

Carbohydrate Disorders

Beverly Hird

*Biochemistry Department
Royal Manchester Children's Hospital*

Introduction

Carbohydrates are important energy stores, fuels and metabolic intermediates

Routine biochemistry tests e.g. lactate, glucose and second-line metabolic tests e.g. amino acids are essential for the investigation of disorders of carbohydrate metabolism. However, definitive diagnosis is usually achieved by measurement of the activity of the affected enzyme.

The easiest sample type to obtain is blood (erythrocytes, leucocytes, lymphocytes) but the choice of tissue depends on the pattern of expression of the enzyme in question. For some assays, cultured skin fibroblasts (from a punch biopsy) or liver/muscle biopsies are required.

Inborn Errors of Carbohydrate Metabolism

Galactosaemia

Glycogen storage diseases

Pyruvate carboxylase deficiency

Fructose-1,6-bisphosphatase deficiency

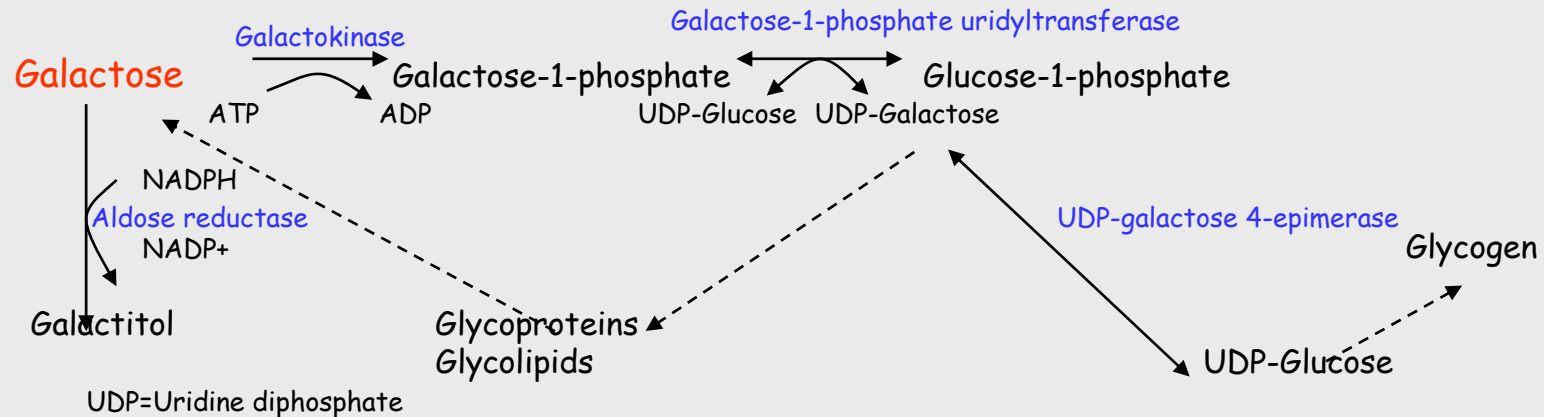
Hereditary fructose intolerance

Glucose-6-phosphate dehydrogenase deficiency

Galactosaemia

Lactose from milk is hydrolysed by intestinal lactase to produce glucose and galactose.

The main pathway of galactose metabolism



There are three inborn errors of galactose metabolism:

Galactokinase deficiency

Galactose-1-phosphate uridylyltransferase (Gal-1-PUT) deficiency

Uridine diphosphate-galactose 4-epimerase deficiency

The Pyrophosphorylase Pathway is an alternative route for production of UDP-galactose (essential for incorporation of galactose into proteins and lipids). The generation of galactose-1-phosphate by this pathway explains why galactose-1-phosphate can still be synthesised in patients with galactosaemia who are on strict dietary galactose restriction

Classical Galactosaemia (1)

