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PharmGKB summary: very important pharmacogene information for G6PD

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Introduction

Glucose-6-phosphate dehydrogenase (*G6PD*) was one of the first genes found to be associated with variable drug response. It is also very polymorphic, with G6PD deficiency found in more than 300 million people worldwide [1]. Here, we provide an overview of *G6PD* as a very important pharmacogene, and detail genetic variants and haplotypes associated with drug response (Although most G6PD variants are caused by single nucleotide polymorphisms (SNPs) in the coding region of the G6PD gene at the X chromosome, due to the heterogeneity of alleles causing G6PD deficiency; here, we use the term ‘haplotype’ to define the set of linked SNPs in a G6PD variant that are inherited together and that may or may not produce G6PD deficiency). A more in-depth report, with interactive links to individual literature annotations, can be found at <http://www.pharmgkb.org/gene/PA28469>.

Molecular structure and function

The G6PD enzyme is conserved throughout evolution, with human G6PD sharing approximately 93% amino acid identity with rat and 37% with *Escherichia coli* [2,3]. G6PD is encoded by a gene on the X chromosome (Xq28) [4-6], contrary to an early report describing the G6PD enzyme as a fusion protein encoded by genes on chromosomes 6 and X [7]. The *G6PD* gene is around 18 kb in length and consists of 13 exons and 12 introns, and was originally cloned in 1986 [8-10].

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Conflicts of interest

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The promoter region of the *G6PD* gene has some sequence homology with other housekeeping genes, and contains elements for tissue-specific expression that regulate transcription in response to oxidative stress, hormones, nutrients, and growth factors [3,10]. Alternative transcriptional start sites and mRNA splice variants have been described [8,10-13]. The G6PD mature peptide of 514 amino acids in length (59 kDa) is active as a dimer or tetramer, and one molecule of NADP⁺ is bound per protein subunit [13-17]. The binding of NADP⁺ is thought to be integral to the enzyme's stability, and thus, its function, as point mutations close to the NADP⁺ and dimer interface result in severe G6PD deficiency, revealed by the crystal structure of the Canton variant [14] and site-directed mutagenesis studies [18].

G6PD is a cytoplasmic protein and has two main roles within the cell: the production of NADPH and ribose-5-phosphate (reviewed in [19,20]). Both are synthesized by steps within the pentose phosphate pathway (PPP), also known as the hexose monophosphate shunt (for example [21]); reviewed in [19,22]). NADPH is essential to maintain the redox state of the cell and relieves oxidative stress through the reduction of glutathione, which in turn reduces hydrogen peroxide and oxidative free radicals (reviewed in [3,19,20,22,23]). Ribose-5-phosphate is required for glycolysis and for DNA and RNA biosynthesis (reviewed in [3,19,20,22,23]). Alternative pathways can be utilized for the biosynthesis of nucleic acids, but G6PD is essential for a cell's ability to cope with oxidative stress [24]. Tumor suppressor protein p53 has been shown to regulate the PPP by binding to G6PD, preventing dimer formation and thus NADP⁺ binding, inhibiting NADPH production [25]. Several p53 mutants associated with tumors were shown to lack this inhibitory property, and therefore dysregulation of G6PD in cancer cells may result in increased cell growth through unregulated glucose biosynthesis and the production of NADPH [20,25].

G6PD is expressed in all cells, but its role is particularly important in red blood cells (RBCs), which do not have mitochondria and are therefore dependent on G6PD as the only source of NADPH to relieve oxidative stress and protect the hemoglobin β chain from oxidation (reviewed in [19,22,23]). In addition, enzyme levels decrease during the RBC lifespan [23]. When the required levels of NADPH cannot be maintained, the amount of reduced glutathione decreases, resulting in oxidative damage, which can ultimately lead to lysis of RBCs (reviewed in [1,19,22]). Under normal conditions, G6PD activity in RBCs is only around 2% of its capacity, inhibited through a negative feedback loop with NADPH (reviewed in [23,26]). However, under oxidative pressure, oxidation of NADPH releases the inhibitory effect, and G6PD enzyme activity increases, enabling enhanced reducing activity to deal with the additional stress (reviewed in [23,26]). In G6PD-deficient RBCs, where enzyme activity can be below 10% of the normal value, homeostasis can be maintained and most G6PD-deficient individuals remain asymptomatic [23]. However, the deficiency becomes apparent under oxidative stress conditions when an increased demand in NADP⁺/NADPH turnover cannot be met (reviewed by the WHO Working Group [23]).

