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NLM Citation: Kane M. Hydroxychloroquine Therapy and *G6PD* Genotype. 2023 May 2. In: Pratt VM, Scott SA, Pirmohamed M, et al., editors. Medical Genetics Summaries [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2012-.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/

Hydroxychloroquine Therapy and *G6PD* Genotype

Megan Kane, PhD[1]

Created: May 2, 2023.

Introduction

Hydroxychloroquine, which is closely related to chloroquine, can be used for the prevention and treatment of some forms of malaria and rheumatic conditions such as systemic lupus erythematosus (SLE) and rheumatoid arthritis. Malaria is an infection caused by the *Plasmodium* parasite, transmitted via mosquito bites. Hydroxychloroquine sulfate is indicated for the prevention and treatment of uncomplicated malaria due to sensitive strains of *Plasmodium falciparum (P. falciparum), Plasmodium vivax (P. vivax), Plasmodium malariae (P. malariae), Plasmodium ovale (P. ovale),* and *Plasmodium knowlesi* (*P. knowlesi)* by both the US Centers for Disease Control (CDC) and World Health Organization (WHO) (1, 2). Resistance to chloroquine and hydroxychloroquine has been reported in *Plasmodium* species, thus hydroxychloroquine therapy is not recommended if the infection arose in a region with known resistance. Most *P. falciparum* infections are resistant to the 4-aminoquinolines (chloroquine and hydroxychloroquine), and as such these drugs are no longer used widely for these infections. Hydroxychloroquine must be co-administered with an 8-aminoquinoline compound for the radical cure of *P. vivax* or *P. ovale* infection to eliminate the hypnozoite forms of these parasites. (3) Additionally, hydroxychloroquine is indicated for the treatment of many rheumatoid conditions in adults, including chronic discoid lupus erythematosus, systemic lupus erythematosus, as well as acute and chronic rheumatoid arthritis. Hydroxychloroquine has also been used in an off-label capacity for the management of Sjögren syndrome (4).

Hydroxychloroquine accumulates in cellular acidic compartments such as the parasitic food vacuole and mammalian lysosomes, leading to alkalinization of these structures. Among antimalarial medications, hydroxychloroquine is less likely than other medicines to cause hemolysis in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals; however, the U.S. FDA-approved drug label states there is still a risk of acute hemolytic anemia (AHA) (Table 1) (3). In contrast, the Clinical Pharmacogenetics Implementation Consortium (CPIC) performed a systematic review of the available clinical literature and found low-to-no risk of AHA for individuals with G6PD deficiency who take hydroxychloroquine (5). It should be noted that G6PD deficiency has a range of severity; CPIC advises caution for all medications when used by an individual with a severe G6PD deficiency with chronic non-spherocytic hemolytic anemia (CNSHA) (Table 2) (5). Regardless of G6PD phenotype, chronic use of hydroxychloroquine can cause irreversible retinal damage and regular visual exams are recommended by the FDA (3).

hydroxychloroquine therapy, hydroxychloroquine response, G6PD, lupus, malaria, rheumatoid arthritis

Author Affiliation: 1 NCBI; Email: mgs@ncbi.nlm.nih.gov.

Corresponding author.

Table 1: The FDA Drug Label for Hydroxychloroquine Sulfate (2022)

Phenotype	Warnings and precautions
G6PD deficiency	Hydroxychloroquine should be administered with caution in individuals that have G6PD deficiency.

G6PD - Glucose-6-phosphate dehydrogenase. This FDA table is adapted from (3).

Table 2: The CPIC Guidelines for Hydroxychloroquine based on G6PD Phenotype

Predicted G6PD phenotype based on genotype	Implication for phenotypic measures	Therapeutic recommendation	Classification of recommendation[a]
Normal	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status	Strong
Deficient	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status at standard doses	Moderate
Deficient with CNSHA	High risk of acute exacerbation of chronic hemolysis	Use all drugs cautiously in this group; if a drug is used, close monitoring for acute exacerbation of chronic hemolysis is recommended	Optional
Variable	Low-to-no risk of acute hemolytic anemia	No reason to avoid low-to-no risk drugs based on G6PD status at standard doses	Moderate
Indeterminant	Unknown risk of acute hemolytic anemia	To ascertain G6PD status, enzyme activity must be measured. Drug use should be guided per the recommendations based on the activity-based phenotype	Moderate

CNSHA - Chronic non-spherocytic hemolytic anemia, G6PD - glucose-6-phoshate dehydrogenase, CPIC - Clinical Pharmacogenetics Implementation Consortium
[a] Rating scheme from (5) Supplement.

