentration).

- Consider
  - CSF (freeze immediately, if possible, at -80°C)
  - Bile (spot on filter paper for acylcarnitines analysis)
  - Skin biopsy (store at ambient temperature in culture medium, send for fibroblast culture. DO NOT FREEZE)
  - Fine needle biopsy of muscle, heart, liver.

Management of an asymptomatic newborn but at risk of having potentially treatable IEMs

- High risk scenario includes:
  - A previous child in the family has had an IEM.
  - Multiple unexplained early neonatal deaths.
  - Mother has HELLP/ fatty liver disease during pregnancy.
    (HELLP – Haemolytic Anaemia, Elevated Liver Enzymes, Low Platelets).
- Consider to transfer the expected newborn in-utero or soon after delivery to a medical centre with facilities to diagnose and manage IEM.
- If potential/index patient diagnosis is known: screen for the specific condition, e.g. urea cycle disorders – monitor ammonia and plasma amino acid, MSUD – monitor plasma leucine (amino acids).
- If potential/index patient diagnosis is unknown: Collect dried blood spots for acylcarnitine profile, plasma amino acid and urine organic acid on 2nd or 3rd day after feeding, send it immediately and get result as soon as possible.
- Other essential laboratory monitoring may include: blood NH_4^+, VBG, blood glucose. Please discuss with the Metabolic team.
- To prevent decompensation before the disease status of the newborn is known:
  - Provide enough calories (oral/IV)
  - Dietary protein restriction may be necessary especially if index case presented very early (before 1 week). Protein-free formula should be given initially and small amount of natural protein (e.g. breast milk) can be introduced gradually after 48 hours depending on baby's clinical status.
- If the index patient presents after the first week, the newborn should be given the minimum safe level of protein intake from birth (approximately 1.5 g/kg/day). Breast feeding may be allowed under these circumstances with top-up feeds of a low protein formula to minimise catabolism, under the supervision of the Metabolic team.
- Get the metabolic tests results as soon as possible to decide whether the baby is affected or not.

Chapter 7

GENETIC COUNSELLING FOR INBORN ERRORS OF METABOLISM

Genetic counselling is the process of providing individuals and families with information on the nature, mode(s) of inheritance, recurrence risk and implications of genetic disorders to help them make informed medical and personal decisions. It also aims to provide relevant support resources and help families cope and adapt to the genetic condition.

It is normally provided by qualified healthcare professionals including clinical geneticists and genetic counsellors.

Patients with IEMs and their families require unique and comprehensive clinical care including management of acute illnesses, screening for long term complications, discussion of the aetiology of the condition, connections to various social support and resources, clarification of the recurrence risks and discussion of reproductive options and treatment options. Ideally, every patient and family receiving a new diagnosis of an IEM should be provided with genetic counselling at the time of the new diagnosis and periodically over time, throughout the lifespan of the patient.

Mode of inheritance

IEMs (except Primary mitochondrial diseases (PMDs)) follow the principles of Mendelian genetics and follow autosomal recessive, autosomal dominant, and X-linked patterns of inheritance.

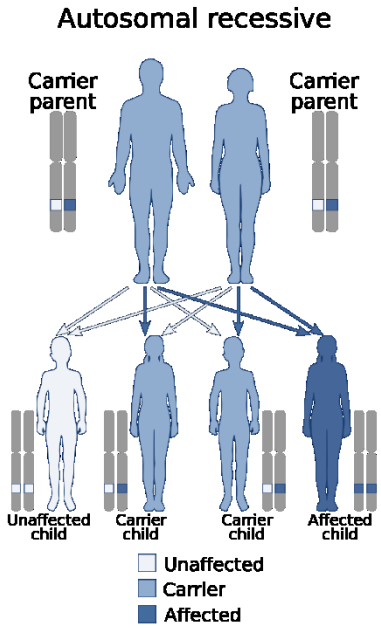
A full family history should be obtained, assessed, and discussed with the family to promote their awareness and understanding. A full family history includes a three-generation pedigree. This is necessary in the assessment of recurrence risk for each family member.