G6PD as an important pharmacogene

We have known for more than 2000 years that the ingestion of fava beans can have adverse consequences in some individuals, and could indeed be why Pythagoras imposed abstinence from beans among his followers [27,28]. However, it was not until the 20th century that a deficiency in the G6PD enzyme was discovered to be the underlying cause of 'Favism', and the connection that agents other than fava beans can cause similar adverse events in G6PD-deficient individuals (discussed in Cappellini and colleagues [22,29]). In the 1950s, it was observed that a subset of African-American soldiers were more likely to develop an adverse reaction to the antimalarial drug primaquine compared with their Caucasian counterparts

[30,31]. This susceptibility to primaquine-induced intravascular hemolysis led to the discovery of a deficiency in G6PD enzyme activity in RBCs [32].

More than 400 variations of the G6PD enzyme have now been described on the basis of clinical manifestations and biochemical properties, and G6PD deficiency is the most prevalent enzyme deficiency in the world (reviewed in [19,22,33-35]), affecting an estimated 4.9% of the world's population (more than 300 million people) [1]. Polymorphic variants in *G6PD* are those of significant frequencies (1–70%) in specific human populations: these fall into World Health Organization (WHO) classes II and III (see Table 1) [19,23,34,46]. Different polymorphic variants have arisen in different geographical areas; for example, the Canton variant is predominantly found in Chinese and South-East Asian populations [35,40,47,48], reviewed in [19]. Higher population densities of humans resulting from the development of agriculture may have facilitated malaria endemics, and introduced a selective pressure for the spread of G6PD variants (reviewed in [19]). Nevertheless, G6PD-deficient variants can also occur sporadically due to *de-novo* mutations (reviewed in [19]). Most of the genetic variants tend to be single-point mutations, and the lack of large or out-of-frame deletions may indicate that the total absence of enzyme activity is fatal [19,23,33,34,38]. In-frame deletions are usually associated with the most severe clinical manifestations (class I; reviewed in [19,33,34]), such as the novel G6PD Tondela variant, a six-amino acid deletion identified in a heterozygous woman with chronic hemolytic anemia (see Table 1) [36].

The mechanism underlying the G6PD-deficient phenotype may vary depending on the location of the mutation in the enzyme's three-dimensional structure, and include alterations to protein assembly, dimer formation and stability, interaction with substrates, and protein turnover (reviewed in [49]). Studies suggest that most *G6PD* variants result in G6PD enzyme instability (reviewed in [19,46]), and variants that cause the most severe deficiency (resulting in congenital nonspherocytic hemolytic anemia [CNSHA]) are found predominantly at or near the dimer interface of the G6PD protein in exon 10 [50], although not exclusively. Different gene variants can confer similar effects on enzyme function and clinical manifestations, and conversely, the same genetic variation can result in different molecular and clinical phenotypes [33,35,51]. Therefore, G6PD variants based on enzyme biochemistry may have been characterized differently and yet have the same underlying genetic mutation [41,52,53]. The term 'haplotype' is used in this review to define a set of linked alleles in *G6PD* that are inherited together. The B haplotype is considered the 'wild-type' precursor sequence, as alignment of human and chimpanzee DNA sequences have shown [41]. Variants that differ from the B wild type are often named with the region where the population in which the variant was found [35,54]. Although attempts have been made to standardize nomenclature since 1967 [54], there are still instances where haplotype names have been used to refer to more than one set of variations (see text on haplotype A-). It should be noted that many studies do not screen the whole *G6PD* gene, and therefore some genetic variants may be unreported and may be a factor underlying differences in biochemical properties.

Exogenous agents can trigger hemolytic anemia in G6PD-deficient individuals by inducing oxidative stress in RBCs (reviewed in [19,22,55]). These include certain food items, therapeutic drugs, infections, and exposure to chemicals (e.g. hair dye containing naphthol) [19,22,37,55]. G6PD variants have been classified into five WHO categories according to the severity of clinical manifestation resulting from the genotype (see Table 1), with classes II and III being the most common type of polymorphic G6PD-deficient variant [23,33]. Due to lower RBC G6PD activity, patients carrying class I sporadic variants (associated with CNSHA) are highly susceptible to hemolytic anemia caused by the same drugs that can induce adverse reactions in carriers of polymorphic G6PD variants (reviewed in [19]).