Drug: Hydroxychloroquine

Hydroxychloroquine is a 4-aminoquinoline used for the treatment of specific forms of malaria, rheumatoid arthritis, SLE, and Sjögren syndrome, with potential utility in additional rheumatoid and autoimmune disorders (3, 6, 7, 8). Hydroxychloroquine is a chloroquine derivative and both medications have significant overlap in clinical use, pharmacodynamics, and metabolism. However, hydroxychloroquine is more frequently prescribed for rheumatoid conditions. Hydroxychloroquine and chloroquine have additionally been investigated as an adjuvant anticancer therapy (8, 9, 10, 11). Because of widespread use to treat and prevent malaria between the 1940s and 1980s, resistance to chloroquine and hydroxychloroquine has arisen in many strains of the malaria-causing parasite (12). Additional antimalarial medications have since been developed and can be used to treat those resistant strains; however, in many countries chloroquine and hydroxychloroquine remain first-line treatments.

In March of 2020, hydroxychloroquine and chloroquine were granted emergency use authorization by the FDA for the treatment of 2019 coronavirus disease (COVID-19) caused by infection with the severe acute respiratory syndrome coronavirus 2 (13). This authorization was revoked on 15 June 2020 due to risk of cardiac adverse events and other potential serious side-effects, which were determined to outweigh the potential benefit of these medications in treating COVID-19 (14). Experimental data suggested hydroxychloroquine and chloroquine may inhibit viral entry to the cell by suppressing angiotensin-converting enzyme 2 protein glycosylation and reduce viral particle release within cells due to neutralization of endosomal pH (15). However, the potential risk of cardiac adverse events and other serious side-effects were determined to outweigh the potential benefit of these medications in treating COVID-19 (14, 16). For up-to-date information please see the latest information from the FDA and NIH.

Hydroxychloroquine inhibits the heme polymerase enzyme in the malarial parasite, resulting in a fatal accumulation of toxic heme as well as accumulation in the lysosomes raising the pH. Within human cells, hydroxychloroquine also accumulates in lysosomes. The lysosome is the site of cellular autophagy, the mechanism whereby cells clear damaged organelles and protein masses as well as degrade foreign material. Inhibition of autophagy and lysosomal enzymes occurs once hydroxychloroquine or chloroquine raise the internal pH of the lysosomes. This results in altered antigen processing and presentation, and prevents major histocompatibility complex components from dimerizing, thus reducing the inflammatory response (15). Chloroquine and hydroxychloroquine both inhibit recognition of nucleic acids by the toll-like receptors, the major histocompatibility complex class II-mediated antigen presentation, inflammation-induced cell proliferation, and antiphospholipid antibody activity, making them useful for the treatment of autoimmune disorders such as SLE (17). Chloroquine may be of more potential benefit as an oncology adjuvant therapy compared with hydroxychloroquine (4, 9, 10, 18, 19).

Hydroxychloroquine sulfate is not indicated for use in the treatment of complicated malaria nor should it be used in individuals with known hypersensitivity to 4-aminoquinoline compounds (3). Concomitant medication with an 8-aminoquinoline is necessary to treat the hypnozoite life cycle stage of certain *Plasmodium* parasites (see below for more details). Additionally, malaria that is resistant to chloroquine will also not respond to hydroxychloroquine.

Chronic hydroxychloroquine sulfate use may cause irreversible retinal damage. If the medication is to be prescribed for an extended period, the FDA-approved drug label states that a baseline visual exam must be taken within the first year of therapy to monitor for any changes in vision. The baseline exam should include "best corrected distance visual acuity, an automated threshold visual field (VF) of the central 10 degrees (with retesting if an abnormality is noted), and spectral domain optical coherence tomography (SD-OCT)" (3). These exams should be performed annually in individuals taking daily doses of hydroxychloroquine sulfate greater than 5 mg/kg of actual body weight, a duration of use greater than 5 years, experiencing subnormal glomerular filtration, or using concomitant drug products such as tamoxifen citrate (3).

The American Academy of Ophthalmology (AAO) also advises that in addition to the automatic VF exams, SD-OCT should be performed (20). The American College of Rheumatology, American Academy of Dermatology, Rheumatologic Dermatology Society and AAO recently issued a joint statement regarding hydroxychloroquine and retinal toxicity(21). In this joint recommendation, it is stated that the initial baseline examination should be completed within the first few months of hydroxychloroquine use and further clarifies that in the absence of risk factors (such as daily dose above 5 mg/kg body weight or tamoxifen co-medication), the next retinal exam can be deferred for 5 years, but afterwards should be performed annually. They further advise that hydroxychloroquine therapy should not be stopped prematurely based on borderline findings from visual testing, rather there should be a team-based approach and clear communication between the eye care providers and prescribing care provider with the treated individual regarding the risks, screening importance, and treatment options (21).The Royal College of Ophthalmologists in the United Kingdom has also published recommendations for visual screening with regular monitoring for individuals on prolonged chloroquine and hydroxychloroquine therapy (22). If ocular toxicity is suspected, immediate discontinuation of use is recommended, although visual changes may continue to progress after withdrawal due to the prolonged systemic half-life of chloroquine (11).

