Glucose-6-phosphate dehydrogenase deficiency

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme defect, being present in more than 400 million people worldwide. The global distribution of this disorder is remarkably similar to that of malaria, lending support to the so-called malaria protection hypothesis. G6PD deficiency is an X-linked, hereditary genetic defect due to mutations in the *G6PD* gene, which cause functional variants with many biochemical and clinical phenotypes. About 140 mutations have been described: most are single base changes, leading to aminoacid substitutions. The most frequent clinical manifestations of G6PD deficiency are neonatal jaundice, and acute haemolytic anaemia, which is usually triggered by an exogenous agent. Some G6PD variants cause chronic haemolysis, leading to congenital non-spherocytic haemolytic anaemia. The most effective management of G6PD deficiency is to prevent haemolysis by avoiding oxidative stress. Screening programmes for the disorder are undertaken, depending on the prevalence of G6PD deficiency in a particular community.

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme that catalyses the first reaction in the pentose phosphate pathway, providing reducing power to all cells in the form of NADPH (reduced form of nicotinamide adenine dinucleotide phosphate). NADPH enables cells to counterbalance oxidative stress that can be triggered by several oxidant agents, and to preserve the reduced form of glutathione (figure 1). Since red blood cells do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore, defence against oxidative damage is dependent on G6PD.[1]

G6PD deficiency is an X-linked, hereditary genetic defect caused by mutations in the *G6PD* gene, resulting in protein variants with different levels of enzyme activity, that are associated with a wide range of biochemical and clinical phenotypes. The most common clinical manifestations are neonatal jaundice and acute haemolytic anaemia, which in most patients is triggered by an exogenous agent.[1] The striking similarity between the areas where G6PD deficiency is common and *Plasmodium falciparum* malaria is endemic provides circumstantial evidence that G6PD deficiency confers resistance against malaria.[2] The highest frequencies are detected in Africa, Asia, the Mediterranean region, and in the middle east; owing to recent migrations, however, the disorder is also found in North and South America and in northern European countries.[3]

A pathological disorder linked to ingestion of fava beans (*Vicia faba*), later identified as G6PD deficiency, has been recognised for centuries. The Greek philosopher and mathematician, Pythagoras, forbade his followers from eating fava beans, possibly because of their pathological effects.[4] At the beginning of the 20th century, several doctors in southern Italy and Sardinia drew a clinical picture of so-called favism.[5] However, because the response to fava bean ingestion is inconsistent, popular theories on the pathogenesis of favism were related to toxic effects or allergy.[6,7] In 1956, Carson and colleagues discovered that individuals developing haemolytic anaemia caused by the antimalarial drug primaquine had a very low level of G6PD activity in their red blood cells.[8,9] After a trip to Sardinia, Crosby noted a similarity between the severe haemolytic anaemia associated with ingestion of fava beans, or even inhalation of the plant's pollen, and the haemolytic anaemia induced by primaquine.[10] A low activity of G6PD in people with a history of favism was subsequently reported in Italy and Germany.[11,12] We now know that G6PD deficiency is the most common human enzyme defect, present in more than 400 million people worldwide.[13,14] Panel 1 summarises the history of our understanding of G6PD deficiency.

Search strategy and selection criteria

We searched PubMed, without any date restrictions, for the keywords "red cell metabolism", "G6PD deficiency", "inherited haemolytic disorders", "neonatal jaundice", "favism", and "G6PD and malaria". We also referred to important books on these topics. When more than one report described a specific point, the most representative paper was chosen.

Structure and function of G6PD

G6PD catalyses the first reaction in the pentose phosphate pathway, in which glucose is converted into the pentose sugars required for glycolysis and for various biosynthetic reactions. The pentose phosphate pathway also provides reducing power in the form of NADPH (figure 1), by the action of G6PD and 6-phosphogluconate dehydrogenase. NADPH serves as an electron donor for many enzymatic reactions essential in biosynthetic pathways, and its production is crucial to the protection of cells from oxidative stress. G6PD is also necessary to regenerate the reduced form of glutathione that is produced with one molecule of NADPH.[15,16] The reduced form of glutathione is essential for the reduction of hydrogen peroxide and oxygen radicals and the maintenance of haemoglobin and other red-blood-cell proteins in the reduced state.[1]

The monomer of G6PD consists of 515 aminoacids, with a molecular weight of about 59 kDa.[1] A model of the three-dimensional structure of G6PD was published in 1996 (figure 2),[17] and subsequently the crystal structure

of human G6PD has been elucidated.[18] The enzyme is active as a tetramer or dimer, in a pH-dependent equilibrium. Every monomer consists of two domains: the N-terminal domain (aminoacids 27–200), with a β–α–β dinucleotide binding site (aminoacids 38–44); and a second, larger, β+α domain, consisting of an antiparallel nine-stranded sheet. The dimer interface lies in a barrel arrangement, in this second part of the G6PD molecule. The two domains are linked by an α helix, containing the totally conserved eight-residue peptide that acts as the substrate binding site (aminoacids 198–206).[14,17,18] Viewing the structure, at 3 Å (0·3 nm) resolution, reveals an NADP+ (a coenzyme) molecule in every subunit of the tetramer, distant from the active site but close to the dimer interface.[18] Stability of the active quaternary structures is crucial for normal G6PD activity.

