Glucose-6-Phosphate Dehydrogenase Deficiency

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KEYWORDS

- Glucose-6-phosphate dehydrogenase • Hemolytic anemia • Favism
- X-linked genetic polymorphism • Malaria selection

KEY POINTS

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency, expressed in red cells, is mostly asymptomatic; however, G6PD-deficient persons develop acute hemolytic anemia (AHA) when exposed to fava beans, to infection, or to certain drugs, including primaquine.
- The gene encoding G6PD maps to the X chromosome. Therefore, full-blown G6PD deficiency is more common in males, but female heterozygotes are also at risk of hemolysis.
- G6PD deficiency is widespread in the entire world and its epidemiology correlates with that of malaria; different mutant alleles underlie G6PD deficiency in different populations.
- Primaquine is still the only drug that can eradicate *Plasmodium vivax* hypnozoites; to promptly prevent or to treat hemolytic anemia, it is important to test for G6PD before administering primaquine.

INTRODUCTION

G6PD was discovered and biochemically characterized in 1932 by Otto Warburg and Walter Christian[1] in yeast and in red cells as an enzyme with a redox function. It was one of the first enzymes of glucose metabolism to be identified, but, although Warburg did not know that, the clinical manifestations of what later became known as G6PD deficiency had been already described. In the nineteenth century, pediatricians in Greece, Portugal, and Italy observed severe anemia and hemoglobinuria in children who had eaten fava beans – hence, the term *favism*[2]; it was noticed that favism tended to recur in the same persons and also that it ran in families. Subsequently, since the 1920s, it was observed[3] that an adverse side effect of 8-aminoquinolines (primaquine and plasmoquine), used for the treatment and the prophylaxis of malaria, was AHA. No connection to favism was suspected at the time, but again it was reported that it was

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only in certain people that this side effect occurred, and in those people it could happen again on rechallenge with the same drug; this became known as the *primaquine sensitivity syndrome*.

In 1956, Paul Carson's group in Chicago[4] reported that red cells from primaquine-sensitive persons were deficient in G6PD (enzyme activity <15% of normal), and in 1958 Gennaro Sansone's group in Genoa, Italy,[5] found the same deficiency in children with a previous history of favism. It was promptly proved that G6PD deficiency was genetically determined and that its inheritance was X-linked.[6] Almost as soon as Mary Lyon[7] discovered the X-chromosome inactivation phenomenon in mice, Ernie Beutler's group[8] found independently, using G6PD as a marker, that the same applied to humans. At the time this was the first example of a hemolytic anemia due to an inherited abnormality expressed in red cells; hence, the term *enzymopathy* was coined, in analogy to hemoglobinopathy. Reassuringly, however, it was clear that in the absence of an exogenous trigger, G6PD-deficient persons had no pathology; hence, primaquine-induced or fava bean–induced AHA became a prototype of a disease arising from a specific interaction between a gene and an environmental factor, just at the time when the term *pharmacogenetics* was coined.[9]

At approximately the same time, Tony Allison[10] and Arno Motulsky[11] hypothesized that genetically determined G6PD deficiency might have been favored by malaria selection; this spurred a flurry of studies aiming to determine the frequency of this trait in many countries. It quickly emerged that G6PD deficiency was widespread in human populations in all continents; a wealth of epidemiologic data were tabulated by David Livingstone as early as 1967.[12] In the meantime, the World Health Organization (WHO) Human Genetics, then headed by Italo Barrai, was prompt in taking on board the public health implications of such a widespread genetic abnormality; in 1966, a study group was arranged with the remit to review available data and to agree on a measure of standardization for the study of G6PD deficiency.[13]

This article focuses on the essentials of G6PD deficiency as a global health problem and on the essentials of its clinical manifestations, which are a paradigmatic example of a highly specific interaction between an inherited abnormality and exogenous agents that trigger hemolysis. Space does not permit a comprehensive coverage, particularly with respect to management, for which existing literature is referred to.[14,15]

BIOCHEMISTRY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

G6PD is a housekeeping enzyme, expressed in all cells of the body, that catalyzes the oxidation of glucose 6-phosphate (G6P) to 6-phosphoglucono-δ-lactone (**Fig. 1**), which is then hydrolyzed to 6-phosphoglucono-∂-lactone this, in turn, through the action of the enzyme phosphogluconate dehydrogenase (6PGD), is further oxidized and decarboxylated to the pentose sugar ribulose 5-phosphate.[16] Both G6PD and 6PGD have NADP as coenzyme, and therefore 2 molecules of NADPH are formed per molecule of G6P oxidized by G6PD (see **Fig. 1**). Because the product of these reactions is pentose, G6PD is commonly referred to as the first enzyme of the pentose phosphate pathway. On the other hand, from targeted inactivation of G6PD in embryonic stem cells[17] and from other lines of evidence it became clear that the prime physiologic role of G6PD is the production of NADPH.

In most cells of the human body NADPH is the key electron donor required for many biosynthetic processes, including several reactions in the pathways of fatty acid synthesis, cholesterol, and steroid hormone synthesis, as well as in the formation