It should be noted that in individuals of Asian descent, retinal toxicity may present first outside of the macula, thus the FDA recommends the VF screening be performed in the central 24 degrees (rather than 10) in this population (3). The AAO also recommends SD-OCT testing should look beyond the central macula in Asian individuals (20). The specific mechanism underlying this difference in disease presentation is unknown, though genetics are suspected to play a role (20).

Hydroxychloroquine use may be associated with rare adverse effects on cardiac, neurological and muscle tissues (4, 17). Cardiac tissue toxicity can present as cardiomyopathy with conduction defects including prolonged QT interval, torsades de pointes, or ventricular arrhythmias. Some studies have indicated that QT prolongation can present within 3 to 5 days of treatment with hydroxychloroquine, though arrythmias and conduction disorders are more common with chloroquine versus hydroxychloroquine (17, 23). Acute cardiotoxicity is dose-dependent and often associated with overdose, while chronic cardiomyopathy is primarily a concern for individuals after chronic, high-level dosing. The arrythmia risk is increased by co-medication with other arrhythmogenic drugs such as moxifloxacin. However, other studies have reported limited to no increased risk for arrythmia associated with hydroxychloroquine therapy alone (24, 25). One study found that after an acute course of hydroxychloroquine, drug levels remained at or above therapeutic goal levels for an average of 16 days following cessation of treatment, which may delay the timeline to safely administer other arrhythmogenic medications (26). Overall, the literature suggests standard dosing protocols (which vary between indications) for chloroquine and hydroxychloroquine have minimal risk of inducing either chronic or acute cardiotoxicity, with reported cardiotoxicity occurring in <1 out of 100 individuals (27, 28).

Additional side effects or risks include muscle weakness, increased risk of psoriatic attack or worsening of porphyria, and a potential elevated risk of convulsions among individuals with a history of epilepsy (3, 29). Rare instances of acute intermittent porphyria have been reported in some individuals with SLE on long-term hydroxychloroquine therapy (29). Cutaneous toxicity has also been reported, including pruritus, alopecia, and pigmentation changes (3, 7, 17). Much of these off-target tissue toxicities are predicted to result from the alkalinization of lysosomes, modulation of immune reactions and, in some cases, off-target activation of cellular receptors (17). Neuromuscular toxicity is rare, but there have been reports of proximal symmetric muscle deficits and polyneuropathies (17). Hydroxychloroquine can also cause severe hypoglycemia, both with and without concomitant antidiabetic medication. (3)

There have been reports of psychiatric adverse reactions with hydroxychloroquine use. Rare instances of suicidal behavior have been associated with hydroxychloroquine sulfate (3). However, in one study of long-term use for rheumatoid arthritis, hydroxychloroquine showed no increased risk of psychiatric side effects compared with sulfasalazine (30). When they do occur, psychiatric side effects seem to predominate in the context of short-term use (31) and may be the result of multiple predisposing risk-factors, with few studies of these risks in an elderly population (32).

Individuals with G6PD enzyme deficiency may experience hemolysis following treatment with hydroxychloroquine (3). However, other medications, including the antimalarials primaquine and tafenoquine, have a much higher risk of AHA in G6PD-deficient individuals (5, 33). The CDC advises that individuals with G6PD deficiency who may not tolerate other antimalarial medications may be prescribed a prophylactic dose of chloroquine for one year following acute malarial infection with *Plasmodium* species with hypnozoites, as most relapses from reactivation occur within this timeframe; hydroxychloroquine is not specifically recommended (34). The drug regulatory agency of Switzerland (Swissmedic) states that G6PD deficiency is a contraindication for hydroxychloroquine therapy (35).

Hydroxychloroquine is metabolized by the cytochrome P450 family of enzymes. First, hydroxychloroquine is N-dealkylated to N-desethylhydroxychloroquine. This is achieved primarily through the action of CYP3A4, with additional contributions from the enzymes CYP3A5, CYP2D6, CYP2C8, and CYP1A1 (15, 36). Hydroxychloroquine and desethylhydroxychloroquine have elimination half-lives of 40 to 50 days, primarily via renal excretion. This long half-life is due to extensive tissue uptake and storage rather than slow excretion (3). It is hypothesized that the mechanism of retinal damage with prolonged hydroxychloroquine therapy is due to its ability to bind to the melanin pigment in the iris, ciliary body, and retinal pigment epithelium. Notably, some animal research data suggests that chloroquine is more toxic than hydroxychloroquine. (11, 37, 38, 39)

Although hydroxychloroquine can freely diffuse into cells, it is also a substrate of several transporter proteins. Hydroxychloroquine is derived from chloroquine and shares many of the same enzyme interactions. Chloroquine is a substrate, inhibitor, and inducer of the multidrug resistance-associated protein 1 (MRP1) and a substrate of organic anion transporting proteins (OATPs) (36). Hydroxychloroquine has been shown to inhibit OATP1A2, which in turn can affect all-*trans*-retinol uptake in retinal cells (36). Various drug interactions have been documented with hydroxychloroquine due to their metabolism by cytochrome P450 enzymes described above. Altered plasma level of digoxin and cyclosporin have been correlated with hydroxychloroquine co-medication. Based on studies in chloroquine, it is possible that ampicillin, praziquantel, and cimetidine could have similar drug-drug interactions with hydroxychloroquine (3).

