Review Article

Glucose-6-phosphate dehydrogenase deficiency

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most commonly known inherited disorder in man, and is estimated to affect 400 million people worldwide.¹ The highest prevalence rates (with gene frequencies in the range of 5–25% are found in tropical Africa, the Middle East, tropical and sub-tropical Asia, some parts of the Mediterranean, and in Papua New Guinea. The spectacular advances in molecular biology of the last decade are helping to unravel the molecular basis of its biochemical and clinical diversity. It serves as a model for the clinician of the importance of the environment in determining the clinical expression of genetic disease. For the population biologist, its study has yielded important insights into the interaction of host red cells with the malaria parasite and the influence of this interaction on human genetic polymorphism. Most human pathology due to G6PD deficiency is preventable by population screening and avoidance of precipitants, thus posing a challenge for epidemiologists and community physicians. There are several excellent recent reviews.^{2–5}

Biochemical and genetic basis

G6PD is a cytoplasmic enzyme that is distributed in all cells. It catalyses the first step in the hexose monophosphate pathway, to produce NADPH, which is required for reactions of various biosynthetic pathways as well as for the stability of catalase and the preservation and regeneration of the reduced form of glutathione (GSH). Catalase and GSH are essential for the detoxification of hydrogen peroxide, and the defence of cells against this compound depends ultimately and heavily on G6PD. This is especially true in red cells, which are exquisitely sensitive to oxidative damage and in which other NADPH-producing enzymes are lacking. G6PD in its active enzyme form is made up of

either two or four identical subunits, but the three-dimensional structure of the protein remains to be determined. The complete primary sequence of 515 amino acids has been determined from the cDNA sequence, which was determined in 1986.⁶ The gene encoding G6PD is on the long arm of the X chromosome (band Xq28), and spans 18 kb. G6PD deficiency is genetically heterogeneous and about 400 different variant enzymes have been reported. These have been categorized according to criteria established by the World Health Organization,^{7,8} and are divided into five classes (Table I) according to residual enzyme activity. DNA sequence analysis of these mutants has been completed for about 65 mutants⁹ and a number of interesting features have emerged:

1. The overwhelming majority result from single point mutations resulting in amino-acid substitution. Only three deletions have hitherto been reported and the largest is only eight amino acids.^{10,11} This is in keeping with the notion that G6PD is a 'house-keeping' gene that is ubiquitously expressed and a small amount of G6PD activity is essential for all cells.
2. Many variants that were regarded as distinct based on their biochemical features have emerged as being identical; whereas some that were thought to be identical have been found to be different. No clear structure–function relationships have emerged thus far, though the cluster of Class I variants around residues 386 and lysine 205 have led to the suggestion that these are the NADP⁺ and G6P binding sites, respectively.²
3. Very few of the severely deficient variants are polymorphic, whereas most of the polymorphic variants are associated with mild deficiency. This is further evidence that the selective pressure for the emergence of these mutations is the relative protection afforded against *Plasmodium falciparum* malaria. At least five polymorphic and mildly deficient 'double' mutants share the mutation found in the non-deficient A+ variant.

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Table I Summary of G6PD variants

Class	Number	Electrophoretic mobility				Altered electrophoretic mobility (%)	Total
		Polymorphic (%)	Fast	Normal	Slow		
I	1	1	24	34	39	64	97
II	37	30	31	37	54	70	122
III	22	21	41	16	46	84	103
IV	12	23	20	3	29	94	52
V	2	0	2			100	2
Total	72	19	118	90	168	76	376

The WHO classification is based on residual G6PD activity in red cells and on clinical manifestations as follows: Class I variants are associated with CNSHA; Class II variants have a G6PD activity of less than 10% normal; Class III variants have an activity of 10–60% normal; Class IV variants have near normal activity and no clinical manifestations; Class V variants have increased activity.