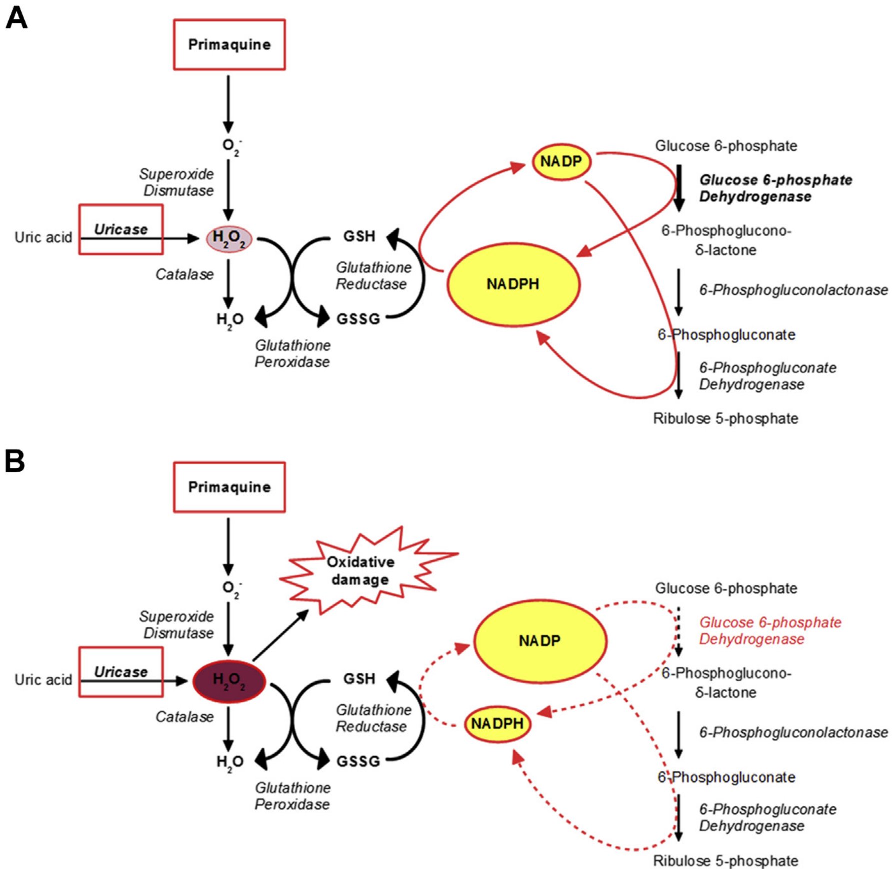

Fig. 1. Role of G6PD in protection against oxidative damage. (*A*) In G6PD-normal red cells, G6PD and 6-phosphogluconate dehydrogenase—2 of the first enzymes of the pentose phosphate pathway—provide ample supply of NADPH, which in turn regenerates GSH when this is oxidized by reactive oxygen species (eg, O_2^- and H_2O_2). O_2^- is one of the most reactive oxygen species that can be generated from the metabolism of pro-oxidant compounds, such as primaquine; uricase, on the other hand, directly produces hydrogen peroxide in equimolar amount to uric acid degraded. (*B*) In G6PD-deficient red cells, where the enzyme activity is reduced, NADPH production is limited and it may not be sufficient to cope with the excess of reactive oxygen species generated in the presence of pro-oxidant compounds.

from ribose of deoxyribose required for DNA synthesis.[16] In most cells there are several enzymes catalyzing dehydrogenase reactions – other than G6PD – that produce NADPH, and therefore even when G6PD is deficient there may be no shortage of NADPH. The situation is radically different in red blood cells, because the other NADPH-producing enzymes have been sacrificed in the course of erythroid cell differentiation; at the same time, these cells do not need NADPH for the biosynthetic pathways (discussed previously) because they do not exist, having been sacrificed as well.[18] On the other hand, red cells have a great need for the other major function of NADPH: defense against oxidative stress or oxidative attack.[19] This defense is largely mediated through the glutathione cycle, whereby a steady regeneration of reduced glutathione (GSH) depends on a steady supply of NADPH (see Fig. 1). Because the red cell is a professional loader, carrier, and unloader of

hemoglobin-bound oxygen, and because free radicals can be formed in the process,[20] it is crucially important that it can defend itself against endogenous oxidative stress, even when there is no exogenous attack in sight.

G6PD deficiency is due to inherited mutations in the *G6PD* gene (discussed later) that are expressed in all cells; and it is already clear from the above that erythrocytes, more than other cells, are vulnerable to the consequences of this defect. But there is an additional important reason for this. As red cells age in circulation, there tends to be a gradual decrease in many of their functions, because individual proteins underlying those functions decay exponentially in these ribosome-less cells that cannot make new protein[21]; as a result, G6PD activity is approximately 50 times less in a normal red cell that is ready to be removed on day 120 compared with when it itself was a reticulocyte.[22] This process is further magnified with those mutations—the large majority—that compromise the in vivo stability of the G6PD protein.[23]

G6PD deficiency is never complete; if it were complete, it would be lethal.[24] Therefore, in most cases, in the steady state, the consequences of G6PD deficiency are not noticeable (see **Fig. 1**); the NADPH produced by the residual G6PD activity and by 6PGD activity is just enough to keep the red cell going, with marginal reduction of its life span. If an exogenous oxidative stress is applied, however, G6PD-deficient red cells are unable to step up NADPH production (which normal red cells do); as a consequence, GSH is rapidly depleted (see **Fig. 1**), hemoglobin and other proteins are damaged, and eventually the red cell becomes prey to macrophages or hemolyzes altogether.[25]

MOLECULAR-GENETIC BASIS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

The *G6PD* gene consists of 13 exons (the first of which is noncoding) and it encodes a 515–amino acids (AAs) protein subunit,[26] the homodimer of which is enzymatically active; the dimer can further dimerize to give an enzymatically active homotetramer.[27] Each subunit has 1 molecule of tightly bound NADP[28] in addition to binding sites for the NADP substrate and the G6P substrate.