The FDA-approved drug label states that hydroxychloroquine has not been determined to be safe or efficacious for pediatric individuals with SLE or juvenile idiopathic arthritis (3). However, the use of hydroxychloroquine is recommended for managing both pediatric and adult SLE (40, 41), for selected cases of juvenile idiopathic arthritis (42), for managing lupus nephritis (43), and has been reported to manage pediatric Sjögren disease (44). It should be noted that overdose is a serious risk, particularly for children with accidental ingestion, as these medications are rapidly and completely absorbed. The FDA label states that even one gram of chloroquine may be fatal in children (45); it should be noted that both chloroquine and hydroxychloroquine are dosed based on the body mass of the individual and the severity of poisoning would depend upon the size of the child. Toxicity symptoms include nausea, vomiting, headache, drowsiness, visual disturbances, cardiovascular collapse, convulsions, hypokalemia, cardiac arrythmia and conduction defects and sudden potentially fatal respiratory and cardiac arrest; these symptoms may present within minutes of overdose. Immediate medical attention is required. Thus, it is strongly advised to keep hydroxychloroquine and chloroquine phosphate out of reach of children, as these individuals are particularly sensitive to 4-aminoquinoline compounds. (3)

There are also limited data regarding use in individuals over 65 years of age, though this is an active area of clinical research (46). As kidney function may be decreased in these individuals and thus slow hydroxychloroquine clearance, there is a risk of toxic accumulation in geriatric individuals. The FDA-approved label suggests monitoring of renal function and that care should be taken during dose selection for these individuals (3).

Human studies have not shown an increase in the rate of birth defects associated with hydroxychloroquine use by pregnant mothers, nor evidence of fetal ocular toxicity (3, 47). The CDC states that pregnant women with uncomplicated malaria caused by *P. malariae, P. ovale*, or chloroquine-sensitive *P. vivax* or *P. falciparum* should be treated with hydroxychloroquine or chloroquine (34). The WHO recommends chloroquine as an alternative therapy during pregnancy for infection with sensitive *Plasmodium* strains (2). Furthermore, the CDC advises continued chloroquine prophylaxis for individuals with *P. vivax* or *P. ovale* infection for the duration of pregnancy. If, upon delivery, the mother intends to breastfeed, the infant should be tested for G6PD deficiency. If neither the infant nor mother are G6PD deficient, primaquine phosphate is the recommended therapy for the mother (tafenoquine is not recommended during breastfeeding). Otherwise, women who cannot take tafenoquine or primaquine should continue weekly chloroquine prophylaxis for one year following acute malarial infection (34). Small amounts of hydroxychloroquine are excreted in breast milk (3). However, studies have found no adverse effects on growth, vision, or hearing, leading international experts to state that hydroxychloroquine is acceptable during breastfeeding (48).

Disease: Malaria

Malaria is a serious tropical disease caused by a parasite (*Plasmodium*) that spreads to humans by infected mosquitos. The only available vaccine is moderately effective and acts only against the *P. falciparum* species (49). Widely recommended antimalarial drugs such as mefloquine or atovaquone-proguanil can be used for prevention, which is known as chemoprophylaxis. The type of chemoprophylaxis recommended depends upon

the individual taking the prophylaxis (namely, age, pregnancy status, and medical and psychiatric comorbidities) and the nature of travel –– specifically, the countries travelled to, the length of stay, the species of *Plasmodium* that are most prevalent, and the level of drug resistance. For individuals residing in malaria-endemic regions, the WHO recommends a variety of preventative chemotherapies that can be used in infants, children, during pregnancy or collectively for the population of endemic areas (50).

Despite chemoprophylaxis, travel to malaria-endemic areas is not without risk. Individuals at elevated risk for malaria complications include pregnant women (34) and adults who have had their spleen removed (51). If travel cannot be avoided, chemoprophylaxis should be combined with additional precautions to avoid mosquito bites, such as bed nets and repellents. In 2021, the WHO estimated 247 million cases of malaria occurred worldwide, and malaria was responsible for at least 619,000 deaths. (52)

Malaria is found in over 100 countries and occurs throughout most tropical regions in the world. These regions include large parts of Africa, Asia, Central and South America, and parts of the Middle East and Pacific islands (52, 53). Individuals who are heterozygous carriers for sickle cell disease or G6PD deficiency have a protective advantage against malaria, and as a result, the frequency of such genetic conditions is higher in countries where malaria is endemic (54).