G6PD is present in all cells; however, its concentration varies in different tissues.[19] In healthy red blood cells, the enzyme operates at only 1–2% of its maximum potential (even under oxidative stress generated by methylene blue): a large reserve of reductive potential exists, which is substantially decreased in G6PD-deficient red-blood cells, leading to pathophysiological features.[20] After G6PD deficiency was established as a clinical disorder, its phenotypic expression was noted to be heterogeneous. More than 140 mutations of the *G6PD* gene have been identified, suggesting genetic heterogeneity.[21]

In 1967, WHO made initial recommendations for the characterisation of G6PD deficiency, which have subsequently been updated.[22,23] Initially, the G6PD deficit was characterised biochemically, by measuring residual enzyme activity and electrophoretic mobility. More than 400 biochemical variants of G6PD deficiency have since been defined according to other criteria, including physicochemical properties (thermostability and chromatographic behaviour), and kinetic variables (the concentration of substrate needed for an enzymatic reaction at half the maximum speed [Km] for glucose-6-phosphate, Km for NADP [nicotinamide adenine dinucleotide phosphate], pH dependence, use of substrate analogues [ie, any enzyme that will react with glucose-6-phosphate]).[24] Variants of G6PD deficiency were grouped into five classes based on enzyme activity and clinical manifestations (panel 2).[23] Variants can also be classified as sporadic or polymorphic.[1] The G6PD enzyme deficit can be caused by a reduction in the number of enzyme molecules, a structural difference in the enzyme causing a qualitative change, or both. Examination of G6PD variants shows that, in most cases, G6PD deficiency is due to enzyme instability, implying that aminoacid substitutions in different locations can destabilise the enzyme molecule.

Genetics and molecular basis of G6PD deficiency

The inheritance of G6PD deficiency shows a typical X-linked pattern, which was identified through favism having a higher incidence in males than in females, long before G6PD deficiency was identified as the cause. Males are hemizygous for the *G6PD* gene and can, therefore, have normal gene expression or be G6PD-deficient. Females, who have two copies of the *G6PD* gene on each X chromosome, can have normal gene expression or be heterozygous; in some populations, in which the frequency of the G6PD-deficient allele is very high, homozygous females are not rare. Heterozygous females are genetic mosaics as a result of X-chromosome inactivation (in any cell, one X chromosome is inactive, but different cells randomly inactivate one chromosome or the other) and the abnormal cells of a heterozygous female can be as deficient for G6PD as those of a

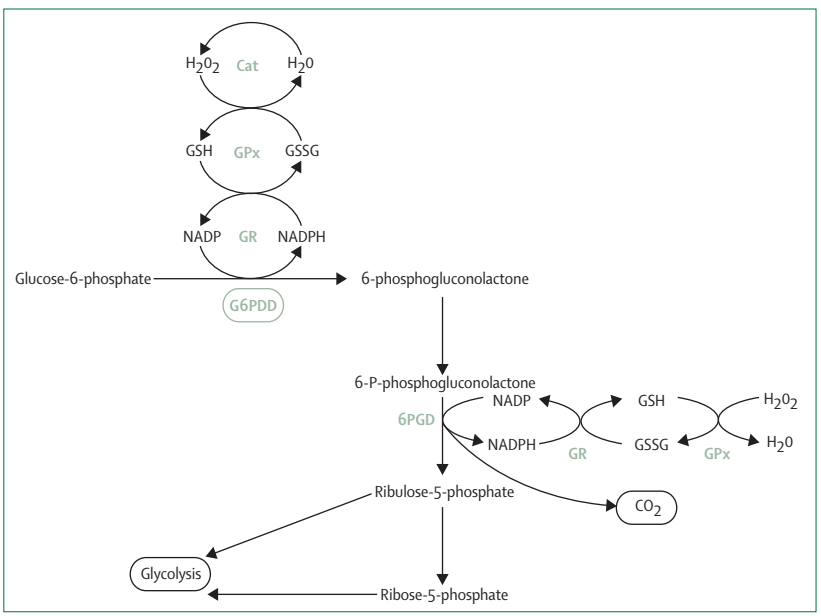

Figure 1: **Pentose phosphate pathway**
NADPH is produced by the action of G6PD and 6-phosphogluconate dehydrogenase. It serves as a proton donor for the regeneration of reduced glutathione, and as a ligand for catalase. NADPH also acts as an electron donor for many other enzymatic reactions essential in reductive biosynthesis. Cat=catalase. GPX=glutathione peroxidase. GR=glutathione reductase. G6PDD=glucose–6–phosphate dehydrogenase. 6PGD=6-phosphogluconate dehydrogenase. GSH=reduced glutathione. GSSG=oxidised glutothione.

Panel 1: **History of understanding of G6PD deficiency**

1956: Discovery of G6PD deficiency
1966: Standardisation of procedures for the study of G6PD deficiency (WHO scientific group)
1966–86: About 400 biochemical variants of G6PD deficiency characterised
1986: Cloning and sequencing of *G6PD* gene
1986–2006: About 140 molecular variants of the *G6PD* gene identified
1994: Crystallisation of G6PD protein from *Leuconostoc mesenteroides*
1995: Targeted disruption of *G6PD* gene
1996: Three-dimensional model of human G6PD protein developed

G6PD-deficient male: therefore, such females can be susceptible to the same pathophysiological phenotype.[25] Although heterozygous women, on average, have less severe clinical manifestations than G6PD-deficient males, some develop severe acute haemolytic anaemia.[26]

The *G6PD* gene is located at the telomeric region of the long arm of the X chromosome (band Xq28), close to the genes for haemophilia A, congenital dyskeratosis, and colour blindness (figure 3).[27,28] The gene was cloned in 1986,[29] and consists of 13 exons and 12 introns, spanning nearly 20 kb in total (table 1); it encodes 515 aminoacids, and a GC-rich (more than 70%) promoter region. The 5′ untranslated portion of the mRNA corresponds to exon I and part of exon II; the initiation codon is in exon II.[30] In the promoter region, there are several binding sites for the transcription factor SP1—GGCGGG and CCGCCC sequences—similar to those in other housekeeping gene promoters.[31,32] Wild-type *G6PD* is referred to as *G6PD B*.