4. The only ‘nonsense’ mutation, that is, one that introduces a premature stop codon, has been found on one of the two X chromosomes of a female with partial G6PD deficiency.² The now classical concept that one of the two X chromosomes in female cells is inactivated was conceived because of the variable expression of G6PD deficiency in females¹² and had long been the basis of the application of the use of G6PD expression as a clonal marker.¹³ The availability of molecular techniques for detecting G6PD polymorphisms will lead to a wider application of these techniques in the future.¹⁴

The phenomenon of X-chromosome inactivation also offers a clue as to why female heterozygotes appear to have greater protection against malaria than do deficient male hemizygotes. *In vitro* culture studies have shown that the growth of malaria parasites is impaired upon first passage from normal to G6PD-deficient red cells but that through subsequent passages they can adapt and grow normally.¹⁵ Such ‘adaptation’ occurs as the parasite’s own G6PD gene is induced to protect it from oxidant stress.¹⁶ The female heterozygote’s red cell population is a mosaic of deficient and normal cells, and ‘adaptation’ does not occur in these circumstances.¹⁷

Clinical manifestations

Most G6PD-deficient individuals are entirely asymptomatic and the overwhelming majority of the remainder only develop symptoms in response to oxidant stress. The commonest clinical manifestations are neonatal jaundice and acute haemolytic anaemia related to drugs, infection or ingestion of fava beans. The critical role of

environmental precipitants has been recognized since the earliest descriptions of G6PD deficiency. Pythagoras is said to have warned his disciples against the dangers of eating fava beans (*Vicia faba*; broad beans). Observant practitioners had noticed that favism appeared to ‘run in families’.¹⁸ It was also clear that only some individuals were susceptible to haemolytic anaemia caused by drugs before the discovery by Carson *et al.*¹⁹ in Chicago that primaquine-sensitive people had a very low level of G6PD activity in their red cells.

Mechanism of haemolysis

The detailed mechanism of haemolysis is not fully known but it undoubtedly results from the inability of G6PD-deficient red cells to withstand the oxidative damage produced, directly or indirectly by an exogenous trigger. The identity of the G6PD variant, and hence the residual enzyme activity, is clearly an important variable. Residual activity is below a critical level in Class I, NADPH production is inadequate for the steady-state requirements of the red cell, and chronic non-spherocytic haemolytic anaemia (CNSHA) results. Neonatal erythrocytes have depressed levels of vitamin E, glutathione reductase and catalase,^{20,21} making them more susceptible to oxidant haemolysis. Certain drugs²² and infectious agents (for example, influenza A virus²³) stimulate the hexose monophosphate shunt pathway in normal red cells, indicating that in their presence increased NADPH production is required. Hydrogen peroxide is generated by activated polymorphonuclear neutrophils.²⁴ Based on studies of the effect of fractionated extracts on erythrocyte metabolism, the toxic components of fava beans have been suggested to be the pyrimidine aglycones, divicine and isouramil^{25,26} in combination with ascorbic acid.

A reasonable model for all of these situations is that the red cell GSH level becomes so low that critical sulphhydryl groups in some key proteins are not maintained in reduced form, and intramolecular or intermolecular disulphides are formed. Such aggregates decrease red cell deformability,²⁷ and they may alter the cell surface sufficiently to make it recognizable by macrophages as abnormal (much like an aged red cell), thus leading to extravascular haemolysis within the reticulo-endothelial system. Disturbed erythrocyte calcium homeostasis (specifically, reduced activity of the membrane Ca^{2+} -ATPase, leading to increased intraerythrocytic calcium and decreased intraerythrocytic potassium) has been suggested to mediate activation of proteolytic activity within erythrocytes of favic subjects.²⁸

Chronic non-spherocytic haemolytic anaemia

Some G6PD variants are characterized by overt chronic haemolytic anaemia, which is further exacerbated by oxidant stress. Such variants have been described (almost invariably in males within a single kindred) in many parts of the world, regardless of whether the common types of G6PD deficiency are endemic in the region. Most patients present with or give a history of neonatal jaundice, often requiring exchange transfusion^{29,30} and go on to develop infection and drug-induced haemolysis. Gallstones may be a prominent feature and splenomegaly is usually present, G6PD activity is low in all tissues and, in rare cases, deficiency in granulocytes is associated with granulocyte dysfunction³¹ and haemolysis is worsened by increased susceptibility to infection.

Drug-induced haemolytic anaemia

A critical analysis of the data whereby individual drugs have been implicated in the causation of haemolysis in G6PD-deficient subjects has been conducted by Beutler³² who uncovered a discrepancy between the relatively small list of drugs for which there is strong evidence linking them to haemolytic anaemia (Table II) and a much larger list of agents for which the evidence is less secure. The degree of haemolysis is also influenced by the activity of the host G6PD activity, the dose and duration of therapy, and the presence of additional oxidant stress, for example, infection. Furthermore, clinical and haematological assessment of haemolysis has notoriously low sensitivity, in that even a two- to three-fold increase in red cell destruction may not produce a significant anaemia or reticulocytosis. Clinical haemolysis and jaundice typically begin within 2 to 3 days of starting the drug. The haemolysis is largely intravascular and it is characteristically associated with haemoglo-