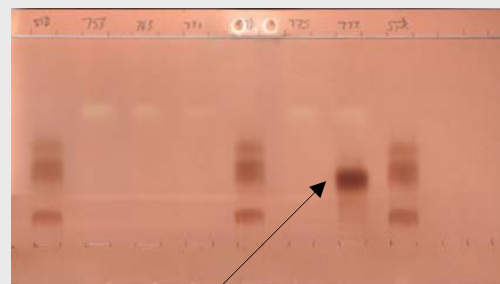
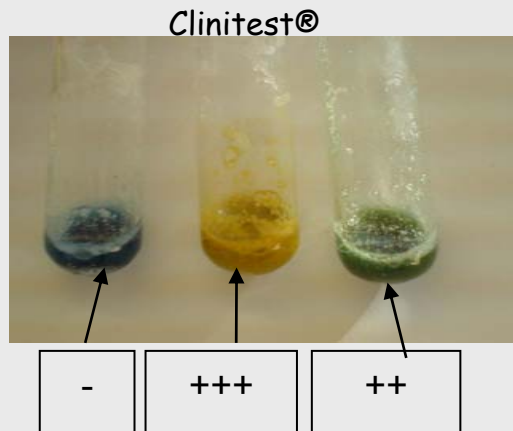
This disorder is due to galactose-1-phosphate uridylyltransferase deficiency

There is accumulation of galactose-1-phosphate and galactose and secondary formation of galactitol.

It typically presents by the end of the first week of life with poor feeding, vomiting, lethargy, jaundice, hepatomegaly, neonatal cataracts and renal tubular disease and is often associated with *E. coli* septicaemia.

Biochemically there is hyperbilirubinaemia, raised alanine aminotransferase (ALT), generally elevated plasma and urine amino acids, albuminuria, glycosuria and galactosuria.

A positive urine Clinitest® for reducing substances with a negative Clinistix® test (specific for glucose) may provide a clue to the diagnosis but is not specific. However in galactosaemia a negative Clinitest® may occur if there has been insufficient galactose intake due severe vomiting or reduced milk intake. Furthermore the Clinitest® and Clinistix® may be positive due to the glycosuria due to the renal tubular dysfunction. Urine sugars can be identified by Thin Layer Chromatography (TLC).



a generalised aminoaciduria

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Classical Galactosaemia (2)

Milk feeds should be stopped whilst awaiting the definitive test results.

The definitive test is quantitative assay of galactose-1-phosphate uridylyltransferase ('Gal-1-PUT') activity in red cells or the qualitative Beutler test which is a fluorescent spot test for 'Gal-1-PUT'.

Beutler Test

Mins	Control	Affected
0	●	●
60	●	●
120	●	●

The diagram shows a table with three columns: 'Mins', 'Control', and 'Affected'. The rows represent time points: 0, 60, and 120 minutes. At 0 minutes, both Control and Affected have a red dot. At 60 minutes, Control has a green dot and Affected has a red dot. At 120 minutes, Control has a green dot and Affected has a red dot. The entire table is enclosed in an oval.

Whole blood and a reaction mixture (UDP-glucose, galactose-1-phosphate, NADP) is spotted onto filter paper at 0 minutes, after 60 minutes incubation & after 120 min incubation

Normal transferase activity results in the production of NADPH, which is fluorescent under UV light.

No fluorescence indicates galactose-1-phosphate uridylyltransferase deficiency

False negative results may occur if the patient has had a blood transfusion up to 6 weeks before the sample was taken. False positive results may occur if the patient has glucose-6-phosphate dehydrogenase deficiency as the endogenous activity of this enzyme is used to generate NADPH.

This can be confirmed with the quantitation of galactose-1-phosphate in erythrocytes or DNA analysis for common European mutation: Q188R (77% of mutant alleles in UK).

Treatment: If galactose is excluded from diet then life-threatening illness usually resolves quickly.

Long-term complications e.g. low IQ, growth delay, ovarian dysfunction, still occur despite early diagnosis and treatment possibly due to endogenous galactose production.

Partial enzyme deficiencies occur and several variant alleles exist e.g. Duarte; usually benign (identified on newborn screening in some European countries & USA)

Galactokinase Deficiency

This is a rare disorder where there is an inability to phosphorylate galactose and galactose and galactitol are excreted in urine

Neonatal (but not congenital) cataracts occur-often bilateral due to the accumulation of galactitol in the lens.

There is a positive urine reducing substances due to galactose in urine usually with a negative Clinistix®.

Diagnosis is by measurement of galactokinase activity in red cells.

Treatment is with a galactose-free diet. The cataracts are reversible if milk excluded in first few weeks of life

UDP-galactose 4-epimerase deficiency

Mild form

A partial enzyme deficiency due to reduced protein stability and more pronounced in cells with long lifespan e.g. erythrocytes.

Normal growth and development
No treatment appears necessary

Severe form

Very rare

A similar presentation to classical galactosaemia.