As discussed previously, G6PD deficiency was known from formal genetics to be inherited as an X-linked trait,[6] and the *G6PD* gene maps to the long arm of the X chromosome (band Xq28).[29] X-linkage has important implications with respect to G6PD deficiency. First, in males there are only 2 genotypes: hemizygous normal and hemizygous G6PD deficient. In females there are 3 genotypes: homozygous normal, homozygous deficient, and heterozygous. Second, although it is often stated that G6PD deficiency is more common in males, this is not correct; according to the fundamental principle of population genetics (the Hardy-Weinberg equilibrium), homozygous females are much more rare than hemizygous males, but heterozygous females are much more numerous (**Table 1**). A bonus of X-linkage is that male frequencies indicate directly allele frequencies (see **Table 1**). Third, as a result of X-chromosome inactivation, heterozygous females are genetic mosaics[8,30]; on average, one-half of their red cells are G6PD normal and one-half are G6PD deficient. There is a wide distribution around this average, whereby in some females the enzyme activity phenotype overlaps with normal, whereas in others it overlaps with the G6PD deficiency as seen in homozygotes[31]; this has obvious clinical implications.

All *G6PD* mutations known (**Table 2**), except *G6PD* A, are associated with more or less severe enzyme deficiency but never with complete loss of activity; there are no frameshift mutations in the database (such mutations presumably are lethal; discussed previously and later), and the only nonsense mutation has been found in a heterozygous woman.[32] The mutations that underlie G6PD deficiency are spread

Table 1
The frequency of glucose-6-phosphate dehydrogenase deficiency is markedly different in males and in females (Hardy-Weinberg rule)

Glucose-6-Phosphate Dehydrogenase Deficiency Allele Frequency, *q*	Glucose-6-Phosphate Dehydrogenase–Deficient Hemizygous Males, %	Glucose-6-Phosphate Dehydrogenase–Deficient Homozygous Females, %	Glucose-6-Phosphate Dehydrogenase–Deficient Heterozygous Females, %
0.01	1	0.01	2
0.05	5	0.25	9.5
0.25	25	6.25	37.5

In the first column, q is the conventional symbol for the rarer allele in a 2-allele polymorphism. The frequency of G6PD-deficient males in column 2 is simply the value of q expressed in %. The frequency of G6PD-deficient homozygous females (column 3) is q^2, and the frequency of G6PD-deficient heterozygous (column 4) is $2(1-q)q$, as from the Hardy-Weinberg rule. It is seen that at low allele frequency heterozygous females are approximately double the hemizygous males, whereas homozygous females are rare.

throughout the coding region (Fig. 2). The most recent compilation[33] lists 186 *G6PD* alleles in addition to the normal or wild-type gene, referred to traditionally as *G6PD* B.

A large majority of mutations (159) are missense mutations due to single nucleotide substitutions causing single AA replacements; however, there are also multiple missense mutations within the same allele as well as in frame deletions and rare mutations that affect splicing. The frequency of single and multiple nucleotide substitutions is approximately the same among variants that are or may be polymorphic and those that are not. In contrast, deletions are only found within the nonpolymorphic variants (see Table 2).

Not surprisingly, different mutations cause both quantitative and qualitative changes in the enzyme. This has led to a classification of G6PD variants based on the degree of deficiency and on clinical manifestations (Table 3). A cluster of mutations, most of them in exon 10, encode AAs that are in the dimerization domain[34]; they affect markedly the stability of the dimer and they produce class I variants (see Table 3), causing chronic nonspherocytic hemolytic anemia (CNSHA).

A remarkable feature at the genomic level is that the *G6PD* gene overlaps with the *IKBKG/NEMO* gene, which is transcribed in the opposite direction and mutations of

Table 2
Molecular basis of allelic variants of glucose-6-phosphate dehydrogenase[a]

	Class	Single Nucleotide Substitutions	Multiple Nucleotide Substitutions	Deletions	Intronic Mutations	Total Number
Polymorphic	II, III, and IV	27	4	0	0	31
Perhaps polymorphic[b]	II and III	55	6	0	0	61
Nonpolymorphic	I	69	5	10	1	85
Undefined	—	8	0	0	1	9
Total number	—	159	15	10	2	*186*

[a] *G6PD* B is regarded as the human wild type and, therefore, is not included in the count.
[b] Not every G6PD variant in class II or III has been proved polymorphic, but this is probable.

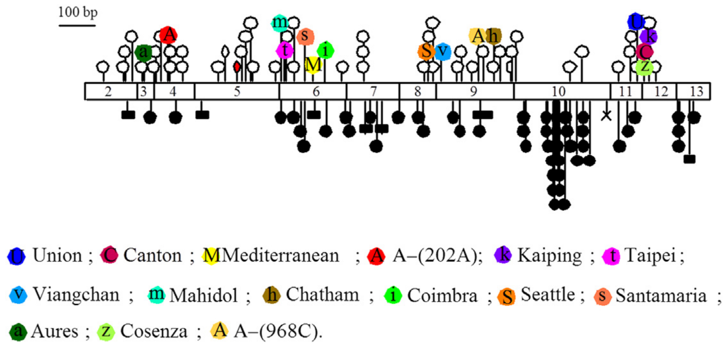

Fig. 2. Many structural mutations are found within the coding region of the human *G6PD* gene. All variants shown, except *G6PD* A, are associated with enzyme deficiency. Below the diagram with numbered exons are mutations associated with the most severe clinical phenotype (class I: see Table 3), all of which are sporadic and rare. Above the exon diagram are mutations associated with a milder phenotype (class II or III), many of which are polymorphic; and some of those that have been more extensively investigated are shown by initialed lollipop symbols. A− is heterogeneous because it can result from a combination of the N126D replacement with any of three additional mutations, the most common of which is V68M. (*From* Luzzatto L, Poggi, VE. Glucose 6-phosphate dehydrogenase deficiency. In: Orkin SH, Nathan DG, Ginsburg D, et al, editor. Hematology of infancy and childhood. Philadelphia: Saunders; 2009. p. 887; with permission.)