Malaria is transmitted to humans by the bite of an infected *Anopheles* mosquito. Only female mosquitos spread the infection (females feed on blood, males feed on nectar). Although malaria can also be spread by sharing contaminated needles or via a contaminated blood transfusion, these are rare means of transmission.

There are several different *Plasmodium* species, but only a few species cause most human malaria cases:

- *P. falciparum*
  - The most common cause of malaria, and death from malaria
  - Predominates in sub-Saharan Africa
  - Also found in regions of Australasia (Papua New Guinea, Southeast Asia), and the Caribbean (Haiti and the Dominican Republic)
- *P. vivax*
  - A common cause of malaria outside of Africa
  - Most frequent species found in Central and South America, and South-East Asia
  - Parasite has a dormant, hypnozoite stage in the liver
  - Early gametocytes that infect mosquitos
- *P. malariae*
  - Less common
  - Found in most areas where malaria is endemic
- *P. ovale*
  - Less common
  - Parasite has a dormant, hypnozoite stage in the liver
- *P. knowlesi*
  - Less common
  - Found in some areas of Southeast Asia

The first stage of malaria infection begins when an infected mosquito bites the human host. Typically, mosquitos bite at dusk, or during the night. As the mosquito feeds, infective parasite sporozoites (the motile spore-like stage in the life cycle of this parasitic sporozoan, which is the infective agent) are inoculated into humans. The sporozoites travel to the liver, where they invade liver cells and asexually reproduce to form schizonts. The liver schizonts contain daughter merozoites. This process is asymptomatic, and because it occurs outside of the red blood cell (erythrocyte), it is known as the exoerythrocytic stage.

Some species of the parasite (*P. vivax* and *P. ovale*) have an additional dormant stage in the liver–– the hypnozoite. These parasites can stay in the liver for weeks or months without causing any clinical symptoms.

The second stage of malaria infection is the erythrocytic stage. It begins when the liver schizonts rupture and release the daughter merozoites into the bloodstream. The merozoites invade red blood cells, digest hemoglobin, produce a toxic metabolite (hemozoin), and damage red blood cell membranes. Infected, brittle red blood cells are rapidly broken down (hemolysis) and if too many damaged red blood cells get trapped in the spleen, the spleen can rapidly enlarge (splenic sequestration).

Some of the daughter merozoites differentiate into male or female gametocytes (sexual forms). When they are ingested by a mosquito, they mature, fertilize, and reproduce, and develop into sporozoites. When the mosquito feeds again, the sporozoites are inoculated into another human host and the cycle of malaria transmission is complete.

The erythrocytic stage of malaria is usually associated with fever, and malaria should always be suspected in anyone with a fever who has recently returned from a malaria-endemic region, even if antimalarial chemoprophylaxis was correctly followed. Other symptoms and signs include nausea, vomiting, abdominal pain, tachycardia (fast heart rate), diaphoresis (sweating), chills, and myalgia (muscle pain). The complications of malaria infection include severe anemia, cerebral malaria, and multi-organ failure. Without correct diagnosis and prompt treatment, malaria can be fatal.

Disease Class: Rheumatic and Autoimmune Disorders

Several rheumatic and autoimmune disorders are treated with long-term hydroxychloroquine administration. These disorders include acute and chronic rheumatic arthritis, SLE, chronic discoid lupus erythematosus, and Sjögren syndrome, with potential use in many more conditions (4). In these conditions, there is a common theme of altered immune reactions leading to the immune system attacking endogenous tissues and cells. This causes cellular death and breakdown of the affected tissues, with potentially widespread systemic symptoms. The specific etiology of auto-antigen production may vary by specific conditions and individuals, but activation of toll-like receptors, release of interferon, or other inflammatory cytokines can all drive inflammation and activate innate and adaptive immunity. This can lead to auto-antigen presentation in lupus, (7) aberrant B-cell maturation in primary Sjögren syndrome, (55) and contribute to pathogenesis of rheumatoid arthritis (56). Antimalarial drugs, including the 4-aminoquinoline compounds like hydroxychloroquine have been utilized to manage these wide-ranging inflammatory processes (4, 17).

Gene: *G6PD*

The G6PD enzyme is encoded by the *G6PD* gene, which is located on the long arm of the X chromosome (Xq28). Variants in the *G6PD* gene that eliminate enzymatic activity are not viable; variants observed in living humans impact the stability of the enzyme. As such, males can only be hemizygous (have one *G6PD* allele) while females randomly inactivate one X chromosome during development, resulting in a mosaic expression of either one X chromosome or the other in their somatic cells. This mosaicism can occur in the hematopoietic progenitor cells that give rise to red blood cells, resulting in mixed expression of *G6PD* alleles. Females with Turner syndrome (45, X) have only one X chromosome and thus are also hemizygous for the *G6PD* gene. Males with Klinefelter syndrome have an additional X chromosome (47, XXY) and thus 2 *G6PD* alleles. Thus, it is important to consider the number of X chromosomes for an individual when determining *G6PD* genotype or phenotype.