Testing for G6PD deficiency

G6PD variants that result in enzyme deficiency confer a G6PD-deficient phenotype in hemizygous men (with one copy of the *G6PD* gene) and homozygous women (for example [56,57]). To diagnose a phenotype of G6PD deficiency in heterozygous women is more difficult, as the extent of enzyme deficiency activity can vary considerably within heterozygous individuals, due to X-linked mosaicism [34,57,58]. This pattern of gene inactivation is random; therefore, female heterozygotes will have G6PD-deficient RBCs combined with those expressing normal G6PD activity, and the population sizes of these cells can vary from 50 : 50 to minimal levels or a majority of G6PD-deficient cells [23,34,58]. Genotyping is therefore essential to establish heterozygosity in women; however, this can make prediction of drug response difficult without phenotypic information of G6PD enzyme activity levels. For example, 75% of women genetically heterozygous for the Mediterranean variant had normal G6PD activity, whereas 25% were enzyme deficient, as assessed by a colorimetric test [56]. Testing for both genotype and enzyme function is the ideal method; however, due to various factors, including the impracticalities and costs of genotyping in the field, many studies and clinics have solely tested G6PD enzyme activity, making the association of causative variants difficult. In a meta-analysis of 280 studies, less than 8% used DNA analysis to assess G6PD deficiency [1].

G6PD and therapeutic drug response

The WHO recommends testing of drugs to predict the risk of hemolysis in G6PD-deficient individuals if the drugs are to be prescribed in areas of high prevalence of G6PD deficiency [23]. As a consequence of adverse reactions in individuals with G6PD deficiency, the Food and Drug Administration (FDA) has introduced warnings or precautions on the drug labeling of primaquine, chloroquine, dapsone, rasburicase, avandaryl tablets (glimepiride + rosiglitazone maleate), and glucovance tablets (metformin + glibenclamide) [59]. These highlight the possible risk of hemolytic anemia in G6PD-deficient individuals upon drug intake [59]. Numerous factors can contribute to drug-induced hemolytic anemia in G6PD-deficient individuals, including high dosage, other drugs taken in combination, concurrent infections, and other genetic variants, as discussed in Cappellini and colleagues [22,61,62]. Therefore, it may be that drugs (including aspirin, vitamin C, and chloroquine) reported to cause hemolysis can actually be taken safely by G6PD-deficient individuals. These drugs, however, should be administered with caution, especially in combination with other drugs or at high doses, potentially with monitoring of RBC or hemoglobin levels [61,62]. Readdressing the safety of drugs in these individuals could make effective therapeutics available (as discussed in Youngster *et al.* [62]). More information and comprehensive advice on unsafe drugs in G6PD-deficient individuals can be found at www.favism.org [63]. Several drug subsets and the possible consequences of drug intake for G6PD-deficient individuals are outlined below.

G6PD deficiency and antimalarial drugs

It is hypothesized that variants associated with G6PD deficiency have remained prevalent in the human population due to positive selective pressures, in particular resistance to uncomplicated and severe malaria (reviewed in [19,22]). At the same time, G6PD deficiency confers susceptibility to hemolytic anemia triggered by some antimalarial drug treatments [1]. The prevalence of G6PD deficiency in malaria endemic regions where antimalarial drugs are required is an important public health issue [1,64]. Hemolysis is also a phenotype induced by malaria infection; therefore, distinguishing a disease from a drug-induced effect may be difficult (as discussed in Allouche and colleagues [65,66]). In areas where malaria is endemic, the financial and practical costs of G6PD screening are important considerations

[22,56]. However, the wide-scale drug-based eradication of malaria is likely to require G6PD deficiency testing [64], and thus the application of pharmacogenomics in the treatment of G6PD-deficient individuals.

Race-specific differences in sensitivity to hemolytic anemia after treatment with aminoquinolines have been reported since the 1920s (reviewed in [29]). In 1956, a deficiency in the G6PD enzyme was determined as the underlying cause of 'primaquine sensitivity' [30-32]. Metabolism of primaquine mediated by CYP proteins is thought to be one of the mechanisms behind the drug sensitivity, as the resulting metabolites induce the formation of methemoglobin and reactive oxygen intermediates [67]. Alternatives to primaquine have been sought for use in areas where G6PD deficiency is prevalent. However, unfortunately, numerous antimalarial drugs can also induce hemolysis in G6PD-deficient individuals, including dapsone which has FDA drug labeling precautions [59,66,68,69] (reviewed in [62]). The WHO released guidelines after a technical consultation in 2004 for the use of Lapdap (a combination of chlorproguanil and dapsone) due to safety concerns in G6PD-deficient individuals, recommending use only if malaria infection is confirmed, testing for G6PD deficiency and avoiding drug treatment in these individuals [70], as discussed in Luzzatto *et al.* [71].