Table 3
Classification of glucose-6-phosphate dehydrogenase variants

Current Classification				Proposed Revision	
Class	Residual Glucose-6-Phosphate Dehydrogenase Activity (% of Normal)[a]	Clinical Manifestations	Examples of Genetic Variants	Class	Residual Glucose-6-Phosphate Dehydrogenase Activity (% of Normal)[a]
I[b]	<10[c]	CNSHA[d] (NNJ, acute exacerbations)	Guadalajara, Nara, Sunderland	I	<10
II	<10[c]	None in the steady state	Mediterranean, Canton, Union	II + III	<30[e]
III	10–60	None in the steady state	A−, Mahidol, Seattle		
IV	100	None	A, B	IV	>85
V	>100	None	—	—	—

[a] Levels of residual G6PD activity in hemizygous males.
[b] The definition of class I variants is not biochemical but clinical (ie, class I variants cause CNSHD).
[c] The range of G6PD activity is similar in class I and class II variants, which may seem strange because the clinical phenotype is significantly different. It must be considered, however, that (1) in CNSHD, there is always reticulocytosis, which increases G6PD levels, and (2) in some class I variants, the residual G6PD activity may be similar to a class II or even a class III variant, but the enzyme kinetics may be unfavorable.
[d] When hemolysis is not compensated, chronic anemia is present and blood transfusions may be necessary at times or even at regular intervals.
[e] Cutoff is indicated as 30%, because all G6PD variants in class II and III described so far have a residual activity of less than 30%.

which are responsible for the serious disease *incontinentia pigmenti*.[35] Some of these mutations are lethal in males, but in females they include large deletions of *G6PD*, which are compatible with life thanks to selection for cells in which the active X chromosome has an intact *G6PD* allele.

EVOLUTION

From full genome databases, it is inferred that G6PD is not present in *Archaebacteria*, which, because they live in environments with low or no oxygen, hardly need defense against oxidative stress.[36] In all other living organisms, G6PD is highly conserved; the AA sequence similarity from microorganisms to mammals ranges from 43% to 98%. This high degree of conservation must mean that the G6PD protein has been shaped by evolution early and robustly to perform its enzymatic function well. In many plants, there are 2 *G6PD* genes—1 encodes cytosolic G6PD and the other G6PD present in plastids[37]—and a human pseudogene is known.[38]

In the alignment of *G6PD* coding sequences from all organisms, those of *Plasmodia* stand out because they have a long 5′ extension,[39] which encodes the metabolically related enzyme 6-phosphoglucono-δ-lactonase.[40] It has been suggested that the protein product of the parasite's bifunctional gene[41] might be a new target for antimalarials.[42,43]

It is often thought that in an enzyme protein, functionally critical AA residues are less likely to change over evolutionary times; as a result, mutations that cause disease in general are more likely to affect the most evolutionarily conserved residues.[44,45] This simple correlation, however, is not always seen,[46] and it seems that it does not exactly hold for G6PD. An analysis of 103 *G6PD* mutants causing G6PD deficiency in humans, as against 52 *G6PD* sequences from 45 different organisms, has shown that most mutations (74%) are in highly and moderately conserved (50%–99% of similarity) AAs, whereas few mutations are in fully conserved or in poorly conserved AAs (**Fig. 3**).[36] This distinct relationship suggests that mutations in poorly conserved AAs may remain inconspicuous because they do not cause significant G6PD deficiency, whereas mutations in fully conserved AAs might be lethal, which is not

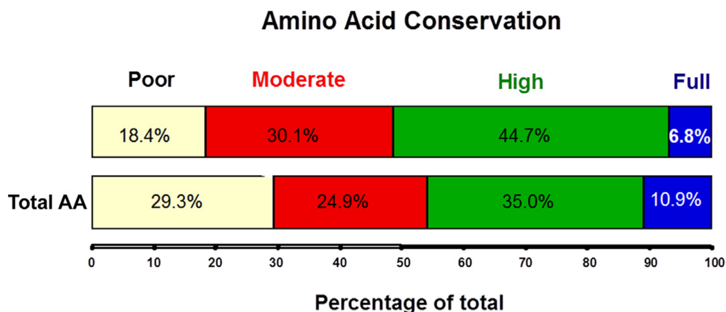

Fig. 3. Conservation and human G6PD mutants. The upper bar reports the distribution of G6PD mutants among evolutionary AA conservation categories: full, 100% of similarity; high, 76%–99% of similarity; moderate, 50%–75% of similarity; and poor, less than 50% of similarity. The lower bar reports the distribution of the 515 AAs of the human G6PD among the conservation categories. The distribution among evolutionary conservation categories of the mutants is statistically different from the distribution of AAs: $\chi^2 = 9.36$; $P<.03$. (*Adapted from* Notaro R, Afolayan A, Luzzatto L. Human mutations in glucose 6-phosphate dehydrogenase reflect evolutionary history. FASEB J 2000;14:491; with permission.)

surprising in the case of a gene, such as G6PD, present in a single functioning copy and indispensable for life.

EPIDEMIOLOGY AND MALARIA SELECTION

The geographic distribution of G6PD deficiency is spectacular, because it spares no continent[47] (Fig. 4), yet population frequencies are highly variable, because they reflect 2 major factors in the epidemiology of a genetic abnormality: environmental selection and migration. The correlation with the epidemiology of malaria, discussed previously, is obvious, for instance, in areas as distant as tropical Africa, Southeast Asia, and the Vanuatu archipelago in the Pacific; and parts of Southern Europe can be included, where malaria was endemic until 2 to 3 generations ago.[48] G6PD deficiency is also common in the Americas, however, including areas that have never had malaria; this is largely accounted for by migrations, voluntary or otherwise, from Africa, Asia, and Europe. G6PD deficiency has no significant frequency in native American populations.