Accumulation of UDP-galactose & galactose-1-phosphate.

The Beutler test is normal and red cell galactose-1-phosphate is increased. The definitive test is assay of UDP-galactose-4-epimerase activity in erythrocytes.

Glycogen Storage Diseases

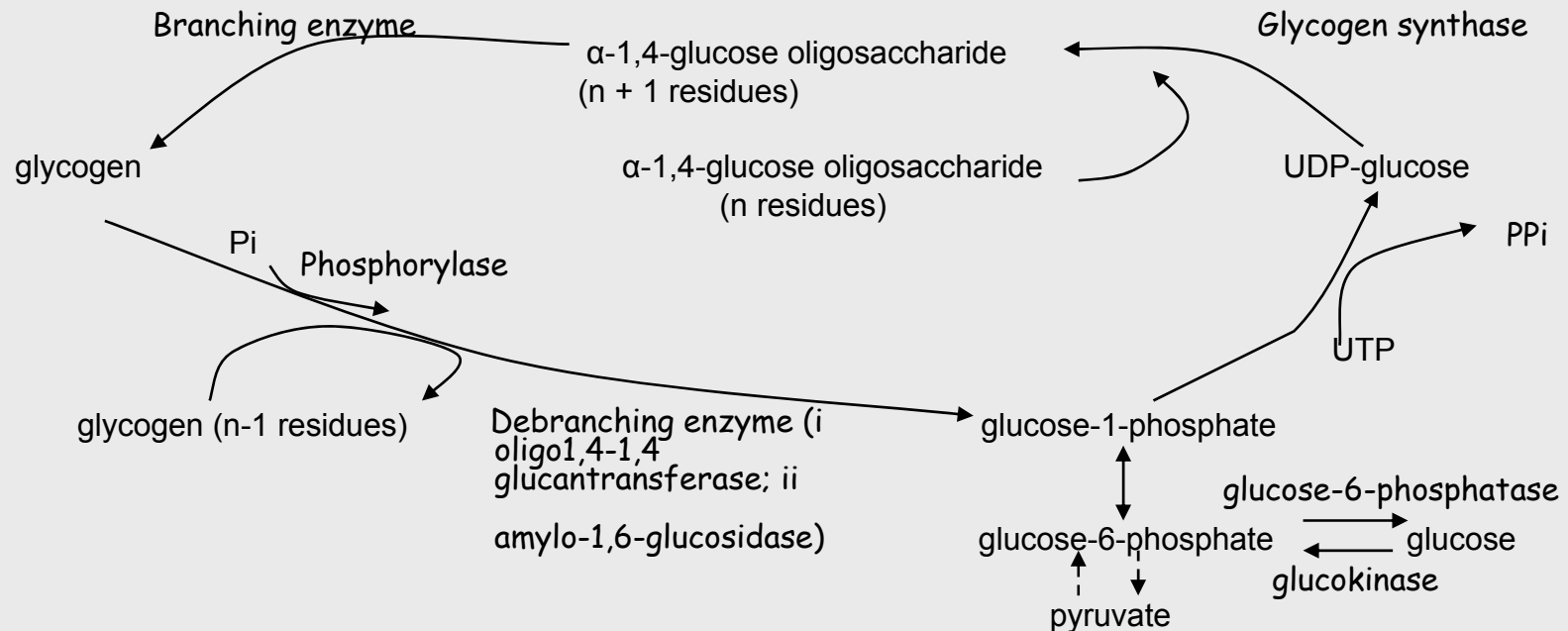
Pathway

Glucose is stored in the form of glycogen, mainly in liver and muscle

Glycogen is made up of straight chains of glucose residues with α -1,4 linkages, branched at intervals with α -1,6 linkages

Glycogen metabolism is highly regulated by several amplifying reaction cascades.

Adrenaline and glucagon stimulate glycogen breakdown. Glycogen synthesis is increased by insulin.



In glycogen storage diseases, liver (hepatomegaly, hypoglycaemia) and muscle (exercise intolerance, weakness) are the most affected tissues

GSD I

GSD I accounts for approximately 25% of GSD cases

Glucose-6-phosphatase deficiency (GSD Ia) is a defect in the release of glucose from glucose-6-phosphate and affects both glycogenolysis and gluconeogenesis.