FDA labeling of chloroquine advises that caution should be taken when administering this drug to G6PD-deficient individuals [59], although it is not contra-indicated. Treatment of normal RBCs with chloroquine *in vitro* does not boost the PPP, and does not reduce the survival of G6PD-deficient RBCs transferred into wild-type recipients [72,73]. However, a high dose of chloroquine (600 mg) for the prophylaxis of malaria was reported to induce severe hemolytic anemia in 50 soldiers, all G6PD deficient [74]. G6PD deficiency may also increase the risk of side effects such as pruritus induced by chloroquine [75]. Chloroquine treatment combined with primaquine for *Plasmodium vivax* infection has been associated with significantly decreased hemocrit levels [76], although the development of hemolysis in G6PD-deficient individuals has been affiliated with primaquine administration rather than chloroquine [77]. Severe hemolysis has been reported in several children treated with combinations of chloroquine, chloramphenicol, aspirin, and primaquine [78]. In contrast, chloroquine treatment combined with methylene blue or elubaquine does not induce severe adverse effects in individuals with G6PD deficiency [76,79,80]. To conclude, the safety of chloroquine in G6PD-deficient individuals is unclear, and seems to depend on numerous factors including dosage, concurrent drugs and infections, as discussed in Beutler and colleagues [61,62].

When comparing G6PD-deficient and G6PD 'normal' children with uncomplicated malaria infection in a trial of artesunate and amodiaquine or artemether-lumefantrine (also known as artemisinin-based combination therapy ACT), no significant differences in adverse events were observed, indicating that these drug combinations are a promising alternative (see PharmGKB Artemisinin and Derivatives Pharmacokinetics Pathway <http://www.pharmgkb.org/pathway/PA165378192> and PharmGKB Amodiaquine Pharmacokinetics Pathway <http://www.pharmgkb.org/pathway/PA165815256>) [81].

G6PD deficiency and cancer therapeutics

Several agents involved in the treatment of cancer patients have the potential to result in severe adverse side effects in G6PD-deficient individuals, due to induction of oxidative stress in RBCs. Carmustine [3-bis(chloroethyl)-1-nitrosourea (BCNU)] treatment results in a deficiency in glutathione reductase in erythrocytes, platelets, and leukocytes [21,82]. This lowers the levels of reduced glutathione and leads to insufficient removal of hydrogen peroxide and susceptibility to oxidative hemolysis, particularly in RBCs that are G6PD

deficient [21,82]. Doxorubicin (adriamycin) stimulates the PPP, but to a lower extent in G6PD-deficient RBCs, and results in oxidative stress through the accumulation of hydrogen peroxide due to an inability to increase G6PD activity [see the PharmGKB Doxorubicin Pathway (Cancer Cell), Pharmacodynamics <http://www.pharmgkb.org/pathway/PA165292163>] [21,83]. This response can also be mirrored in 'normal' RBCs when cells are pretreated with carmustine before doxorubicin treatment, resulting in diminished glutathione stability and enhanced susceptibility to oxidative stress [21]. A number of cases of methemoglobinemia and acute hemolysis after treatment with rasburicase in G6PD-deficient individuals have been described [84-86]. The FDA labeling has contraindicated rasburicase for G6PD-deficient individuals [59,60].

The anti-leukemia drug daunorubicin is metabolized into the less potent form daunorubicol, a process dependent on NADPH via G6PD [87]. This biotransformation was considerably reduced in RBCs from A- or Mediterranean G6PD-deficient individuals, and thus may have implications in the clinic, raising the issue of whether or not the drug is more effective in these individuals due to prolonged exposure to the more active form and possible toxicity concerns [87]. NADPH inhibitors, including primaquine aldehyde, were also shown to reduce daunorubicin metabolism and daunorubicol appearance [87].