Glucose-6-phosphate dehydrogenase deficiency affects 400 million people worldwide, with a worldwide prevalence of approximately 5% (57). Glucose-6-phosphate dehydrogenase deficiency appears to be protective against malaria infection leading to a higher prevalence (more than 25%) in countries where malaria is, or once

was, endemic; for example, tropical Africa, tropical and subtropical Asia, the Middle East, and some parts of the Mediterranean (58, 59, 60). In the US, G6PD deficiency is more common among individuals of African descent, affecting approximately 12% (61).

The G6PD enzyme catalyzes the first step in the hexose monophosphate shunt (HMP) pathway, which converts glucose to pentose sugars for nucleic acid synthesis. In this step nicotinamide adenine dinucleotide phosphate (NADP+) is reduced to NADPH, which protects cells from oxidative stress. In mature red blood cells, the HMP pathway is the only source of NADPH. This promotes the generation of reduced glutathione that protects the sulfhydryl groups of hemoglobin, which are susceptible to oxidation by hydrogen peroxide and oxygen radicals. (62)

Red blood cells that are G6PD deficient have a normal function but are more susceptible to increased oxidative stress (for example, by reactive oxygen species and hydrogen peroxide). Oxidative stress occurs naturally, but is increased during illnesses, such as infections and diabetic ketoacidosis. Oxidative stress can also follow the ingestion of fresh fava beans (favism), and is an adverse effect of several drugs, for example, the antimalarial drugs primaquine and tafenoquine, the antibacterials dapsone and sulfamethoxazole, the skin cancer drug dabrafenib, and the uric acid lowering drugs pegloticase and rasburicase.

Most individuals with G6PD deficiency are asymptomatic –– they have a normal lifespan and may not know they have G6PD deficiency. At birth, they are at a higher risk of developing neonatal jaundice, and throughout their lives will be sensitive to oxidizing agents. All individuals with G6PD deficiency should avoid exposure to oxidizing agents when possible, including drugs such as tafenoquine.

Symptomatic individuals with G6PD deficiency may suffer from episodes of AHA or, the more severe condition, CNSHA. The management of hemolytic episodes depends on the severity of hemolysis, with more severe cases requiring blood transfusions.

More than 200 genetic variants of the *G6PD* gene have been identified so far (63). Most known *G6PD* variants are missense, and variants that are in cis as a haplotype have also been described, including the A- variant that is most common in individuals of African and South-American genetic ancestry (64). Large deletions are rare, and a complete lack of G6PD activity is fatal in utero.

The normal (wild type, referred to as B) copy of the *G6PD* gene is found in most individuals of European descent, individuals of Asian descent, and individuals of African descent. Common *G6PD* variants include:

- *G6PD* A (p.Asn126Asp) has normal enzyme activity and is not associated with hemolysis, and is found in up to 30% of individuals of African descent and approximately 1.5% of Latinos (65, 66)
    - *G6PD* A- (p.Asn126Asp with p.Val68Met) is associated with mild to moderate hemolysis, and is found in up to 15% of African-Americans (67). Additional *A-* haplotypes have also been identified, both with the A+ variant with a second single nucleotide polymorphism (p.Asn126Asp with p.Arg227Leu; and p.Asn126Asp with p.Leu323Pro. See Nomenclature table below for additional information) (68)
    - *G6PD* Mediterranean (p.Ser218Phe) can cause severe hemolysis, and is a common pathogenic variant in individuals of European descent (69)
    - *G6PD* Canton (p.Arg489Leu) can cause severe hemolysis, and is found in individuals of Asian descent (70)
- *G6PD* Viangchan (p.Val291Met) is the most common *G6PD* variant among Thais, Laotians, Cambodians, and Malaysians (based on common genetic ancestry) (71, 72)

The WHO recently updated its categorization of *G6PD* variants into 4 classes based on the median residual enzyme activity in males (expressed as a percentage of normal activity) (73). Class A variants have <20% activity and are associated with chronic hemolytic anemia. Most individuals with G6PD deficiency have variants that

belong to class B (enzyme activity less than 45%). Class B variants are associated with intermittent hemolysis, usually triggered by infection or drugs, but most of the time, affected individuals are asymptomatic. Class C variants show median G6PD activity from 60–150% and are not associated with hemolysis. In class U are the variants with any activity and unknown clinical significance. The CPIC has assigned G6PD phenotypes based on *G6PD* genotypes under the previous classification system; the updated WHO categories are also provided (Table 3) (5).