It presents with hepatomegaly, short stature and truncal obesity

Biochemically there is hypoglycaemia, lactic acidemia, hyperuricaemia and hyperlipidaemia

Histological analysis shows excess of fat and glycogen in hepatocytes

GSD Ib is a defect in a transporter protein and has the above features plus neutropenia, recurrent bacterial infections and inflammatory bowel disease

The diagnosis is nowadays confirmed by DNA analysis in the majority of patients

It is also possible to measure the activity of glucose-6-phosphatase and the transporter protein function in a fresh liver biopsy with comparison of fresh and frozen results to distinguish type Ia from Ib

Glycogen Storage Disease Overview (1)

Disorder (approximate % GSD cases)*	Enzyme defect	Most affected tissue(s)	Clinical Features	Diagnostic tests	Sample
GSD II (Pompe's) (15%)	Lysosomal α 1,4- glucosidase	Generalised; accumulation of glycogen in lysosomes	Infantile form: cardiomegaly, hypotonia; Juvenile & adult form: skeletal myopathy	Enzyme assay	Leucocytes (with inhibitor)
GSD III (24%)	Debranching enzyme	Liver & muscle (IIIa), liver only (IIIb); storage of large amounts of abnormal glycogen with short outer branches	Hepatomegaly, hypoglycaemia, hyperlipidaemia, growth retardation, muscle weakness	Enzyme assay (& red cell glycogen concentration)	Leucocytes
GSD IV (3.3%)	Branching enzyme	Liver; accumulation of glycogen with fewer branch points and longer chains (poor solubility)	Hepatosplenomegaly, failure to thrive, liver cirrhosis	Enzyme assay	Leucocytes

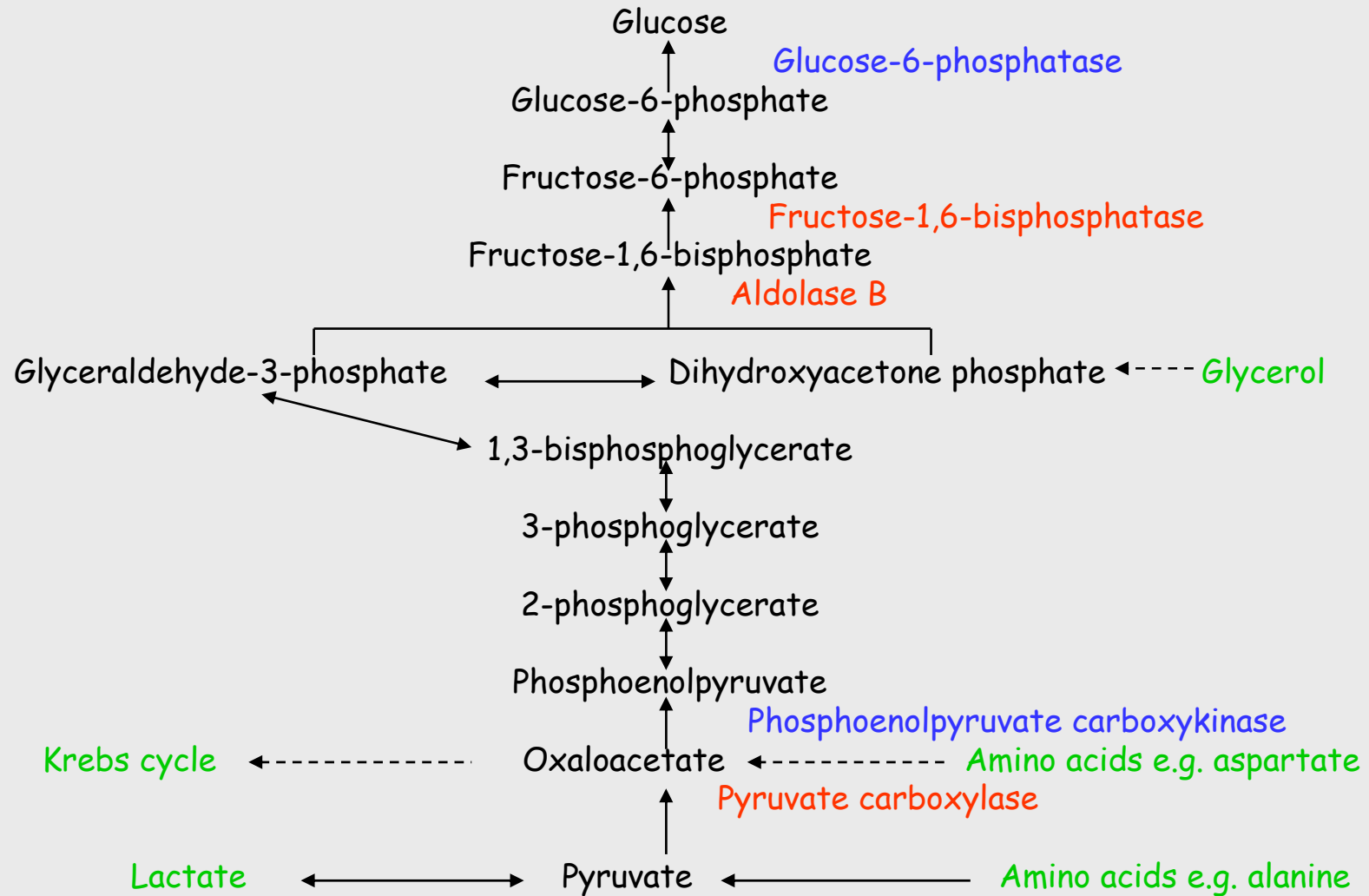
*Note muscle GSDs likely under-diagnosed