G6PD deficiency and Aspirin

A child carrying the Mediterranean variant who was diagnosed with systemic arthritis and prescribed a daily dose of 100 mg/kg of aspirin subsequently developed severe hemolytic anemia, with no sign of viral or bacterial infection [88]. Hemolysis was reported in a male administered 1.5 g of aspirin for a fever (likely a viral infection), who was later found to be G6PD deficient and had a family history of Jaundice [89]. However, in 22 healthy G6PD-deficient individuals, normal therapeutic doses of aspirin for 4 days (50 mg/kg daily) had no effect on the RBC count or the hemoglobin levels [90]. Therefore, the effect of aspirin on G6PD-deficient individuals may be dependent on the variant type and pre-existing clinical conditions (such as an infection or inflammatory disease) [91]. Dosage is also likely to contribute, as exemplified in the above studies and demonstrated by *in-vitro* studies in which higher levels of aspirin were required to observe a drop in reduced glutathione levels in the blood from sensitive patients [89].

G6PD deficiency and diabetes mellitus treatment

Glibenclamide (glyburide) has been shown to induce acute hemolysis in diabetic patients carrying the A- haplotype or the Mediterranean variant [92,93], although it should be noted that these seem to be sporadic case studies, as discussed in Youngster *et al.* [62]. FDA labeling of glucovance tablets, which contain glyburide and metformin HCl, cautions use in G6PD-deficient individuals as sulfonylurea agents can result in hemolytic anemia, and advise using a non-sulfonylurea alternative [60].

G6PD variants and haplotypes

A selection of the most studied *G6PD* variants and known haplotypes are discussed below, and their association with drug response is summarized in Table 2.

Canton (rs72554665)

The Canton variant was first identified in 1966 [96] and is encoded by a G to T nucleotide change at position 1376 (NM_001042351.1:c), resulting in the amino acid change Arg 459 Leu (NP_001035810.1:p.) [35,39]. The same genetic variant is also known as Taiwan-Hakka, Gifu-like, and Agrigento-like [34,35], all G6PD enzyme variants that have been

reported to display differences in thermostability, enzyme activity, and substrate utilization but share the same nucleotide change 1376T [40]. G6PD Canton is commonly found in China, with one study reporting incidence of the variant in 2% of men and 0.9% of women, and is also found in South-East Asia [35,40,47,48]. G6PD Canton is a category II variant, associated with hemolytic anemia and Favism, resulting in less than 10% enzyme activity and reduced thermostability (see Table 1) [33,40,97,98]. This variant was used to determine the first crystal structure of G6PD [14].

A (rs1050829)

The A variant is an A > G change at nucleotide position 376, resulting in an Asn to Asp missense mutation at amino acid 126 [35,38,43,99]. The A variant results in normal thermostability and enzyme activity levels, between 60 and 150%, and is a class IV phenotype according to the WHO classification, not associated with acute hemolytic anemia (see Table 1) [23,38,57]. The polymorphic A variant is found at frequencies between 0.1 and 0.34 in Sub-Saharan African populations (as reviewed in [100]) and is part of the A-haplotype.

A- haplotype

The A- haplotype is a combination of two SNPs, both of which confer missense mutations in the protein sequence [12,38,41]. This is complicated by the fact that what was previously defined as the A- G6PD enzyme form by biochemical properties is encoded by three possible different haplotypes (a set of linked SNP alleles that are inherited together) [35,41]. Each is made up of two variants: the A variant coupled with a second SNP to yield the A- haplotype [35,41]. Here, to avoid confusion, we have distinguished the three different haplotypes according to the second variant nucleotide position:

1. A^{-202A/376G}: is a G > A nucleotide change at position 202 (rs1050828) resulting in the missense mutation Val to Met at amino acid 68, combined with the A variant.
2. A^{-680T/376G}: is a G > T nucleotide change at position 680 (rs137852328) resulting in the missense mutation Arg to Leu at amino acid 227, combined with the A variant.
3. A^{-968C/376G}: is a T > C nucleotide change at position 968 (rs76723693) resulting in the missense mutation Leu to Pro at amino acid 323, combined with the A variant.

Haplotype A^{-202A/376G} is found at frequencies up to 0.24 in African populations, and has also been described as Distrito Federal, Matera, Betica, Castilla, Alabama, Tepic, Ferrara, Laghouat, and Kabyle, found in populations from around the world [35,38,41,51,57,100,101]. The A^{-968C/376G} haplotype has also been named Betica, Guantanamo, and Selma [35,41].

The Santamaria haplotype (542T/376G) is an A > T nucleotide change at position 542 (rs5030872) resulting in a missense mutation Asp to Val at amino acid 181, combined with the A variant [102,103]. Although associated with G6PD deficiency, the Asp to Val does not result in a change of charge and hence the electrophoretic properties of the Santamaria protein are not the same as those defined for the G6PD A- enzyme [102,103]; therefore, this is not included here under the A-haplotype description (as discussed in Beutler *et al.* [102]). The Sierra Leone haplotype (311A/376G) is another example where the A variant is found to be combined with a second variant, resulting in G6PD deficiency [104].