Table 3. Assignment of likely G6PD Phenotype based on Genotype/Diplotype (CPIC, 2022)

Likely phenotype	Definition[a]	Genotype	WHO class for *G6PD* variants[b]	Example of diplotype[c]
Normal	Very mild or no enzyme deficiency no less than 60% of normal enzyme levels (60–150% of normal activity)	An X chromosome hemizygote who has a nondeficient (class IV) allele	IV (C)	B, Sao Borja
		An individual who has 2 nondeficient (class IV) alleles	IV/IV (C)	B/B, B/Sao Borja
Deficient	Less than 10–60% of normal enzyme activity (20–45% of normal activity)	An X chromosome hemizygote who has a deficient (class II–III) allele	II, III (B)	A–, Orissa, Kalyan-Kerala, Mediterranean, Canton, Chatham
		An individual who has 2 deficient (class II–III variants) alleles	II/II, II/III, III/III (B)	A–/A–, A–/Orissa, Orissa/Kalyan-Kerala, Mediterranean/Mediterranean, Chatham/Mediterranean, Canton/Viangchan
Deficient with CNSHA	Severe enzyme deficiency (<10% activity) and associated with CNHSA (<20% of normal activity)	An X chromosome hemizygote who has a class I allele	I (A)	Bangkok, Villeurbanne
		An individual who has 2 deficient (class I variants) alleles	I/I (A)	Bangkok/Bangkok, Bangkok/Villeurbanne
Variable[d]	Normal or deficient enzyme activity[c]	An individual who has one nondeficient (class IV) and one deficient (class I–III variants) allele	IV/I, IV/II, IV/III (U)	B/A–, B/Mediterranean, B/Bangkok

Table 3. continued from previous page.

Likely phenotype	Definition[a]	Genotype	WHO class for *G6PD* variants[b]	Example of diplotype[c]
Indeterminant	Uncertain		(U)	

CNSHA - chronic non-spherocytic hemolytic anemia, WHO - World Health Organization, G6PD - glucose-6-phosphate dehydrogenase

[a] The traditional (Class I-IV) and updated (A, B, C, and U) activity levels are both provided, with the updated activity ranges provided in parentheses where relevant.

[b] WHO classifications were under revision at the time of CPIC publication, updated classification (using A, B, C and U designations) have been proposed based on enzyme activity levels and are provided in parenthesis here (73).

Class I alleles are extremely rare; the distinction between class II and III alleles is not clear. Almost all individuals will have class II, III, or IV alleles. It should be noted that the class of a variant may have been assigned only by the clinical manifestations of an individual in which the variant was subsequently identified.

[c] Due to the large number of *G6PD* variants, many other diplotypes may be possible besides those given as examples here; see Supplementary data from (5) for a more comprehensive list of alleles with their assigned WHO class. For Human Genome Variation Society terms, please see the Nomenclature table below. The alleles and diplotypes provided here are based upon the historic class I-IV definitions and may not fit the updated WHO classification.

[d] Due to X-linked mosaicism, females heterozygous for one nondeficient (class IV) and one deficient (class I–III variants) allele may display a normal or a deficient phenotype. It is therefore difficult to predict the phenotype of these individuals (Supplementary Material online (G6PD heterozygotes)) (5).

This table is adapted from (5).

Additional Genes of Note

Hydroxychloroquine is metabolized by the enzymes encoded by *CYP2C8*, *CYP3A4,* and *CYP2D6*, all of which are classified as "very important pharmacogenes" by PharmGKB (74, 75). Variability in the *CYP* genes can lead to reduced or increased enzyme function. Classification of individual phenotype as either ultrarapid metabolizers, normal metabolizers, intermediate metabolizers, or poor metabolizers is carried out on a predictive basis from genotype results of known alleles. Allele classifications for various *CYP* genes and other pharmacogenes are available from CPIC (76) and the Pharmacogene Variation Consortium (77). Allele frequencies for these pharmacogenes vary across global and even regional populations, where populations are defined as groups with shared genetic variants due to ancestry (76, 78, 79, 80, 81).

Interaction of hydroxychloroquine with these CYP enzymes (for example, CYP2D6) leads to varying degrees of enzyme inhibition depending on the method of testing used. In vivo measurement of CYP2D6 inhibitory activity ranges from moderate to no inhibition. Thus, drug-drug interactions may occur due to co-medication of hydroxychloroquine with other CYP substrates(36). This results in phenoconversion, a phenomenon where an individual's genetically predicted CYP metabolism is altered to a different activity level. Drugs competing for the same metabolic enzyme will often lead to phenoconversion with lower effective enzyme activity.