Table II Drugs causing haemolytic anaemia in G6PD deficiency*

Anti-malarials†	Primaquine, pamaquine, pentaquine
Sulphonamides	Sulphanilamide, sulphacetamide, sulphapyridine, sulphamethoxazole
Sulphones	Dapsone
Nitrofurans	Nitrofurantoin
Miscellaneous	Nalidixic acid, naphthalene (moth-balls), niridazole, ciprofloxacin, methylene blue

Note: The genetic heterogeneity of G6PD deficiency means that a drug found to be safe in some deficient subjects is not necessarily safe in all. The risk and severity of haemolysis is usually dose related.

*For references and further information see references 1, 32, 73 and 74; †quinine, chloroquine and quinidine are all acceptable for the treatment of acute malaria, and chloroquine, mefloquine, halofantrine, proguanil and pyrimethamine (but not Maloprim®, which contains dapsone, or Fansidar®, which contains a sulphonamide) are acceptable for malaria prophylaxis.

binuria. The anaemia worsens until the seventh to eighth day, a reticulocyte response then sets in, and the haemoglobin level begins to recover on the eighth to tenth day. *In vitro* tests^{33,34} have been developed aiming to predict whether a drug will cause haemolysis *in vivo* and they should be carried out before a new drug is introduced to a population in which G6PD deficiency is prevalent.

Infection-induced haemolysis

Infection is probably the most common cause of haemolysis in subjects with G6PD deficiency. Numerous bacterial, viral and rickettsial infections have been reported as precipitants, but particularly important are infectious hepatitis,^{35,36} pneumonia³⁷ and typhoid fever.³⁸ Viral infections affecting either the upper respiratory tract or the gastrointestinal tract are reported³⁹ to cause more severe haemolysis than bacterial infections in G6PD-deficient children.

Haemolysis is again largely intravascular and renal failure is a well-recognized complication in adults,^{40,41} whereas it is rare in children.

Favism

All patients with favism are G6PD deficient; however, not all G6PD-deficient subjects are sensitive to fava beans, and even those who are sensitive show striking variability from one exposure to the next. The reason for this discrepancy is not clear, and it seems likely that one or more factors in addition to G6PD deficiency are required for the development of favism^{42,43} and to determine the severity of the individual attack.

Clinical favism presents characteristically with

sudden onset of acute haemolytic anaemia within 24–48 hours of ingestion of the beans. Pallor, jaundice and haemoglobinuria are the hallmarks. Acute renal failure may supervene in adults, but it is very rare in children; however, fatalities in children were not uncommon prior to the availability of transfusion therapy. The highest incidence is in boys aged 2–6 years. It is well-documented that heterozygous girls are affected, although the condition is usually milder in these subjects.⁴⁴ Favism occurs after ingestion of fresh, dried or frozen beans, but fresh beans are by far the commonest offender and therefore favism is commonest during the spring season. Haemolysis in breast-fed babies whose mothers have eaten fava beans is well documented.⁴⁵

The mainstay of prevention is avoidance of fava beans. Experience in Sardinia has demonstrated the value of neonatal screening and health education in reducing the incidence of favism within that community.⁴⁶ The mainstay of treatment remains blood transfusion in severe cases. The original observation suggesting arrest of haemolysis by desferrioxamine⁴⁷ has been disputed,⁴⁸ but a recent larger study appears to confirm that a single bolus of desferrioxamine may be useful as an adjunct to red cell transfusion.⁴⁹ The proposed mechanism is that desferrioxamine reduces iron-dependent formation of damaging oxidant radicals (for example, hydroxyl ions).

It has been widely held that favism is only associated with the more severely deficient amongst the polymorphic variants of G6PD (particularly G6PD Mediterranean); and specifically that G6PD A- in not associated with favism. This is not correct, as typical attacks of favism have been well documented in subjects of African origin with the A-variant.⁵⁰