Most IEMs are inherited in an autosomal recessive pattern. Autosomal recessive conditions are recognizable on a pedigree when the patient is affected and both parents are unaffected (no vertical transmission). The majority of patients and their parents with autosomal recessive IEMs will have no family history of the condition. As they have no personal experience with the condition, they often need more information about the disease itself and its impact on health and life, which makes genetic counselling very important for them.

Autosomal recessive inheritance: recurrence risk to family members

(i) Parents of a proband

- The parents of an affected child (proband or index case) are obligate heterozygotes (carriers), meaning that they have a pathogenic variant in one of their two copies of the gene causing that IEM. Heterozygous carriers are asymptomatic and are not at risk of developing the disorder.
- The chance for a disease to recur in a family is determined by the mode of inheritance for that particular gene and condition. In the case of autosomal recessive conditions, unaffected carrier parents of an affected child have a 25% chance in each pregnancy to have another affected child.



Autosomal recessive inheritance pattern

(ii) Siblings of a proband

- For an autosomal recessive IEM, each sibling of the proband has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. They should be offered genetic counselling in an age-appropriate manner, and after discussion with parents. Testing of their carrier status should only be offered when they are legally an adult.

(iii) Offspring of a proband

- The offspring of an individual with an autosomal recessive IEM are obligate heterozygotes (carriers). The chance for an offspring to be affected would be determined by the carrier status of the patient's partner. Genetic counselling is recommended for patients with IEMs who have reached reproductive age and their partners prior to and during the family planning process to allow for informative carrier testing based on the diagnosis.

(iv) Other family members

- Other family members, may also be carriers. Carriers have an increased risk to have a child with the condition compared to the general population.
- Each sibling of the proband's parents is at a 50% risk of being a carrier of an autosomal recessive IEM.
- In consanguineous families, the chance for a relative of a patient with an IEM to be a carrier is often higher than that estimated based on the degree of relationship due to the fact that consanguinity is often present in multiple generations. In these cases, it's appropriate to offer genetic counselling and carrier screening to anyone in the family, regardless of degree of relationship.

Carrier detection

- Carrier testing for at-risk relatives requires prior identification of the pathogenic variants in the family. Biochemical testing e.g. quantitative plasma amino acids and fibroblast enzymatic analyses are not recommended for detection of heterozygotes.

- Ideally, carrier testing should occur prior to achieving a pregnancy to allow an individual or couple to fully understand their options regarding carrier detection and/or prenatal genetic diagnosis.
- Performing carrier testing prior to a pregnancy also gives individuals and couples the option of preimplantation genetic testing for monogenic disorders.
- Carrier testing of minors has important implications concerning the minor patient's present and future autonomy and best interest. In keeping with ethics guidance, testing of carrier status for any children should only be offered when he/she reaches 18 years-old, when he/she has the ability to understand the implications of genetic testing.

- When family members are considering carrier testing, they should be offered genetic counselling by qualified healthcare professionals to ensure that the appropriate test is performed based on his or her specific risk, and that they are informed of all of their testing options and potential outcomes and implications of testing. A written informed consent is required. Pre-test genetic counselling and post-test genetic counselling should adhere to the latest ethical guidelines issued by the Malaysia Medical Council.

Reproductive options

- These discussions should include all options available to that family which may include: natural conception without prenatal genetic diagnosis (with the option of high-risk newborn screening and early medical intervention for treatable IEMs), preimplantation genetic testing, prenatal genetic diagnosis, the option of foregoing having additional children, and adoption. The options of surrogacy and egg donation may be controversial ethically, culturally and religiously to be included in the discussion.
- In order to perform preimplantation genetic testing (PGT) or prenatal genetic diagnosis, the specific gene and pathogenic variants causing the proband's IEM must be known. Prenatal diagnosis is performed by chorionic villus sampling (CVS) at approximately 11-13 weeks' gestation or amniocentesis at approximately 15-17 weeks' gestation. Couples who want to pursue prenatal genetic diagnosis should be counselled to prepare for carrying an affected child, including deciding on location of delivery and neonatal care plan, or, may choose to terminate an affected pregnancy in accordance with Malaysian law. They must also weigh prenatal genetic diagnosis with a risk of procedure-related miscarriage, generally quoted as about 1/300.
- PGT involves in-vitro fertilization and genetic testing of cells removed from the embryos at a very early stage in order to identify unaffected embryos which can then be transferred for pregnancy. High cost and access to these technologies can be a barrier for some families.
- Healthcare professionals should also respect the family's cultural and religious beliefs, and their autonomy in making the informed decision.