The wealth of data on the frequency of G6PD deficiency (reflected in Fig. 4) originates from many population surveys, carried out by a variety of methods; therefore, a margin of error must be allowed in the classification of individual samples. Also, in collecting data there may have been several sources of inadvertent sampling bias (blood donors, school children, and so forth). The most common type of confusion relates, however, to some cases of a single figure reported as the overall frequency of G6PD deficiency, observed in a mixed group of males and females, despite that the *G6PD* gene is X-linked. Most tests for G6PD deficiency classify as deficient all G6PD-deficient hemizygous males, all G6PD-deficient homozygous females, and some (variable and difficult to estimate) portion of heterozygous females. It is

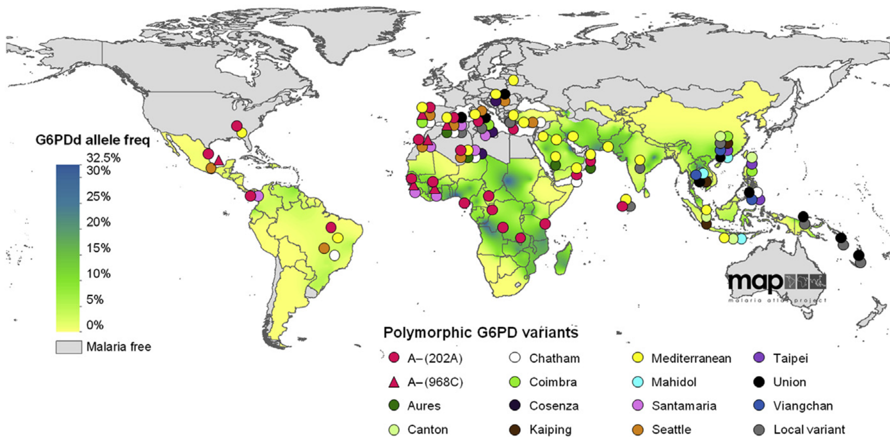

Fig. 4. Global distribution of G6PD deficiency. This map is a combination of 3 previous maps.[47,49,59] Color shades on the map indicate the median predicted allele frequency of G6PD deficiency in malaria-endemic and malaria-eliminating countries, according to the geostatistical model designed by Howes and coworkers.[49] Each colored circle illustrates the geographic distribution of 1 polymorphic *G6PD* allele present in more than 1 population. (triangles used for G6PD A− [968C, L323P] to distinguish it from *G6PD* A− [202A, V68M]; note that both of these mutations are always found associated with 376G, N126D). Dark gray circles indicate local polymorphic variants that have been detected only in 1 population. (*Data from* Refs.[47,49,59])

preferable to carry out a population survey by testing males only; from the G6PD frequency value obtained, the frequencies of the female genotypes can be calculated by using the Hardy-Weinberg rule (see **Table 1**).

The striking geographic similarity between the distribution of G6PD deficiency and that of malaria[49] does not itself constitute proof that the latter has selected for genes that cause the former, but more pieces of evidence exist.[50] Natural selection has to do with prereproductive mortality, and in malaria-endemic countries, malaria mortality is mostly in children. Numerous field studies in Africa have tested this evolutionary hypothesis by comparing, in G6PD-normal children and G6PD-deficient children, the rate of malaria incidence, the levels of parasitemia, or the severity of malaria[51–54]; an additional large multicentric study has been published recently.[55] All studies have been concordant in finding that G6PD deficiency is malaria protective (especially against severe malaria); however, again it is highly relevant that the *G6PD* gene is X-linked. The dynamics of natural selection for an X-linked gene are such that if both males and females with the protective gene have increased fitness, the gene tends to fixation: however, there is no instance where, despite high levels of malaria, the entire population has become G6PD deficient. This is a strong argument identifying females who are heterozygous for G6PD deficiency as the genotype that is most protected.[56]

Until recently it was thought that among malaria parasites, *P falciparum* was the selective agent for G6PD deficiency; however, recent data suggest that *P vivax*, where present, may have played a role as well.[57,58] Finally, a strong argument in favor of malaria as the main force that has selected for G6PD deficiency is that many different G6PD deficiency alleles have reached polymorphic frequencies in populations in disparate parts of the world, a glaring example of convergent evolution.[59]

CLINICAL MANIFESTATIONS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

Perhaps the most important point to consider about the clinical implications of G6PD deficiency is that this genetic abnormality remains largely or totally asymptomatic throughout life; thus, what is outlined previously is the epidemiology of a genetic abnormality, not of a disease. G6PD-deficient persons do develop a disease only under specific circumstances.

- In newborns, G6PD deficiency entails an increased risk of neonatal jaundice (NNJ), including severe NNJ.[60] The reason for this has not been fully elucidated, but in countries where G6PD deficiency is prevalent this is probably the most frequent cause of NNJ, which, if not appropriately treated, can lead to invalidating neurologic consequences. Thus, serious complications can take place not only in G6PD-deficient hemizygous baby boys but also in G6PD-deficient heterozygous baby girls.[15] Also, in this type of NNJ, the peak bilirubin is usually on day 3; this implies that if the onset of NNJ is not taken seriously enough, it may get worse after the baby is discharged.
- Ingestion of fava beans (*Vicia faba*), also known as broad beans, can trigger AHA (ie, *favism*) in G6PD-deficient persons.[61,62] It was thought that favism was an allergic reaction, which could be triggered not only by ingestion of the beans but also even just by inhalation of pollen; this is not correct, as it is now known that the offending chemicals, present at a high concentrations in fava beans, are nonvolatile glucosides (vicine and convicine), the aglycones of which produce free radicals.[63] These glucosides are not present in other beans, which, contrary to mistaken if well-meaning counseling, are safe for G6PD-deficient

persons. Favism can happen at any age, but it is more common and more commonly severe in children. The AHA can be brisk; a fall in hemoglobin from normal levels to 4 g/dL can take place over 48 hours or less, and it is associated with macroscopic hemoglobinuria, indicating that much of the hemolysis is intravascular.