Neonatal jaundice

G6PD deficiency is the commonest red cell enzymopathy to cause neonatal haemolysis and jaundice. The best population data are available from West Africa,⁵¹ the Mediterranean^{52,53} and the Far East (for example, Thailand⁵⁴) and it is clear that perhaps as many as one third of all males with neonatal jaundice have G6PD deficiency, and a similar proportion of male children with G6PD deficiency develop neonatal jaundice (NNJ). Kernicterus has been described in all population groups. G6PD deficiency is a less frequent cause of NNJ among subjects of African descent in the USA, and of Greek ancestry in Australia, than in the countries of origin of these populations,¹ although the differences are perhaps less marked than originally thought. Environmental factors that may account for this include maternal exposure to oxidant drugs and use of herbal

remedies that may precipitate or exacerbate NNJ. Gestational age and maturity is an important consideration, as NNJ is more common, severe and potentially harmful in premature infants.⁵⁵ Environmental factors will also affect the incidence of neonatal infection, hypoglycaemia, acidosis and the normal level of neonatal haemoglobin within a population. Cultural factors, including exposure to icterogenic agents, have been identified as important precipitants of NNJ amongst the G6PD-deficient population of Nigeria.⁵⁶ Of genetic factors, the type of G6PD variant that is prevalent within a population is likely to be relevant, and is clearly of importance with respect to unusual or sporadic variants in the USA.^{57,58} In Sardinia, where at least three polymorphic variants are associated with NNJ, the severity of NNJ does not correlate with red cell G6PD activity,⁵⁹ suggesting that additional variables (for example, expression and activity of the G6PD-deficient variant in the liver⁶⁰) may be important.

Diagnosis

This is based on the clinical history and haematological findings, including anaemia, reticulocytosis and characteristic red cell changes (for example, 'bite' cells and Heinz bodies, produced by adherence of oxidized and denatured red cell proteins, and haemoglobins to the cell membrane).

Assays of G6PD activity^{61–63} depend on measuring the rate of production of NADPH from NADP in red cells, and the assay may be performed on a sequestrene (EDTA) or heparinized blood sample. Enzyme activity declines with red cell age and is highest in reticulocytes. Assay results obtained after an acute haemolytic episode should always be confirmed during the steady state, as a reticulocytosis may rarely lead to a false-negative result. Most haematology laboratories in the UK utilize screening tests, which are rapid and can reliably distinguish between affected men and heterozygous females; formal biochemical characterization involves enzyme purification from red cells, assay of activity by spectrophotometry and enzyme electrophoresis, and is only necessary in selected cases (for example, if a new variant is suspected).

Treatment

The most important aspect of management is to avoid precipitating causes of haemolysis (for example, drugs, fava beans). Haemolysis is usually self-limiting, but in severe cases red cell transfusion may be required. Red cells from G6PD-deficient

donors are acceptable for transfusion purposes⁶⁴ except in the case of exchange transfusion for neonatal jaundice, which should be performed after screening the donor unit for G6PD deficiency.⁶⁵ Folic acid (5 mg daily) is required long term for all patients with chronic haemolysis and should be given for 2–3 weeks following an acute haemolytic event. Patients with CNSHA are variably anaemic in the steady state and, if they are symptomatic or require transfusion therapy, splenectomy should be considered. Long-term therapy with vitamin E and oral selenium are not of proven value.^{66,67} All other patients with G6PD deficiency should not be anaemic during the steady state and, if they are, an alternative or additional diagnosis (for example, co-existence of G6PD deficiency with another cause of CNSHA such as pyruvate kinase deficiency⁶⁸) must be considered. Renal failure can complicate acute intravascular haemolysis (particularly due to drugs and fava beans). Neonatal jaundice arising as a result of haemolytic anaemia requires closer monitoring and earlier therapy than jaundice due to other causes, for example, breast milk jaundice.⁶⁹ In the term infant, phototherapy is required if the bilirubin exceeds 170 $\mu\text{mol/l}$ during the first 2 days of life, or 240 $\mu\text{mol/l}$ on day 4 or later. Exchange transfusion is indicated if the bilirubin exceeds 250 $\mu\text{mol/l}$ during the first 2 days, or 330 $\mu\text{mol/l}$ at any time.

Future prospects

In spite of the enormous increase in our understanding, much remains to be learnt about G6PD deficiency. The lack of detailed knowledge of the tertiary structure of the enzyme hampers attempts to understand structure–function relationships.⁷⁰ Molecular analysis of deficient variants will help but there is an important need to continue to perform full biochemical characterization of variants subjected to DNA sequence analysis. The lack of severe clinical effects in the overwhelming majority of deficient individuals means that antenatal diagnosis (which has already been performed⁷¹) and gene replacement therapy² will remain largely of theoretical interest. Much greater practical benefit will result from the institution of community screening programmes⁷² so that appropriate educational material can be targeted towards affected patients and their carers.

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