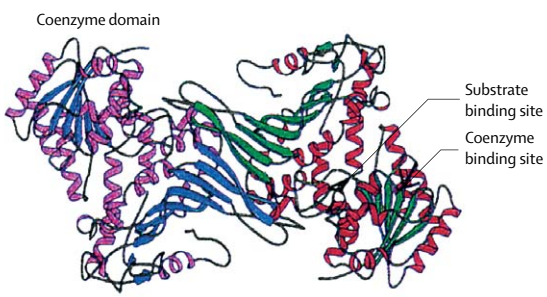

Figure 2: **Three-dimensional model of active G6PD dimer**
The two identical subunits are located across a symmetrical axis. Structure modified from the human G6PD model as proposed by Naylor and colleagues, with permission.[17]

Panel 2: **Classes of G6PD deficiency[23]**

Class I
Severely deficient, associated with chronic non-spherocytic haemolytic anaemia

Class II
Severely deficient (1–10% residual activity), associated with acute haemolytic anaemia

Class III
Moderately deficient (10–60% residual activity)

Class IV
Normal activity (60–150%)

Class V
Increased activity (>150%)

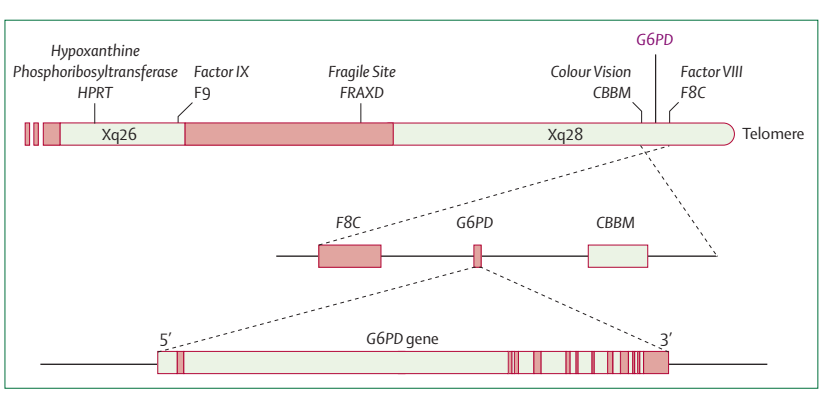

Figure 3: **Location of *G6PD* gene on X chromosome**

All mutations of the *G6PD* gene that result in enzyme deficiency affect the coding sequence (figure 4).[33] About 140 mutations have been reported, most of which are single-base substitutions leading to aminoacid replacements. Rarely, a second mutation is present in cis.[34,35] Small and in-frame deletions are exceptions. The promoter region of *G6PD* has been characterised extensively, by bandshift assays (also known as electrophoretic mobility shift assays) and systematic mutagenesis;[36] however, no mutations have yet been reported in the human promoter, although findings from a mouse model have shown that mutations of GC boxes can affect transcriptional activity greatly.[37] Point mutations are spread throughout the entire coding region; a cluster of mutations that cause a severe phenotype (class I, chronic non-spherocytic haemolytic anaemia) occurs in exons 10 and 11 (aminoacids 380–430, close to the dimer interface).

Analysis of the three-dimensional model of human G6PD enzyme, obtained from the crystallised protein,[17,18] has indicated that the NADP+ binding site is located in a part of the enzyme close to the N terminus, with the highly conserved aminoacid (in 23 species) Arg72 playing a direct part in coenzyme binding.[38,39] The cluster of mutations around exons 10 and 11 designates the subunit interface, which interacts with other important residues located elsewhere but which is brought close to this domain by protein folding . As mentioned above, almost all mutations in and around this domain cause variants of G6PD deficiency associated with chronic non-spherocytic haemolytic anaemia (class I), and affect both hydrophobic and charge–charge interactions or salt bridges (ie, weak ionic bonds). All the variants caused by mutations located in this area show a striking reduction in thermal stability in vitro.

All point mutations in the *G6PD* gene, when grouped according to the gradual decrease in conservation of aminoacids, show diminishing clinical severity.[40] It is noteworthy that many single point mutations have been recorded repeatedly in different parts of the world, suggesting that their origin is unlikely to be from a common ancestor and that they are, therefore, probably new mutations that have arisen independently.[41–43]

Apart from mutations that lead to enzyme deficiency, several polymorphic sites in introns have been identified, enabling the definition of G6PD haplotypes.[44,45] These haplotypes have been used in an attempt to understand

	Number
DNA	
Size of gene	18·5 kb
Total number of exons	13
Introns	12
mRNA	
Size in nucleotides	2269
5′ untranslated region*	69
Coding region*	1545
3′ untranslated region*	655
Protein	
Aminoacids	515
Molecular weight	59·265 kDa
Subunits per molecule of active enzyme	2 or 4
Molecules of tightly bound NADP per subunit	1

*Number of nucleotides.