Psychosocial support

- A new IEM diagnosis, particularly in a child can potentially trigger psychosocial issues in the family including the emotional, social, and economic implications, and cultural beliefs of those involved. IEMs require life-long multidisciplinary medical care, and many chronic psychosocial issues and challenges may arise as below:

(i) Grieving the diagnosis

- Parents of a new IEM patient often experience the traditional grief spectrum including shock, denial, anger, guilt, shame, despair/grief and sadness.

- Over time there is typically acceptance although every parent goes through this process in their own time and pace.
- Parents and family caregivers may grieve in different ways and so supporting each caregiver based on their needs will help the entire family system function.

(ii) Feelings of guilt and shame

- The parents of a child with an IEM may experience feelings of guilt and shame, particularly when learning of their obligate carrier status (autosomal recessive disorders).
- This situation can prove even more challenging to the family dynamics for the few IEMs inherited in an X-linked manner, in which only the mother can be the obligate carrier for the condition in the child.
- For many parents, understanding the cause of their child's condition can reduce the feelings of guilt and shame, and so genetic counselling at the time of diagnosis and additionally over time can help enable parents to continue learning new and often complex information.
- The affected child may face shame in relation to the requirements for a strict medical diet, particularly among peers.
- The unaffected siblings may experience a form of 'survivor guilt'. Siblings witness the effects of IEMs on a daily basis. They may hold questions about the underlying cause of the disease, symptoms, and progression. Without adequate information, siblings may be burdened with unanswered questions and misconceptions. This can have a significant impact on personal well-being, as well as their relationship with their siblings and family.

(iii) Psychosocial stress due to unique dietary management

- For many patients with an IEM, their diet is a carefully calculated prescription and foods need to be portioned out, weighed, measured and/or mixed in an exact manner.
- This can be challenging and stressful for many families on a day-to-day basis but even more so during times of holidays or celebrations.
- Stress can be exacerbated due to frequent changes to the medical diet for the child as he/she grows
- Additional challenges for the patient with IEM may be times of fasting related to their religious practices, which can be dangerous for some persons with IEMs.

(iv) Challenges in managing common illnesses

- Common illnesses in childhood such as febrile/viral illnesses, the common cold, and feeding-related issues e.g. frequent vomiting and typical food refusals can be life-threatening in patients with an IEM and require immediate medical intervention.
- These seemingly common childhood events can easily disrupt the family's daily routine and become large stressors for the family.
- Patients with IEM should be provided with an 'Emergency Illness Protocol' that is customized to their diagnosis, age, and other aspects of their health. It should include the immediate/urgent steps any healthcare professional in an emergency department should take to assess and stabilize the patient (e.g. urgent laboratory tests, IV fluids prescription, additional supplements/medications needed, and other recommendations) as well as emergency contact information for the patient's metabolic care team. This protocol should be updated as the child grows and their treatment changes.

(v) Feeling socially isolated

- Many families who have a child with a rare IEM will highlight their feelings of isolation related to this diagnosis.

- Caring for a child with a rare disease can be socially isolating and can impact the psychological health of the parents/caregiver, their long-term employability, and other domains of living.
- Parents are often forced into a position of 'being the expert' for their child when encountering a new healthcare professional, teacher, or other caregiver for the child, a role that many parents are unprepared or inadequate to fill.
- Healthcare professionals with qualified training in genetic counselling are able to connect the parents of the child with an IEM to quality resources and social connections relating to the diagnosis.