- Since the time when G6PD deficiency was discovered through the use of primaquine (discussed previously), several other drugs have been found to entail the risk of hemolysis in G6PD-deficient persons[64] (**Table 4**). Once the list of risky drugs is well known, any drug on the list should be avoided in G6PD-deficient persons; the literature on drug-induced AHA consists almost entirely of individual case reports, most of them with favorable outcome, because, fortunately, after AHA is over, there is full recovery. Drug-triggered AHA is markedly dose dependent; therefore, in rare cases when there is no alternative, a reduced dose may be given deliberately under appropriate surveillance. Two recent mishaps, however, deserve mention.
  - In 2004, a combination of dapsone and chlorproguanil (Lapdap, GlaxoSmithKline, London) was launched as an effective antimalarial and was marketed in 17 African countries where G6PD deficiency is prevalent (male frequency of 10%–23%), in spite of AHA recorded as a serious adverse event in clinical trials.[65–68] There was no mortality in the trials, because the children who developed AHA

Table 4
Drugs that may trigger acute hemolytic anemia in glucose-6-phosphate dehydrogenase–deficient patients

Type of Drug	Evidence Based (Youngster et al,[74] 2010)	Definite Risk of Acute Hemolytic Anemia (British National Formulary, March 2015)	Possible Risk of Acute Hemolytic Anemia (British National Formulary[100])	Additional Possible Association (Other Sources)
Antimalarials	Dapsone-containing combinations Primaquine	Dapsone-containing combinations Pamaquine Primaquine	Chloroquine Quinidine Quinine	—
Other drugs	Methylthioninium chloride[a] Nitrofurantoin Phenazopyridine[b] Rasburicase Tolonium chloride[c]	Ciprofloxacin Methylthioninium chloride[a] Moxifloxacin Nalidixic acid Niridazole Nitrofurantoin[d] Norfloxacin Ofloxacin Rasburicase Sulfamethoxazole/cotrimoxazole	Aspirin[e] Menadiol sodium phosphate Sulfadiazine Sulfasalazine Sulfonylureas	Chloramphenicol[94] Dimercaptosuccinic acid[95,96] Glibenclamide[f],[95] mepacrine[94] Vitamin K analogs[94]

[a] Methylene blue.
[b] Pyridium.
[c] Toluidine blue.
[d] Furadantin.
[e] Acetylsalicylic acid.
[f] Glyburide.
Data from Refs.[74,94–96]

were appropriately treated; it is not known whether there was mortality in the field until 2008, when Lapdap was withdrawn by the manufacturers because of these complications.[69] A thorough post-trial analysis found that AHA developed in all the G6PD-deficient children (**Fig. 5**), including many of the heterozygous girls.[70] It took this epidemic of serious adverse events to persuade the national and international regulatory bodies that G6PD testing is mandatory when a potentially hemolytic drug is administered (discussed later).

- In 2007, rasburicase (the enzyme uricase) was introduced as a potent uricolytic agent, indicated to prevent severe hyperuricemia, particularly as part of the tumor lysis syndrome.[71] Subsequently rasburicase was also used in neonates who had evidence of kidney injury.[72] Unlike with other drugs, for which the biochemical mechanism whereby they produce oxidative damage is incompletely understood, rasburicase is known to produce 1 mol of H_2O_2 for each mole of uric acid catabolized; this produces oxidative damage directly (see **Fig. 1**). A G6PD-deficient newborn may die from rasburicase,[64,73] and it is imperative that a G6PD test is carried out before this drug is administered, especially to a child.

- Infections make it extremely important to be aware of which drugs are potentially capable of causing hemolytic anemia before administration to patients. In the literature, however, there is considerable confusion concerning this topic. One of the main reasons is that infection itself can be a triggering factor for hemolysis; therefore, it is highly probable that many compounds have been considered dangerous because they were administered to patients in whom hemolytic anemia had been triggered by preexisting infection.[74] It has been also observed that after severe trauma, G6PD-deficient persons are at higher risk of sepsis.[75]
- The epidemiology of favism must correspond to the intersection of the prevalence of G6PD deficiency and of the use of fava beans as a foodstuff (**Box 1**). Fava beans are not grown in tropical Africa, but they are popular in the

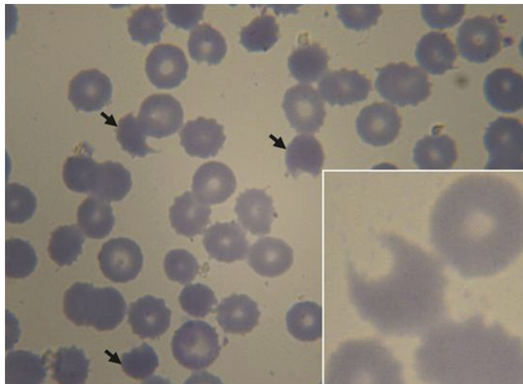

Fig. 5. Blood smear from a 3-year-old boy who was treated for acute malaria with a combination of dapsone and chlorproguanil (Lapdap) and who was G6PD deficient. Three days after starting treatment is the picture of AHA due to severe oxidative damage: (Giemsa staining, original magnification ×63) contracted erythrocytes, spherocytes, and hemighosts (also called bite cells [*arrows*]). (*Inset*) A hemighost at a higher magnification (original magnification ×100). The missing part of the erythrocyte is the negative image of a Heinz body. (*From* Pamba A, Richardson ND, Carter N, et al. Clinical spectrum and severity of hemolytic anemia in glucose 6-phosphate dehydrogenase-deficient children receiving dapsone. Blood 2012;120(20):4129; with permission.)

Box 1
Countries with documented cases of favism

Algeria, Australia, Bulgaria, Chile, China, Croatia, Cyprus, Egypt, France, Germany, Greece, India, Iran, Iraq, Israel, Italy, Japan, Jordan, Lebanon, Morocco, Poland, Portugal, Republic of Macedonia, Romania, Russia, Saudi Arabia, Spain, Taiwan, Thailand, The Netherlands, Tunisia, Turkey, UK, USA.