The G6PD A- enzyme has a class III phenotype according to the WHO classification, conferring moderate enzyme deficiency of between 10 and 60% activity, and has been associated with hemolytic anemia [23,34,57]. Because of differences in both enzyme

kinetics and genetic sequence, it is predicted that the G6PD A- haplotype derived from the A variant, which originally derived from the B wild type [12,105,106]. The A- enzyme is more likely to be found in a tetramer form than the A or B enzyme, which may explain a faster rate of enzyme inactivation [105]. The synergistic interaction of the two mutations spatially close together in the three-dimensional structure of the A- enzyme has been shown to result in defective folding of the subunits, which decreases the stability and thus results in lower levels active enzyme within RBCs [107]. The B, A, and A- G6PD enzymes also have differences in the K_m for glucose-6-phosphate (G6P) at pH7.2 (intracellular erythrocyte conditions) [105].

Numerous associations with G6PD A- and drug response have been described, although the underlying genotype may remain unknown (see Table 2). Some studies do not mention genotyping and only refer to the A- enzyme phenotype, as determined by electrophoretic or biochemical properties. In these cases, we have used the terms A- G6PD-deficient individuals or A- enzyme. In studies which genotype for both variants of the A- haplotype, we indicate the location of the second variation if all three were not screened for. Other studies do not genotype at both loci, screening for only the 202A variant, and yet use the description A- due to the strong linkage disequilibrium that exists between this and the 376G variant in African populations [106] (see Table 2).

The metabolism of daunorubicin into the less potent form daunorubinol was considerably diminished in RBCs from A- or Mediterranean G6PD-deficient individuals [87]. Methylene blue treatment of toxin-induced methemoglobinemia resulted in hemolysis in a patient carrying the A- G6PD deficiency [94]. Large amounts of vitamin C were reported to induce hemolysis in an A- male individual [95]. A woman diagnosed with type 2 diabetes developed acute hemolysis after prescription of glibenclamide and metformin, and was subsequently genotyped homozygous for the A-^{202A/376G} haplotype [93]. Glibenclamide treatment was therefore ended, and hemoglobin levels rose on metformin treatment alone [93]. Combined chlorproguanil-dapsone-artesunate (CDA) treatment is unsafe in carriers of the A- haplotype, resulting in severe reductions in the hemoglobin level, and an increased risk of requiring a blood transfusion [68].

Studies revealing strong linkage disequilibrium between the SNPs 202A and 376G, and haplotype analysis with other polymorphic sites, provide evidence that the G6PD A- variant occurred more recently in evolution, originating from the A variant and expanding in African populations due to selection [12,100,105,106,108]. The pharmacogenetic studies described below only genotype for the 202A variant, but report individuals as carriers of the G6PD A- enzyme (also see Table 2). This is due to the evidence which supports the assumption that in African populations those with the 202A variant also carry the A variant (376G), and thus are defined as having the A- haplotype. A study of artemisinin-based combination therapy (ACT) combined with primaquine revealed that carriers of the *G6PD* A- haplotype are more likely to have a significant reduction in hemoglobin and are at a higher risk of developing moderate anemia compared with those with the wild-type genotype, although a significant reduction in hemoglobin was found in all individuals taking the combination therapy compared to placebo control [66]. Chlorproguanil-dapsone (CD) combination therapy is more efficacious than sulfadoxine-pyrimethamine (SP) treatment, resulting in fewer new malaria infections; however, G6PD-deficient individuals are at a higher risk of a drop in hemoglobin levels when taking the CD combination than SP [65]. Combined chlorproguanil-dapsone-artesunate (CDA) treatment is unsafe in A- G6PD-deficient individuals, resulting in severe decreases in hemoglobin levels and an increased risk of a blood transfusion compared to wild type [69]. A- G6PD-deficient individuals are also more likely to require a blood transfusion when treated with antimalarial SP coadministered with amodiaquine [69].