Glycogen Storage Disease Overview (2)

Disorder	Enzyme defect	Most affected tissue(s)	Clinical Features	Diagnostic tests	Sample
GSD V McArdle's (2.4%)	Muscle phosphorylase	Muscle; Increased amount of glycogen (normal structure)	Exercise intolerance with muscle cramps	Mutation analysis for common mutations, Ischaemic lactate-ammonia test (and/or enzyme assay)	Blood DNA sample or Muscle biopsy for enzyme assay
GSD VI (see IX)	Liver phosphorylase	Liver; Increased amount of glycogen (normal structure)	Hepatomegaly, growth retardation, mild tendency to hypoglycaemia, mild hyperlipidaemia	Enzyme assay	Leucocytes
GSD VII (0.2%)	Phosphofructo kinase	Muscle, erythrocytes (excess glucose leads to increased formation of glycogen)	Exercise intolerance, haemolytic anaemia	Enzyme assay	Muscle biopsy
GSD IX (30% VI + IX)	Phosphorylase b kinase (defect in one of 4 subunits)	Liver and/or muscle	As for GSD VI (functional deficiency of phosphorylase)	Enzyme assay	Erythrocytes for X-linked liver form (muscle biopsy for muscle form)

*Note muscle GSDs likely under-diagnosed

Disorders of Gluconeogenesis



Pyruvate Carboxylase Deficiency

This presents with lactic acidosis, neurological dysfunction (seizures, hypotonia, coma)

It is a defect in the first step of gluconeogenesis which is the production of oxaloacetate from pyruvate. In addition to the effect on gluconeogenesis, lack of oxaloacetate affects the function of the Krebs cycle and the synthesis of aspartate (required for urea cycle function).

In the acute neonatal form the lactic acidosis is severe, there is moderately raised plasma ammonia, citrulline (& alanine, lysine, proline) and ketones. Fasting results in hypoglycaemia with a worsening lactic acidosis.

The diagnosis can be confirmed by assay of pyruvate carboxylase activity in cultured skin fibroblasts

Patients rarely survive >3 months in the severe form

Fructose-1,6-Bisphosphatase Deficiency

The presentation is moderate hepatomegaly, metabolic acidosis (lactate & usually ketones) and hypoglycaemia.

The defect leads to impaired gluconeogenesis and accumulation of precursors of gluconeogenesis: lactate, pyruvate, alanine, ketones. The only glucose source is dietary or via glycogenolysis. The latter may be a problem in neonates as they usually have low glycogen stores.

Acute episodes may be precipitated by fasting or infection

Diagnosis is by assay of Fructose-1,6-bisphosphatase activity in leucocytes or liver homogenate. However there are some reports of normal leucocyte activity in individuals with deficient liver activity.

Treatment involves correction of the acidosis and hypoglycaemia and the avoidance of fasting

Once diagnosed, growth and development are usually normal

Hereditary Fructose Intolerance



Fructose is a monosaccharide found in fruit, honey and many vegetables. The disaccharide sucrose, composed of glucose and fructose, is found in many foods. Sorbitol (also from fruit and vegetables) can be converted into fructose by the liver.