Table 1: **Molecular characteristics of human G6PD**

the evolutionary history of the *G6PD* gene. Looking at linkage disequilibrium in haplotypes themselves, and with coding sequence polymorphisms, dating of the most common mutations and estimation of the timeframe of malaria selection has been possible.[46]

Epidemiology and malaria selection

Deficient *G6PD* alleles are distributed worldwide; a conservative estimate is that at least 400 million people carry a mutation in the *G6PD* gene causing deficiency (figure 5). The highest prevalence is reported in Africa, southern Europe, the middle east, southeast Asia, and the central and southern Pacific islands; however, because of fairly recent migration, deficient alleles are nowadays quite prevalent in North and South America and in parts of northern Europe.[47] For any given population, definition of the quantitative contribution of each allele to the overall prevalence of G6PD deficiency is still difficult, since epidemiological studies based on enzyme activity screening have been imprecise and have not extended to global coverage. In recent years, molecular analysis has been used to map the prevalence of G6PD deficiency.

The worldwide distribution of malaria is remarkably similar to that of mutated *G6PD* alleles,[48] making the malaria hypothesis of G6PD deficiency—that G6PD deficiency is protective against malaria—[2,49] a generally well-accepted notion. Two apparent exceptions to the geographical rule—southern Europe and North America—have clear explanations. Malaria was only eradicated in southern Europe during the 20th century; in North America, the defect is confined to immigrants from regions where malaria is found, and the descendants of these immigrants.[50] Findings of early studies designed to assess the connection between malaria and G6PD deficiency were somewhat contradictory.[51–54] Ruwende and colleagues noted that the *G6PD* A– allele (the most prevalent in Africa) is associated with a reduction in the risk of severe *P falciparum* malaria, for female heterozygotes and male hemizygotes (46% and 58%, respectively).[55] Evidence of protection against malaria also comes from in-vitro work in parasites cultured in red-blood cells with different G6PD genotypes. Several groups, comparing the growth of parasites in *G6PD* A– and *G6PD* Mediterranean mutated red-blood cells (a known G6PD variant) with that in healthy cells, showed that parasite growth is slowest in G6PD-deficient cells.[56–61] Intracellular schizogenesis, rather than invasion, is affected in G6PD-deficient red-blood cells,[58] in which oxidative injury of the parasite can happen.[62] Luzzatto and co-workers showed that red blood cells with normal G6PD activity, taken from *G6PD* A– heterozygous females (who underwent random X-chromosome inactivation), had 2–80 times more parasitic growth than G6PD-deficient red-blood cells.[63] G6PD-deficient red-blood cells infected with parasites undergo phagocytosis by macrophages at an earlier stage of parasite maturation than do normal red-blood cells with parasitic infection, which could be a further protective mechanism against malaria.[61]

In most areas of high prevalence of G6PD deficiency, several polymorphic alleles are found.[64,65] Tropical regions of Africa are one exception, where the variant *G6PD* A– accounts for about 90% of G6PD deficiency. *G6PD* A– is also frequent in North and South America, in the West Indies, and in areas where people of African origin are present. Moreover, *G6PD* A– is quite prevalent in Italy,[65,66] the Canary Islands,[67] Spain, and Portugal, and in the middle east, including Iran, Egypt, and Lebanon.[68] The second most common variant is *G6PD* Mediterranean, which is present in all countries surrounding the Mediterranean Sea,[1] although it is also widespread in the middle east, including Israel,[69,70] where it accounts for almost all G6PD deficiency in Kurdish Jews,[71] India, and Indonesia. In several populations, such as the countries around the Persian Gulf, *G6PD* A– and *G6PD* Mediterranean coexist at polymorphic frequencies.[72] Other polymorphic variants

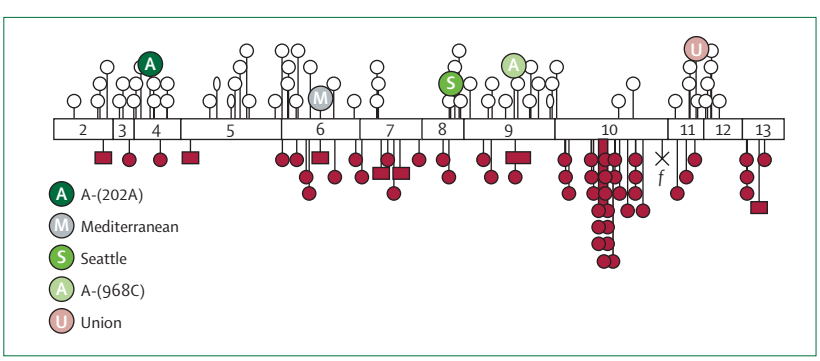

Figure 4: **Most common mutations along coding sequence of *G6PD* gene**
Exons are shown as open numbered boxes. Open circles are mutations causing classes II and III variants. Filled circles represent sporadic mutations giving rise to severe variants (class I). Open ellipses are mutations causing class IV variants. Filled squares=small deletion. Cross=a nonsense mutation. *f*=a splice site mutation. Modified from reference 1 with permission. 202A and 968C are the two sites of base substitution in G6PD-A.