Although A- G6PD deficiency does not confer full protection from *Plasmodium falciparum* (*P. falciparum*) malaria infection, several studies have shown a protective effect of A- G6PD from severe malaria in hemizygous men [109,110]. Contradictory results exist for females carrying A- G6PD, with some studies finding protection from severe and mild malaria in heterozygous females and lower levels of *P. falciparum* parasitaemia [110,111], and yet other studies find no protection from severe malaria [109]. No significant association with protection from severe malaria was found in carriers of the A-^{202A/376G} haplotype; however, when pooled together with data from carriers of A-^{968C/376G} and the Santamaria haplotype, a significant association with reduced risk of severe *P. falciparum* malaria in both men and women was observed [112]. Therefore, the methodology and the extent of alleles examined likely contribute to these mixed reports [112,113]. Increased carbonylation of numerous host membrane proteins involved in oxidative stress and transport of parasite proteins is seen in malaria-infected A- deficient erythrocytes compared with RBCs from G6PD B individuals, and may underlie the mechanism that is thought to confer protection [114].

Asahi (rs1050828)

The Asahi variant is a G > A nucleotide change at position 202, which confers a missense mutation Val to Met in the G6PD protein sequence at position 68 [35,42]. This was originally identified in a Japanese boy admitted to the Asahi hospital with jaundice and anemia, who had been previously diagnosed with neonatal jaundice [42]. Because of its association with acute hemolysis, the Asahi variant is classified as a class III phenotype (see Table 1) [42]. Sequencing of the whole *G6PD* gene in this child also revealed a single base pair deletion in intron 5 [42], which could also contribute to the risk of hemolysis. Asahi has also been reported to occur in Southern China (a single female in the cohort) [47]. Asahi is thought to be a rare variant, occurring independently from the A- haplotype common in Africa, as the A variant and other polymorphic sites found to be in linkage disequilibrium with A-^{202A/376G} were not found in this individual [42,106].

Mediterranean (rs5030868)

The Mediterranean variant is a C > T change at position 563 in exon 6, resulting in an amino acid change Ser to Phe at position 188 [38]. This confers less than 10% residual enzyme activity and is associated with acute hemolytic anemia, and therefore has a class II WHO classification (see Table 1). Compared with the G6PD B wild type, the Mediterranean variant has higher affinity for G6P and reduced thermostability [38]. This variant is also known as Dallas, Birmingham, Sassari, Cagliari, or Panama, on the basis of the place of discovery [35,52,101,115]. An association with protection against *Plasmodium vivax* malaria infection in carriers of the Mediterranean variant has been reported [56], and may be one of the contributing factors why the variant has been retained in areas correlating with endemic regions in human history (as discussed in Leslie *et al.* [56]).

This variant is associated with acute hemolytic anemia in response to therapeutic drugs and fava beans (reviewed in [22]). Sporadic case studies have shown glibenclamide-induced acute hemolysis [92] and severe hemolytic anemia in response to high doses of aspirin [88] in individuals with the Mediterranean variant. This variant has also been shown to have a role in pharmacokinetics of anthracyclines *in vitro*, likely due to NADPH requirements of the metabolizing enzymes (see PharmGKB Doxorubicin Pathway, Pharmacokinetics <http://www.pharmgkb.org/pathway/PA165292177>) [83]. The metabolism of daunorubicin to daunorubinol was considerably diminished in erythrocytes from A- or Mediterranean G6PD-deficient individuals potentially influencing drug efficacy and toxicity [87].

Mediterranean haplotype

In three separate studies, a second SNP was found in all individuals expressing the Mediterranean variant, conferring a C > T nucleotide change at position 1311 which results in a silent mutation of Tyr to Tyr at amino acid position 437 (rs2230037) [52,101,116]. The Mediterranean variant 563T may have arisen in an individual carrying the silent 1311T SNP (as discussed in Vives-Corrons *et al.* [116]). The G6PD^{563T/1311T} haplotype is found in populations of Mediterranean and Middle Eastern countries, but not in Asian individuals with the Mediterranean variant, suggesting the Mediterranean variant 563T arose independently in the two geographical locations [115].

Conclusion

G6PD is a historically important pharmacogene, with variants conferring G6PD enzyme deficiency found in around 4.9% of the world's population [1]. The essential role of the enzyme in preventing oxidative stress in RBCs means that numerous drugs have been reported to induce hemolytic anemia in G6PD-deficient individuals. *G6PD* variants have also been reported to influence pharmacokinetics, by a requirement for NADPH in drug metabolism. However, the wide variety of different G6PD genetic variants, enzyme phenotypes, and clinical manifestations has made it difficult to determine the precise influence of individual genetic variants on the drug response. Confounding factors also include a lack of genotyping data in many studies, and the influence of other exogenous agents such as infection. Teasing apart the genotype–phenotype–drug interaction by applying modern technologies as well as examining more extensive haplotypes could help identify possible effective therapeutics in certain populations, as well as avoid adverse reactions in others.