Mediterranean, in the Middle East as far as Iran, and in Southeast Asia.[76] In a recent report from Gaza, of 80 G6PD-deficient children admitted with AHA, 65 had consumed fava beans.[77] Although no quantitative data are available, on a global basis favism is almost certainly still today the most common form of AHA associated with G6PD deficiency.

MANAGEMENT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

In someone who is known to be G6PD deficient, hemolytic anemia from fava beans or from drugs should not take place, because exposure can be avoided; however, acute infection can trigger an attack. In any known G6PD-deficient person with an acute illness and a fall in hemoglobin, it is not difficult to recognize AHA, and the telltale sign of intravascular hemolysis, namely hemoglobinuria, should be sought not only by asking the patient but also by inspecting the urine. If the G6PD status is not previously known, but a patient spontaneously reports eating fava beans and comes from an area or from a population where G6PD deficiency is common, the index of suspicion for favism ought to be high. Whenever such circumstances do not occur, a diagnosis can be made in most cases with near certainty by an inexpensive approach rarely carried out nowadays—examination of a blood film (see **Fig. 5**)—that often reveals evidence, ranging from suspicious to spectacular, of oxidative hemolysis. A test helpful by virtue of being negative is the direct antiglobulin (Coombs) test.

In terms of treatment, the first priority is to remove the offending agent, if any, and to control infection, if any. Next, in adults, the most important measure is to provide fluids to prevent hemodynamic shock that entails the threat of acute renal failure. In children, more often than in adults, blood transfusion may be indicated; in a child with favism, it may be life-saving.[15] Fortunately, once AHA is overcome, full recovery without sequelae is the rule rather than the exception.

For the rare cases of CNSHA that are associated with G6PD deficiency, the management is different from that discussed previously; it is more similar to that of CNSHA related to other causes (eg, hereditary spherocytosis and pyruvate kinase deficiency). This specialized topic is discussed in other reviews.[15,78]

ANIMAL MODELS AND DRUG SCREENING

Knocking out *G6PD* in mouse embryonic stem cells revealed that the cells were viable, but transfer into blastocysts has proved that a *G6PD*-null mutation is lethal in embryonic life[24]; this explains why *G6PD*-null mutations are never found in humans and also proves conclusively that the irreplaceable function of G6PD is not pentose synthesis but defense against oxidative stress.[79,80] Recently, it has been found in *Caenorhabditis elegans* (**Table 5**) that G6PD RNA interference knockdown was associated with enhanced germ cell apoptosis and oxidative damage to DNA.[81]

Apart from helping to understand physiology, a good animal model would be useful if it predicts whether a new drug will cause AHA in G6PD-deficient persons. In the past, studies performed in vitro on red blood cells from G6PD-deficient donors were

Table 5
Animal models of glucose-6-phosphate dehydrogenase deficiency

Model Type	Methodology	Residual Glucose-6-Phosphate Dehydrogenase Activity (%)	Chemicals Tested	Effects
C elegans[81]	Knockdown of *G6PD* by RNA interference	11	H_2O_2	Enhanced germ-cell death; decreased egg production
Mouse[84]	Genetic cross of Gpdx[a-m1Neu] mice with C57L/J mice	5–10	Naphthalene[a]	AHA[b]
Mouse[85]	G6PD-deficient mice (y/-) on a C3H background	10–20	Primaquine, pamaquine Chloroquine[c]	AHA[b] No AHA
Mouse[86]	NOD/SCID mice, intraperitoneal injection daily for 14 d with human G6PD-deficient red blood cells	5–20[d]	Primaquine, pamaquine[e] Chloroquine[c]	AHA[b] No AHA
Zebrafish[87]	Morpholino targeting of *G6PD*	Visibly reduced	α-Naphthol, primaquine	Significant hemolysis and cardiac edema

[a] The metabolite of naphthalene, α-naphtol, was shown in vitro to cause reduction of GSH in G6PD-deficient mouse red cells.
[b] In general, the doses of drugs causing significant effects have been much higher than those that cause hemolysis in G6PD-deficient humans.
[c] Similar results were obtained with mefloquine, doxycycline, and pyrimethamine.
[d] G6PD activity of human red cells; the G6PD activity of coexisting mouse red cells is normal.
[e] Similar results were obtained with sitamaquine, tafenoquine and dapsone.
Data from Refs.[81,84–87]

disappointing in this respect, partly because such studies poorly mimic hemolysis in vivo and partly because oxidative damage may be caused by metabolites rather than by the drug itself.[82,83]

Recently, Ko and colleagues[84] produced a mouse model (see **Table 5**) with residual G6PD activity comparable to that found in human class II G6PD-deficient subjects. Oxidized glutathione increased and GSH decreased in mouse red cells treated with the pro-oxidative agent α-naphthol, whereas its precursor naphthalene produced dose-dependent AHA in vivo. In a similar model, with characteristics comparable to those of class III G6PD-deficient subjects, Zhang and colleagues[85] obtained dose-dependent AHA through oral administration of primaquine and pamaquine, known to be hemolytic in G6PD-deficient humans, but not with the nonhemolytic drugs chloroquine and mefloquine.

Also, recently, a humanized mouse model of G6PD deficiency has been produced by Rochford and colleagues[86] by injecting human red blood cells from G6PD-deficient donors (G6PD A− or G6PD Med) into nonobese diabetic (NOD)/severe combined immunodeficiency (SCID) mice. When treated with primaquine and pamaquine, these mice hemolyzed their human red cells and developed dose-dependent AHA with (endogenous) reticulocytosis. Moreover, the spleen size increased significantly. On the contrary, nonhemolytic drugs produced no hemolysis.