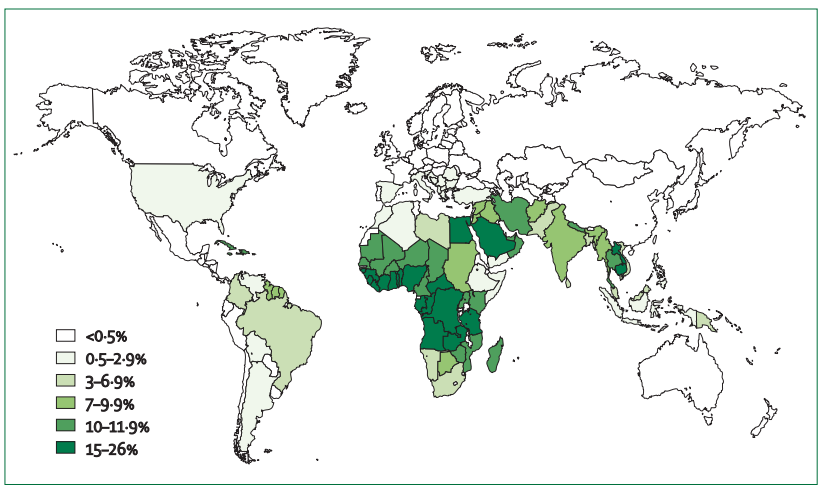

Figure 5: **World map distribution of G6PD deficiency**[23]
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are the Seattle and Union variants, which have been reported in southern Italy, Sardinia,[73,74] Greece, the Canary Islands,[75] Algeria, Germany, and Ireland. *G6PD* Union was also reported in China,[76] and *G6PD* Maewo—which by molecular analysis was shown to be *G6PD* Union—is polymorphic in the corresponding island in the Vanuatu archipelago.[77]

Diagnosis of G6PD deficiency

The definitive diagnosis of G6PD deficiency is based on the estimation of enzyme activity, by quantitative spectrophotometric analysis of the rate of NADPH production from NADP.[24] For rapid population screening, several semiquantitative methods have been applied, such as the dye-decolouration test developed by Motulsky in 1961,[78] and fluorescent spot tests, which indicate G6PD deficiency when the blood spot fails to fluoresce under ultraviolet light.[79] Other semiquantitative tests have been used, but require definitive testing to confirm an abnormal result.[80,81]

Diagnostic issues can arise for G6PD variants when measuring enzyme activity during an episode of acute haemolysis, or in the presence of a high reticulocyte count, because the level of activity in young erythrocytes is higher than in more mature cells, leading to false negative results for G6PD deficiency.[82] Difficulties can also be encountered in the assessment of neonates, who have a young red-blood-cell population. None of the screening tests can diagnose heterozygous females reliably, because X-chromosome mosaicism leads to partial deficiency. Heterozygous females with extremely skewed X inactivation have activity ranging from hemizygote to normal. Blood-film examination of individual cells after dye decolouration is preferable, although this method needs a trained technician.[83] Molecular analysis is the only method by which a definitive diagnosis can be made of a female's status.

Complete biochemical characterisation of G6PD enzyme is needed only for definition of a new variant, as recommended by WHO, although interlaboratory variations have resulted in the new variants being identified in error.[64] The development of simple molecular methods of diagnosis (PCR, direct sequencing, denaturing gradient gel electrophoresis), which allow detection of specific mutations, has enabled population screening, family studies and, in rare, very severe cases, prenatal diagnosis.[14] The most common mutations (Mediterranean, A–, Seattle, Union) can be rapidly detected by restriction enzyme analysis, after PCR amplification of the appropriate *G6PD* exon.[65]

In practical terms, testing for G6PD deficiency should be considered when an acute haemolytic reaction triggered by exposure to a known oxidative drug, infection, or ingestion of fava beans happens, either in children or in adults, particularly if they are of African, Mediterranean, or Asian descent. Moreover, members (especially males) of families in which jaundice, splenomegaly, or cholelithiasis are recurrent should be tested for G6PD deficiency.[84] Newborn babies with severe neonatal jaundice, particularly those of Mediterranean or African ancestry, are quite likely to have G6PD deficiency.

Clinical manifestations

Fortunately, most G6PD-deficient individuals are asymptomatic throughout their life, and unaware of their status. The illness generally manifests as acute haemolysis, which usually arises when red blood cells undergo oxidative stress triggered by agents such as drugs, infection, or the ingestion of fava beans. G6PD deficiency does not seem to affect life expectancy, quality of life, or the activity of affected individuals.[85,86]

G6PD deficiency usually presents as drug-induced or infection-induced acute haemolytic anaemia, favism, neonatal jaundice, or chronic non-spherocytic haemolytic anaemia. Several clinical disorders, such as diabetes[87] and myocardial infarction,[88] and strenuous physical exercise,[89] have been reported to precipitate haemolysis in G6PD-deficient individuals; however, coexisting infection or oxidant drug exposure can be the underlying cause in these instances. The precise mechanism by which increased sensitivity to oxidative damage leads to haemolysis is not fully known; furthermore, the exact sequence of events once an exogenous trigger factor is present is also unknown. Whatever the cause of the acute haemolysis in G6PD deficiency, it is characterised clinically by fatigue, back pain, anaemia, and jaundice.[90] Increased unconjugated bilirubin, lactate dehydrogenase, and reticulocytosis are markers of the disorder.

Drug-induced haemolytic anaemia

As described above, G6PD deficiency was discovered by investigating the development of haemolysis in patients who had received primaquine.[9] Subsequently, several drugs have been linked to acute haemolysis in

G6PD-deficient individuals (table 2).[3] Whether a specific drug directly causes haemolytic crisis in G6PD-deficient patients is often difficult to establish. First, an agent deemed to be safe for some G6PD-deficient individuals is not necessarily safe for all patients—not least because pharmacokinetics can vary between individuals. Second, drugs with potentially oxidant effects are sometimes administered to patients with an underlying clinical condition (such as infection) that could lead to haemolysis. Third, patients are often taking more than one type of medication. Fourth, haemolysis in G6PD deficiency is a self-limiting process and, therefore does not always produce clinically significant anaemia and reticulocytosis (panel 3).[91]

Usually, safe alternative agents are available that doctors should be aware of. If no alternatives exist, treatment decisions are based on clinical judgment of risk. In-vitro tests to establish the likelihood of haemolysis have been developed, but are not yet available in clinical practice.[92] Clinically detectable haemolysis and jaundice typically arise within 24–72 h of drug dosing. Dark urine due to haemoglobinuria is a characteristic sign.[93] Anaemia worsens until days 7–8. After drug cessation, haemoglobin concentrations begin to recover after 8–10 days. Heinz bodies (denatured haemoglobin precipitates) in peripheral red blood cells, detected by methyl violet staining, are a typical finding (figure 6).