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Table 1Classification of *G6PD* variants

World Health Organization class	Enzyme activity	Associated phenotype	Variant example	Genotyping reference
I	Severe deficiency	Congenital nonspherocytic hemolytic anemia (CNSHA)	Tondela	[36]
			Palermo	[37]
II	< 10% severely deficient	Risk of acute hemolytic anemia	Mediterranean	[38]
			Canton	[39,40]
III	10–60% Moderate deficiency	Risk of acute hemolytic anemia	A- Haplotype	[41,42]
			Asahi	[42]
IV	60–150% Normal activity	No clinical manifestations	B (wild type)	
			A	[43]
V	> 150% Enhanced activity	–	Hektoen	[44,45]

Table based on [22,23,33,34,46] reviews.

Table 2Polymorphic *G6PD* variants and haplotypes associated with drug response

Variants genotyped	Drug or treatment	Associated response	Reference ^a
A-Haplotype			
202A/376G (rs1050828 and rs1050829)	Glibenclamide	Acute hemolysis	[93] (case study)
202A (rs1050828)	Sulfadoxine-pyrimethamine and artemisinin and primaquine	Increased risk of developing moderate anemia	[66] ^b <i>n</i> = 562 total population genotyped, 8.4% heterozygous, 3.9% homozygous/ hemizygous.
202A (rs1050828)	Chlorproguanil-dapsone (CD)	Increased risk of a drop in hemoglobin levels, compared with sulfadoxine-pyrimethamine (SP) treatment	[65] (<i>n</i> = 1480 total study group treated with CD, <i>n</i> = 370 treated with SP. <i>n</i> = 237 treated with CD had a > 20 g/L decrease in hemoglobin and of these 35% were carriers of this variant, defined as G6PD deficient), compared with 24% treated with SP
202A (rs1050828)	Chlorproguanil-dapsone-artesunate	Severe decreases in hemoglobin levels and increased risk of blood transfusion	[69] 13% were carriers of this variant and defined as A- G6PD deficient, in <i>n</i> = 343 total genotyped.
202A (rs1050828)	Sulphadoxine-pyrimethamine coadministered with amodiaquine	Increased risk of requiring a blood transfusion	[69] 11% were carriers of this variant and defined as A- G6PD deficient in <i>n</i> = 359 total genotyped.
202A (rs1050828) 376G (rs1050829) 680T (rs137852328) 968C (rs76723693) 542G (rs5030872)	Chlorproguanil-dapsone-artesunate	Severe reduction in hemoglobin levels and an increased risk of requiring a blood transfusion	[68] <i>n</i> = 800 genotyped. G6PD-deficient individuals were defined as A- hemizygous men (17% of <i>n</i> = 388), and homozygous A-/ A- women (4% of <i>n</i> = 412)
NS	Rasburicase	Hemolytic anemia	[84] (case study)
NS	Daunorubicin	Reduced drug metabolism	[87] (<i>in vitro</i>)
NS	Methylene blue	Hemolysis in an individual with methemoglobinemia	[94] (case study)
NS	Vitamin C (high dose of 80 g intravenously, 2 days)	Hemolysis	[95] (case study)
Mediterranean variant			
563T (rs5030868)	Glibenclamide	Acute hemolysis	[92] (case study)
NS	Aspirin (high dose of 100 mg/kg daily)	Severe hemolytic anemia in a child with systemic arthritis	[88] (case study)
NS	Daunorubicin	Reduced drug metabolism	[87] (<i>in vitro</i>)
NS	Rasburicase	Severe G6PD deficiency was revealed during treatment	[86] (case study)

NS, not specified.

^aFor each reference, details of whether the study was a single case study, or total study numbers and percentage of individuals carrying the indicated *G6PD* allele, are given in the reference column – see www.pharmgkb.org website for further annotations of these studies. The *G6PD* gene is found on the minus chromosomal strand. Please note that for standardization, the PharmGKB presents all allele base pairs on the positive chromosomal strand, and therefore, the alleles on the website will differ (in a complementary manner) from those in this review that are given on the minus strand as reported in the literature.

^bPlease note that in this study, heterozygous 202A individuals were considered as G6PD A, and hemizygous/ homozygous 202A individuals were classified as G6PD A-.