(vi) Rebellious behaviour

- Maintenance of strict dietary compliance can be very challenging and is further complicated during periods of normal rebellion and autonomy development such as in adolescence**.**
- For the adolescent patient, there should be additional discussions relating to medical transition of care and for the patient to begin taking more ownership and responsibility for his/her diet and intake, unless the patient has cognitive impairment that prohibits this level of independence.

(vii) Burden of disease not being properly acknowledged

- An IEM diagnosis impacts diet, lifestyle, intercurrent illness management and all aspects of life for many patients and families.
- Acknowledging the family's feelings about their child's IEM can help them cope with the diagnosis and open up communication channels should the caregivers become overwhelmed and need additional support.
- For females with an IEM, there is an additional burden of disease that can be felt prior to and throughout a pregnancy when additional attention is required for strict metabolic control.

(viii) Transition to adulthood

- As treatments for IEMs have become more advanced, the lifespan of these patients has also increased.
- Adolescents with IEMs should receive updated genetic counselling about their diagnosis and their own recurrence risks as they approach adulthood.
- It is important to initiate this discussion prior to the individual reaching the stage of active family planning, as patients may only know the recurrence risks provided to their parents, which are different from their own.
- While many patients live into adulthood, some may not be able to live independently due to cognitive impairment or other complex medical needs. It is important for the caregivers of these patients to obtain guardianship or other legal arrangements (e.g. apply for Pencen Terbitan. Refer to 'Garis Panduan Pendaftaran Anak Kurang Upaya Tanggungan Pesara', https://www.jpapencen.gov.my/Garis%20Panduan%20Pendaftaran%20Anak%20Kurang%20Upaya%20Tanggungan%20Pesara.pdf) based on the needs of the patient to help determine and address long term needs and support systems.

Genetic counselling, prenatal diagnosis, and reproductive options in primary mitochondrial diseases (PMDs)

Genetic counselling depends on whether the PMD is caused by pathogenic variant(s) in mitochondrial DNA or nuclear gene**.**

(i) PMDs due to pathogenic variant(s) of a nuclear gene can be inherited as autosomal recessive, autosomal dominant, or X-linked manner.

- **Autosomal recessive inheritance: refer to above section**

- **Autosomal dominant inheritance:** A patient may have inherited the pathogenic variant(s) from one of the parents who may or may not be symptomatic or it may be de novo in the patient. Parents should be offered testing and if one of them carries the pathogenic variant(s), the risk of each of the patient's siblings of carrying this pathogenic variant(s) is 50% at conception. Each offspring of the patient has a 50% risk of inheriting this pathogenic variant(s).

- **X-linked inheritance:** A patient may have inherited the pathogenic variant from his mother who is usually asymptomatic or it may be de novo in the patient. If the mother carries the pathogenic variant, the risk of transmitting it in each of her pregnancy is 50%. Male siblings of the patient who inherit the pathogenic variant will be affected while females will be carriers. A female inheriting the pathogenic variant may be affected due to non-random inactivation of most of the X chromosomes without pathogenic variant (skewed inactivation) or when the condition is X-linked dominant or semidominant (e.g. pyruvate dehydrogenase deficiency). All daughters of a male patient will be carriers and none of his sons will be affected.

(ii) PMDs due to mtDNA pathogenic variant(s)

Mitochondrial disease can be due to mtDNA deletion or point mutation.

PMDs mtDNA deletion: Mitochondrial DNA deletion is usually de novo. The risk to siblings of a patient is very low. If a male is carrying mitochondrial DNA deletion, his offspring will not inherit the deletion. If a female is affected, there is a small risk (estimated to be about 1 in 24) that she will have an affected child.