	Definite association	Possible association	Doubtful association
Antimalarials	Primaquine Pamaquine	Chloroquine	Mepacrine Quinine
Sulfonamides	Sulfanilamide Sulfacetamide Sulfapyridine Sulfamethoxazole	Sulfadimidine Sulfasalazine Glibenclamide	Aldesulfone Sulfadiazine Sulfafurazole
Sulfones	Dapsone	..	..
Nitrofurantoin	Nitrofurantoin	..	..
Antipyretic or analgesic	Acetanilide	Aspirin	Paracetamol Phenacetin
Other drugs	Nalidixic acid Niridazole Methylthionium Phenazopyridine Co-trimoxazole	Ciprofloxacin Chloramphenicol Vitamin K analogues Ascorbic acid Mesalazine	Aminosalicylic acid Doxorubicin Probenecid Dimercaprol
Other chemicals	Naphthalene 2,4,6-trinitrotoluene	Acalypha indica extract	

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Table 2: **Drugs and chemicals associated with substantial haemolysis in patients with G6PD deficiency[1]**

***Panel 3:* Factors that affect individual susceptibility to, and severity of, drug-induced oxidative haemolysis**

Inherited

Metabolic integrity of the erythrocyte
Precise nature of enzyme defect
Genetic differences in pharmacokinetics

Acquired

Age
Dose, absorption, metabolism, and excretion of drug
Presence of additional oxidative stress—eg, infection
Effect of drug or metabolite on enzyme activity
Pre-existing haemoglobin concentration
Age distribution of red-blood-cell population

Infection-induced haemolytic anaemia

Infection is probably the most typical cause of haemolysis in people with G6PD deficiency. Hepatitis viruses A and B, cytomegalovirus,[94] pneumonia,[95] and typhoid fever are all notable causes.[96] The severity of haemolysis can be affected by many factors, including concomitant drug administration, liver function, and age. The total bilirubin concentration can be increased by hepatitis as well as haemolysis, which is a potential source of diagnostic error when haemolysis is precipitated by hepatitis.[97] In severe haemolysis, prompt transfusions can substantially and rapidly improve the clinical course (figure 7). Acute renal failure is a serious potential complication of viral hepatitis and concomitant G6PD deficiency; pathogenetic factors include acute tubular necrosis due to renal ischaemia, and tubular obstruction by haemoglobin casts. Some patients with haemolysis need haemodialysis.[98] This complication (acute renal failure) is rare in children.[99]

Favism

Clinical sequelae of fava bean ingestion were reported at the beginning of the 20th century, although an association between these beans and a clinical disorder was identified many centuries earlier. So-called favism was noted to be present widely not only in Mediterranean countries, where it was originally noted, but also in the middle east, the far east, and north Africa, where the growth and consumption of fava beans was widespread.[100,101] Favism is now widely believed to be most frequently associated with the Mediterranean variant of G6PD deficiency. Not all G6PD-deficient individuals undergo favism after ingestion of fava beans, and even the same individual can have an unpredictable response, suggesting that several factors affect development of the disorder, including the health of the patient and the amount of fava beans ingested. Favism can develop after ingestion of dried or frozen beans, but is particularly likely to occur after eating fresh beans; the disorder is most frequent in the period when beans are harvested.[102] Divicine, isouramil, and convicine, which are thought to be the toxic constituents of fava beans, increase the activity of the hexose monophosphate shunt, promoting haemolysis in G6PD-deficient patients.[103] Breastfed babies whose mothers have eaten fava beans are also at risk for haemolysis.[104]

Favism presents as acute haemolytic anaemia, usually around 24 h after the beans are eaten. Haemoglobinuria is more severe than that caused by haemolytic crises triggered by drugs or infection, although bilirubin

For more on **safe and unsafe drugs for patients with G6PD deficiency** see http://www.g6pd.org/favism/english/index.mv

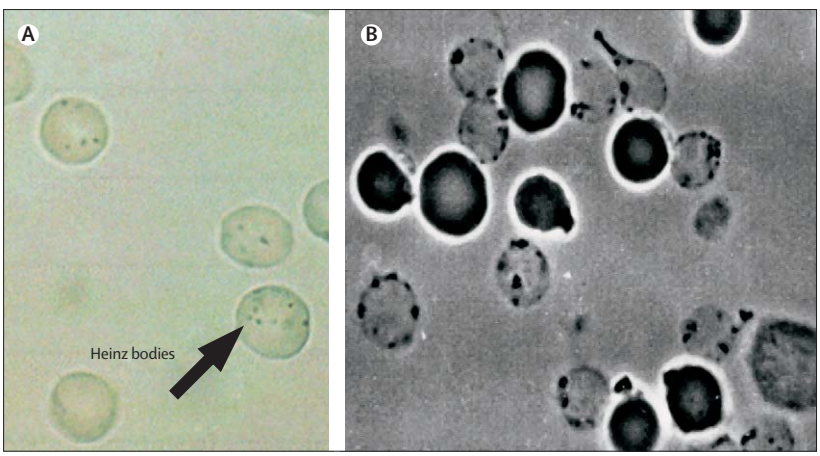

Figure 6: **Blood film from a 24-year-old G6PD-deficient Italian man who had acute haemolytic anaemia after paracetamol ingestion**
(A) Heinz bodies detected by supravital staining with methyl violet. (B) Heinz bodies visualised by electron microscopy.