Recently, Patrinostro and colleagues[87] developed a model of G6PD deficiency in the zebrafish (*Danio rerio*). G6PD expression was knocked down with morpholinos. When exposed to different pro-oxidant compounds, *g6pd* morphants had significant hemolysis and cardiac edema.

It seems reasonable to expect that 1 or more of these models will become validated for preclinical testing of new drugs before they are administered to G6PD-deficient humans.

TESTING FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND MALARIA CONTROL

Even though the term, primaquine sensitivity syndrome (discussed previously), has been appropriately supplanted by the term, G6PD deficiency, the fact remains that primaquine is probably the drug that has caused the largest number of cases of AHA in persons who were G6PD deficient.[88] The well-established indications for primaquine in malaria control are 2 (**Table 6**).[1] With *P vivax*, any of several drugs can successfully terminate an acute attack, but primaquine is the only drug that eradicates the *P vivax* hypnozoites hiding in the liver and thus prevents endogenous recurrence. For this purpose, the recommended dose of primaquine (for an adult) has been 45 mg/d for 14 days.[2] With *P falciparum*, after an acute attack is successfully treated with appropriate medication (currently an artemisinin-containing combination), the only drug that eliminates gametocytes is primaquine; the recommended (adult) dose was 45 mg just once, but recently this has been reduced to 15 mg.[89]

These recommendations have been widely ignored for different reasons. With respect to *P falciparum* malaria, every episode is a threat to life, and the urgency to terminate a clinical attack has understandably obfuscated the public health concern about gametocytes, which are clinically irrelevant for a patient but, through a mosquito, become a threat to the next person bitten by the same mosquito. In addition, in hyperendemic areas there is a huge amount of malaria transmission by people

Table 6
The resurgence of primaquine and the advent of tafenoquine

Drug	Parasite Type	Recommended Use	Standard Adult Dose	G6PD Testing
Primaquine	*P falciparum*	After treatment of the acute disease, to eliminate gametocytes responsible for malaria transmission	Single dose of 15 mg[a]	Not necessary
	P vivax	After treatment of the acute disease or to eliminate liver hypnozoites responsible for relapse	30 mg/d for 14 d	Necessary
Tafenoquine[b]	*P vivax*	Alternative to the use of primaquine (see above)	300–600 mg once only[c]	Necessary

[a] In the past, the recommended dose was 45 mg, but recently this has been lowered to 15 mg.[89]
[b] Not a licensed drug.
[c] Doses tested in 3 clinical trials conducted in G6PD-normal subjects.[90,97,98] In a recent trial in women heterozygous for the G6PD Mahidol variant who had 40% to 60% G6PD-deficient red cells, 100 to 200 mg of tafenoquine caused clinically significant but not severe hemolytic anemia.[99]
Data from Refs.[89,90,97–99]

with asymptomatic malaria; hence, elimination of gametocytes from the minority of patients with clinical malaria has little impact on transmission. With respect to *P vivax*, this infection is endemic in areas where G6PD deficiency is common; with the G6PD status of individual patients unknown, and with the need of a prolonged course of administration, there has been justified concern about causing AHA in those who are G6PD deficient.[88] It might also have been regarded as reasonable to hope that, over decades, an alternative to primaquine would turn up. The only alternative today is tafenoquine[90] (not yet an approved drug) (see **Table 6**), but this too is an 8-aminoquinoline and, although its pharmacokinetics is different from primaquine (it last for weeks in circulation), it causes AHA in G6PD-deficient persons just as primaquine does.

In recent years there have been 2 positive developments. First, more countries are moving toward malaria elimination[91]; therefore, the public health importance of reducing relapse (*P vivax*) and reducing transmission (*P falciparum*) has increased. Second, in spite of screening tests for G6PD deficiency available for half a century, it has been thought by many malariologists and public health professionals that they were too cumbersome or not sufficiently reliable and that, therefore, it was not realistic to adopt their use in endemic areas before giving primaquine. Recently it has become accepted that testing is necessary, and, therefore, there has been a revival of interest by WHO, by public health authorities, and by the diagnostics industry in point- of-care tests for G6PD deficiency. At least 2 kits are on the market[92,93]; they are being extensively validated in the field and, although more expensive than older screening tests, they may come down to less than $1 per test. Having a strip not unlike a pregnancy test that comes in a kit with primaquine tablets can be looked forward to in a not too distant future.

The WHO has worked out recommendations, whereby, for preventing relapse of *P vivax*, primaquine is given whenever indicated to those who have tested G6PD normal, whereas it is not given, or given only under medical/health worker surveillance, to those who have tested G6PD deficient (see http:www.who.int/malaria/mpac/mpac_sep13_erg_g6pd_testing.pdf). For the clearance of *P falciparum* gametocytes, a single adult dose of 25 mg[89] is probably safe for all. The authors expect that implementation of these measures will be straightforward with respect to males; in females, again, the fact that most heterozygotes have intermediate enzyme levels cannot be circumvented. Those who test deficient or doubtful will have to be managed like those who test G6PD deficient; those who test normal may still be heterozygotes, but in their blood the proportion of G6PD-deficient red cells is sufficiently small to make it unlikely that they will develop clinically significant AHA.

CONCLUSION

G6PD is at the crossroads of haematology, pharmacogenetics and malariology. Indeed, one can perceive a remarkable triangular relationship: (1) malarial *Plasmodia* select for (2) G6PD deficient human mutants, (3) primaquine is a potent anti-malarial, but it is dangerous for those G6PD deficient mutants that malaria has selected for. From this triangle we have learnt several of lessons in evolutionary biology and in medicine, but not yet enough: for instance, if we understood fully how G6PD deficiency protects from malaria we might be able to mimic the mechanism in order to protect other people as well. In the meantime, we now do have the means to protect G6PD deficient persons from exposure to fava beans or to iatrogenic risks, and in the interest of global health we have a duty to do so.

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