PMDs due to mtDNA point mutation: Mitochondrial DNA point mutation is maternally inherited. The mother of a patient carries the mutation but may or may not be symptomatic due to a different level of heteroplasmy than the patient. All the siblings of the patient are at risk of inheriting the mutation from the mother but may or may not be symptomatic due to genetic bottleneck resulting in different levels of heteroplasmy among them. If a male is carrying a mitochondrial DNA point mutation, his offspring will not inherit the mutation. If an affected female is heteroplasmic, all her offsprings will inherit the mutation, but they may or may not be affected due to genetic bottleneck resulting in a different level of heteroplasmy between her and her offspring and among her offsprings.

PMDs due to unknown molecular aetiology: Genetic counselling is challenging if the molecular diagnosis is not established. A detailed family history may give clues to the mode of inheritance, but can be misleading sometimes. A maternal inheritance will imply mtDNA mutation while the history of consanguinity will suggest an autosomal recessive inheritance. If muscle biopsy shows isolated complex II deficiency, the inheritance is likely autosomal recessive as complex II is entirely nuclear in origin. It is estimated that approximately 75% of adult-onset mitochondrial disease is caused by mitochondrial DNA mutation while it accounts for only approximately 25% of childhood-onset PMDs.

Preimplantation genetic testing and prenatal diagnosis

- PMDs due to Nuclear Gene pathogenic variant(s):
  PGT and prenatal genetic diagnosis can be offered if the specific gene and pathogenic variants causing the PMD is known.

- PMDs due to Mitochondrial DNA mutation:

- PGT: Heteroplasmy level in cells removed from the embryos at a very early stage is measured. Embryos with heteroplasmy below threshold (18%) are then implanted.
- Prenatal genetic diagnosis: this is challenging due to the concern of variation in heteroplasmy levels between placental tissue and the fetus (chorionic villus sampling) or among cells of early embryo. The possibility of giving birth to an affected child when low heteroplasmy level is detected cannot be completely excluded. The interpretation becomes even more complicated for an intermediate level of heteroplasmy found. Hence, this is rarely recommended.

Reproductive options for a female carrier of pathogenic mtDNA mutation

- Oocyte donation: in-vitro fertilization of donor oocyte with sperm from male partner and implantation of the embryo in the recipient. Ethically controversial.
- Mitochondrial donation
    - This involves removal of nuclear genome from an oocyte or zygote carrying mtDNA mutation followed by transfer to an enucleated donor oocyte or zygote with healthy mitochondria. The resulting embryo will have healthy mitochondria from the donor but nuclear genetic materials from both parents. Ethically controversial. Currently this is only offered in highly specialised overseas centres.
- Adoption is always an option.

Patient support groups in Malaysia

1. **Malaysia Lysosomal Diseases Association (Pertubuhan Penyakit Lisosomal Malaysia)**
No. 30-11, Geo Sense,
Jalan Lagoon Selatan,
Bandar Sunway 47500,
Subang Jaya, Selangor.
Tel: 019-6899620 Email: enquiry.mlda@gmail.com

2. Malaysian Rare Disorders Society (Persatuan Penyakit Jarang Jumpa Malaysia)
No.7, 2nd Floor,
Bangunan Sultan Salahuddin Abdul Aziz Shah,
No. 16 Jalan Utara,
46200 Petaling Jaya, Selangor
Tel:010-9446167 E-mail: info@mdrs.org.my

3. Malaysia Metabolic Society (Persatuan Metabolik Malaysia)
P.O.Box 6098 Pudu 55710 Kuala Lumpur
Tel:013-3901706 Email: care@mms.org.my

Chapter 8

EXPANDED NEWBORN SCREENING FOR INBORN ERRORS OF METABOLISM

Newborn screening (NBS) was pioneered by Robert Guthrie in the early 1960s. In the 60 years since then, NBS has expanded to include dozens of IEMs, genetic and other disorders from a single blood spot using tandem mass spectrometry (TMS). The expanded NBS using TMS gained momentum worldwide in the early 2000s as a health-effective and cost-effective intervention. As for 2024, many Asian countries also have implemented government-funded expanded NBS including Singapore, Philippines, Thailand, Hong Kong, Macau and Taiwan. In Malaysia, our current cord-blood-based NBS program only covers Glucose-6-Phosphate dehydrogenase (G6PD) deficiency and congenital hypothyroidism. Expanded NBS for IEMs is only offered as an opt-in option in University Malaya Medical Centre and some private hospitals. Parents need to pay out of pocket.