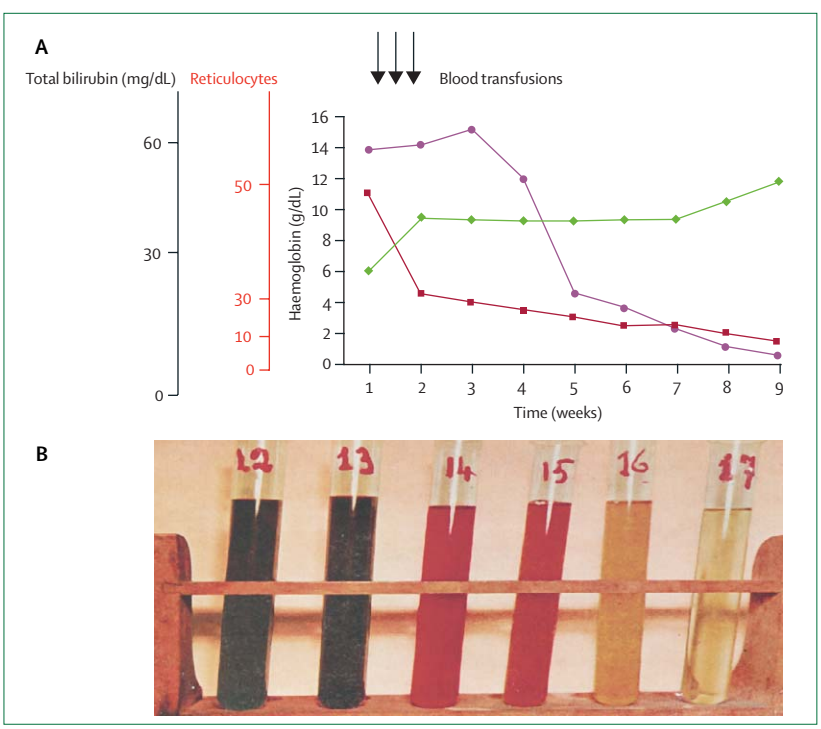

Figure 7: **Clinical course (A) and urine analysis (B) of a 13-year-old G6PD-deficient Italian boy during hepatitis B virus acute infection**
Urine is very dark in colour at the beginning of treatment; as jaundice and haemolysis improve urine becomes progressively clear. Pink=total bilirubin (mg/dL). Red=reticulocytes (%). Green=haemoglobin (g/dL).

concentrations are lower. Anaemia is generally acute and severe, leading to acute renal failure in some patients, due either to ischaemia or to precipitation of haemoglobin casts. The oxidative damage that takes place in patients with favism causes a series of changes to erythrocytes, leading to rapid clearance of these cells from the circulation (figure 8). For this reason, haemolytic events in patients with favism can be either intravascular or extravascular (ie, in the spleen).[105] A patient undergoing a severe haemolytic attack can require a blood transfusion. Prevention campaigns in areas with high prevalence of G6PD deficiency, through neonatal screening and health education, have greatly reduced the incidence of favism.[106]

Neonatal jaundice

Data from a series of studies suggest that about a third of all male newborn babies with neonatal jaundice have G6PD deficiency; however, the deficiency is less common in female neonates with jaundice.[107–109] Jaundice is usually evident by 1–4 days of age, similar to physiological jaundice, but is seen at a later time than in blood group alloimmunisation (ie, rhesus incompatibility). Kernicterus, although rare, can produce permanent neurological damage if not promptly managed.[110–112] G6PD deficiency and neonatal jaundice vary widely in their frequency and severity in different populations. Genetic,[113] cultural,[114] and environmental factors such as maternal exposure to oxidant drugs,[115] herbal remedies, or the effect of naphthalene-camphor balls that are sometimes used to preserve baby's clothes[116] can contribute to these differences. Neonatal jaundice is more typical and more severe in premature infants with G6PD deficiency than in babies born within the normal gestation period.[117]

The mechanism whereby G6PD deficiency causes neonatal jaundice is not yet understood completely. Haemolysis does not seem to contribute as much as impaired bilirubin conjugation and clearance by the liver.[118] G6PD-deficient newborn babies who also inherit a mutation of the uridine-diphosphate-glucuronosyl-transferase 1 (*UGT1A1*) gene promoter, which causes Gilbert's syndrome, are particularly at risk for neonatal jaundice.[119] Where screening for G6PD deficiency is not undertaken routinely, assessment of neonates should be done in those who develop hyperbilirubinaemia (bilirubin concentrations greater than the 95th percentile [150 µmol/L]) within the first 24 h of life, or in those with a history of neonatal jaundice in siblings.[120]

Congenital non-spherocytic haemolytic anaemia

In some patients, variants of G6PD deficiency cause chronic haemolysis, leading to so-called congenital non-spherocytic haemolytic anaemia. These variants have been grouped as class 1 in the proposed WHO classification.[22] The G6PD variants causing congenital non-spherocytic haemolytic anaemia are all sporadic, and almost all arise from independent mutations.[84] Diagnosis of this complication is based on clinical findings; the disorder is usually suspected during infancy or childhood. Many patients with congenital non-spherocytic haemolytic anaemia caused by G6PD deficiency have a history of severe neonatal jaundice, chronic anaemia exacerbated by oxidative stress that typically requires blood transfusions, reticulocytosis, gallstones, and splenomegaly.[1] Concentrations of bilirubin and lactose dehydrogenase are raised and, unlike in the acute haemolytic anaemia described above, haemolysis is mainly extravascular.