Hereditary Fructose Intolerance (HFI), a defect in fructose metabolism (deficiency of aldolase B), only presents after ingestion of foods containing fructose, sucrose or sorbitol. When an infant with HFI is weaned, they suffer from nausea, vomiting, gastrointestinal discomfort and lethargy. They are at risk of liver and kidney failure and death, if fructose is not withdrawn.

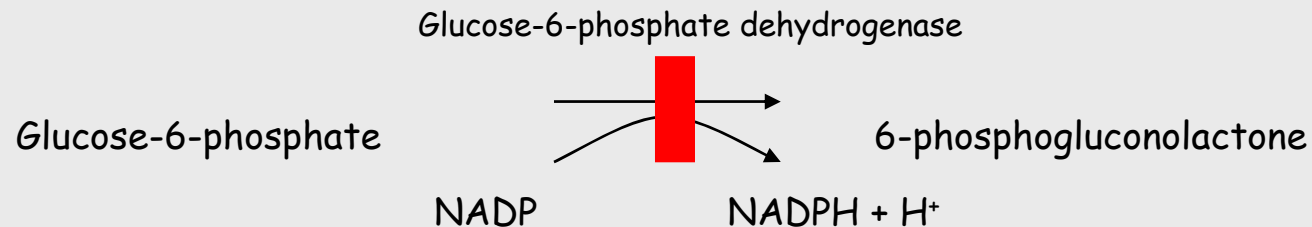
Affected individuals may reach adulthood undiagnosed due to development of an aversion to fructose-containing foods. Around 50% of adults with HFI have no dental caries, so occasionally the diagnosis has been made by dentists.

Biochemical features include hypoglycaemia (accumulated fructose-1-phosphate inhibits glucose production), hypophosphataemia, elevated plasma lactate, positive urine reducing substances, hyperuricaemia and a generalised aminoaciduria.

Diagnostic clues include a rapid improvement on withdrawal of fructose from the diet and a compatible nutritional history. Definitive diagnosis is by mutation analysis (there is one relatively common mutation in northern Europeans: A149P) or measurement of enzyme activity in a liver biopsy. An intravenous fructose tolerance test can be done but this needs to be done in a specialist centre and is not recommended in young children. In affected patients there is a decrease in plasma glucose and phosphate.

Glucose-6-phosphate dehydrogenase deficiency

This is an X-linked defect in the first, irreversible step of the pentose phosphate pathway.



A decrease in NADPH production makes red blood cell membranes vulnerable to oxidative stress, leading to haemolysis.

The most common manifestations are early neonatal unconjugated jaundice and acute haemolytic anaemia. However most individuals with the deficiency are clinically asymptomatic. The haemolytic crises are usually in response to an exogenous trigger such as certain drugs (e.g. antimalarials), food (broad beans) or an infection

Female heterozygotes may have symptoms but the severity varies due to non-random X chromosome inactivation)

The highest frequency is in those of Mediterranean, Asian or African origin

The diagnosis is by measurement of the enzyme activity in erythrocytes either by a qualitative test similar to the Beutler test or by quantitation. However the test is not reliable in detecting female heterozygotes and if required molecular testing is the preferred method. *MetBioNet IEM Introductory Training*

Self assessment questions

Match each disorder to **one** of the features below (Some disorders may have more than one of the features, but only match one per disorder and use each feature only once).

Feature	Disorder
Hyperbilirubinaemia	Hereditary fructose intolerance
Hyperuricaemia	Glycogen storage disease type V
Muscle cramps	Classical galactosaemia
Raised plasma citrulline	Glycogen storage disease type I
Metabolic acidosis	Glucose-6-phosphate dehydrogenase deficiency
Hypophosphataemia	Pyruvate carboxylase deficiency
Acute haemolytic anaemia	Fructose-1,6-bisphosphatase deficiency

Self assessment answers

Feature	Disorder
Hyperbilirubinaemia	Classical galactosaemia
Hyperuricaemia	Glycogen storage disease type I
Muscle cramps	Glycogen storage disease type V
Raised plasma citrulline	Pyruvate carboxylase deficiency
Metabolic acidosis	Fructose-1,6-bisphosphatase deficiency
Hypophosphataemia	Hereditary fructose intolerance
Acute haemolytic anaemia	Glucose-6-phosphate dehydrogenase deficiency