Healthcare professionals in Malaysia have a responsibility to educate our public especially parents-to-be about expanded NBS as an important preventive initiative and internationally recommended standard of care for their newborns. NBS that includes the following IEMs that, without intervention, would cause significant morbidity, mortality, or intellectual disability should be considered by every parents-to-be.

	Primary Marker/Ratio
Organic Acidurias	
Glutaric aciduria type I	↑ C5DC
Isovaleric aciduria	↑ C5, ↑ C5/C2, ↑ C5/C3
Methylmalonic aciduria	↑ C3, ↑C3/C2
Methylmalonic aciduria and homocystinaemia (Cobalamin C deficiency)	
Propionic aciduria	
Multiple carboxylase deficiency	↑ C5OH, ↑ C5OH/C8
Beta-ketothiolase deficiency	↑ C5:1, ↑ C5OH, ↑C5OH/C8
3-hydroxy-3-methylglutaryl-coA lyase deficiency	↑ C5OH, ↑ C6DC, ↑C5OH/C8
Aminoacidopathies	
Argininemia	↑ Arg
Argininosuccinic aciduria	↑Cit
Citrullinemia type I	
Citrullinemia type II	
Phenylketonuria	↑ Phe, ↑ Phe/Tyr Ratio
6-pyruvoyl-tetrahydropterin synthase deficiency	
Homocystinuria	↑ Met, ↑ Met/Phe
Maple syrup urine disease	↑ Xle, ↑Valine
Tyrosinemia type I	↑ Tyr, ↑Succinylacetone
Fatty Acid Oxidation Disorders	
Carnitine-acylcarnitine translocase deficiency	

Carnitine palmitoyltransferase II deficiency	↑C16, C18, ↑C18:1, ↑C18:2, ↓C0/(C16+C18) ↑ (C16+C18:1)/C2
Carnitine uptake deficiency	↓ C0
Glutaric aciduria Type II	↑ C4-C18
Medium chain acyl-CoA dehydrogenase deficiency	↑ C6, ↑ C8, ↑C10, ↑ C10:1, ↑C8/C10
Very long-chain acyl-CoA dehydrogenase deficiency	↑ C14, ↑ C14:1, ↑ C14:2, ↑ C14:1/C16, ↑C14:1/C2, ↑C14:1/C12:1
Other IEMs	
Biotinidase deficiency	↓ BTD activity
Classic galactosemia	↓ GALT activity
Congenital adrenal hyperplasia	↑ 17OHP

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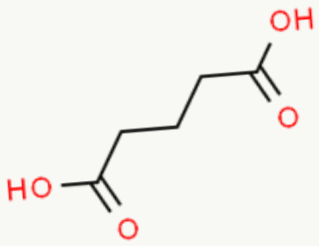
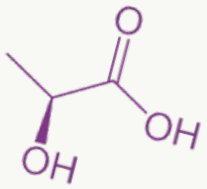
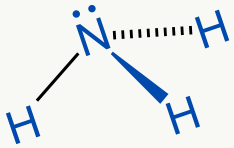
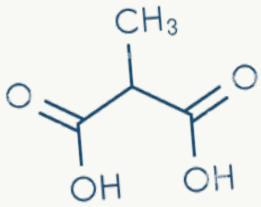
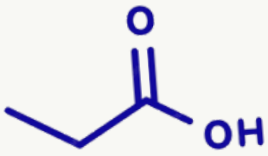




The first publication of a concise outline of the various Inborn Errors of Metabolism encountered in Malaysian hospitals and clinics. This book provides quick access to the various clinical presentations, specific investigations and immediate effective treatment essential for good outcome. It is a resource useful in all specialties as patients with Inborn Errors of Metabolism can present at any age and with any organ involvement.







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