Genetic modifiers of G6PD phenotype

Acute or chronic haemolysis attributable to G6PD deficiency is sometimes exacerbated by coinherited (and unrelated) genetic erythrocyte alterations, such as membrane defects, thalassaemia, glucose-6-phosphate isomerase deficiency, pyruvate kinase deficiency,[121] and congenital dyserythropoietic anaemia.[122] Several reports have been published of G6PD deficiency associated with hereditary spherocytosis.[123,124] Unexpectedly high amounts of unconjugated bilirubin can be seen in the coinheritance of G6PD deficiency and Gilbert's syndrome. In newborn babies and G6PD-deficient adults, the bilirubin concentration is affected by the presence of the $TA_{(7)}$ allele of the uridine diphosphate glucuronosyltransferase (*UGT1A1*) gene, which is G6PD-dose dependent.[125,126]

Management

The most effective management strategy for G6PD deficiency is to prevent haemolysis, by avoiding oxidative stressors (such as drugs and fava beans). This approach, however, requires the patient to be aware of their deficiency, as a result of a previous haemolytic episode or a screening programme. Fortunately, acute haemolysis in G6PD-deficient individuals is usually shortlived, and does not need specific treatment. In rare cases (usually children), acute haemolysis leading to severe anaemia can require transfusions of red blood cells.

Neonatal jaundice caused by G6PD deficiency is treated in the same way as neonatal jaundice of other causes. Some controversy still exists about treatment in relation to bilirubin concentrations.[127] Usually, when the concentration of unconjugated bilirubin approaches or exceeds 150 µmol/L, patients are given phototherapy to prevent neurological damage; at higher concentrations still (>300 µmol/L), a blood transfusion can be necessary. Patients with congenital non-spherocytic haemolytic anaemia sometimes have a well-compensated anaemia that does not require blood transfusions; however, these individuals need to be monitored, because any exacerbating event (such as infection, or ingestion of an oxidant drug) can severely worsen the degree of anaemia. Very rarely, congenital non-spherocytic haemolytic anaemia is transfusion-dependent, so an iron-chelation treatment has to be administered. Antioxidants such as vitamin E and selenium seem to have some effect in patients with chronic haemolysis, but no consistent data to support this strategy are available.[128] Patients with congenital non-spherocytic haemolytic anaemia sometimes develop splenomegaly, but do not usually benefit from splenectomy.[129] Gallstones are a possible complication of haemolysis due to G6PD deficiency.[107] The prenatal diagnosis of G6PD deficiency has been reported, although this approach is questionable when we consider the low mortality and morbidity of G6PD deficiency.[130] For severe cases of the deficiency, which are refractory to other treatments, gene therapy remains a matter for consideration.

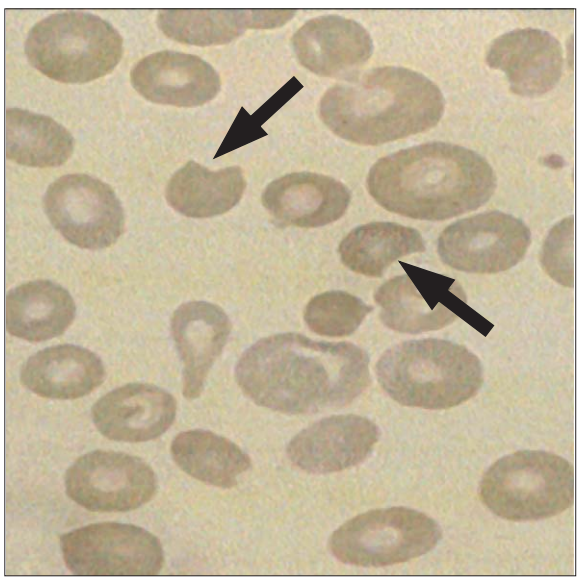

Figure 8: **Morphological erythrocyte changes (anisopoikilocytosis, bite cells) during acute haemolysis in G6PD-deficient patient**
Arrows show bite cells. Anisopoikilocytosis is abnormality in the shape or size of erythrocytes.

Conclusions

At least 400 million people worldwide carry the gene for G6PD deficiency. Fortunately, most of these will remain clinically asymptomatic throughout their lives. However, a proportion of G6PD-deficient individuals develop neonatal jaundice or acute haemolytic anaemia, which, if managed inadequately, can cause death or permanent neurological damage.

The highest frequencies of G6PD deficiency are in tropical Africa and tropical and subtropical Asia, which are also malaria-endemic areas. In areas of high prevalence, clinicians and patients must be alert and prepared to avoid any factors that might trigger severe clinical manifestations of the deficiency. We make three particular recommendations. First, when clinical and haematological findings raise the suspicion of G6PD deficiency, the disorder should be confirmed by quantitative spectrophotometric measurement of red blood cell enzyme activity. If a large screening programme is necessary, the rapid fluorescent spot test can be done initially, after which findings can be confirmed by a quantitative assay if necessary. Second, patients with G6PD deficiency should avoid exposure to oxidative drugs (table 2) and ingestion of fava beans, and they should be informed of any risk of episodes of acute haemolysis, and how to recognise these episodes. Finally, neonates should be tested for G6PD deficiency if they have a family history of haemolysis or are of a particular ethnic or geographic origin, or if the presence of neonatal jaundice suggests the possibility of the disorder.

Conflict of interest statement
We declare that we have no conflict of interest.

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