Transporter proteins encoded by solute carrier organic anion transporter (*SLCO)* genes, also known as OATPs, adenosine triphosphate (ATP)-binding cassette sub-family B member 1 (*ABCB1)* or P-glycoprotein, ATP-binding cassette sub-family C member 1 (*ABCC1)* or MRP1, and ATP-binding cassette sub-family C member 2 (*ABCC2) or* MRP2 have all been shown to have their function impacted by chloroquine, which is likely to extend to hydroxychloroquine as well (36). Both chloroquine and hydroxychloroquine exerted potent inhibition of OATP1A2, but only moderate inhibition of breast cancer resistance protein 1 (BCRP1), human serotonin transporter (hSERT), and OATP2B1, with no inhibition of the bile salt export pump (BSEP) transporter (36).The ATP ABC family of proteins and OATPs are important drug transport proteins and altered function of these enzymes can affect the efficacy of substrate medications and potential side effects. One study found hydroxychloroquine to be a potent inhibitor of the OATP1A2 transport protein, potentially impacting uptake of all-*trans*-retinol in the retinal pigment epithelium (82).

Linking Gene Variation with Treatment Response

Some guidelines report that individuals with G6PD deficiency may be at a higher risk for hemolysis during medication with antimalarials such as hydroxychloroquine and chloroquine than those individuals with normal G6PD enzyme activity (3, 68, 83). However, both the CDC and WHO recommend chloroquine prophylaxis to prevent malaria relapse for pregnant and breastfeeding women, as primaquine is contraindicated during pregnancy and in G6PD deficiency (<30% enzymatic activity) (1). The FDA-approved drug label recommends monitoring individuals on prolonged therapy for signs of hemolysis; the recommended test is complete blood counts (3). Several studies have reported there is no increased risk of hemolysis for G6PD-deficient individuals taking chloroquine or hydroxychloroquine (84, 85, 86, 87, 88) Similarly, multiple studies regarding the safety and efficacy of hydroxychloroquine for a variety of rheumatic conditions did not report significant rates of hemolysis in their cohorts (89, 90, 91). However, despite extensive use of chloroquine in areas with high a high proportion of G6PD deficiency and no evidence of hemolysis, there are case reports documenting hemolysis in individuals with G6PD deficiency who were undergoing experimental treatment for COVID-19 with hydroxychloroquine (92, 93, 94, 95, 96). The specific cause of hemolysis in this context is a matter of scientific debate. Many authors suggest that the systemic response to SARS-CoV2 infection or co-medications or both may be the actual triggers for hemolysis in these cases, with the underlying G6PD deficiency and hydroxychloroquine medication playing minor roles (97, 98, 99).

Studies have reported differences in levels of hydroxychloroquine and its metabolites in individuals with variant *CYP2D6* alleles, however a clear clinical correlation in efficacy or dose requirement has not been demonstrated (100, 101). Medications such as metoprolol and tamoxifen depend upon CYP2D6 metabolism and may be negatively impacted by co-medication with chloroquine or hydroxychloroquine (102, 103). This may lead to altered therapeutic response for all concomitant medications and increase the risk of retinal damage in the case of tamoxifen co-medication (3). There have been reports of different variants in *ABCA4* associating with either increased risk for or protection from hydroxychloroquine toxicity (20, 104, 105).

However, there are no specific actionable guidelines from the pharmacogenetics community or the FDA (3) to alter hydroxychloroquine dosage based on variation of any the genes discussed herein.

The *G6PD* Gene Interactions with Medications Used for Additional Indications

Medications that can induce oxidative stress in red blood cells can trigger hemolysis readily in individuals with G6PD enzyme deficiency. Many of these medications are antimalarial (tafenoquine, for example) but many more medications pose a hazard for G6PD deficient individuals.

- Urate-lowering medications: both refractory gout and tumor lysis syndrome can cause systemic elevation of urate levels, medications such as rasburicase and pegloticase are uricase enzymes that aid in the breakdown of uric acid into more soluble metabolites. These reactions produce hydrogen peroxide as a byproduct, thus increasing oxidative stress in the body.
- Kinase inhibitors: anti-cancer medications such as dabrafenib may also increase oxidative stress.
- Anti-microbial medications: nitrofurantoin, often used for urinary tract infections, was determined to be a medication of moderate risk for AHA in G6PD deficient individuals by CPIC and may call for additional monitoring. In contrast, CPIC found sulfamethoxazole to be a medication with low-to-no risk in G6PD deficient individuals. (5)

Additional information on gene-drug interactions for *G6PD* are available from PharmGKB, CPIC and the FDA (search for "G6PD").

Genetic Testing

Glucose-6-phosphate dehydrogenase deficiency is inherited in an X-linked pattern and most individuals are asymptomatic throughout life. A heterozygous mother has a 50% chance of passing G6PD deficiency to a son and a 50% chance of passing the carrier trait to a daughter. Affected fathers pass the variant *G6PD* to their daughters, but not to their sons. X-linked disorders affect males at a much higher rate than females because their frequency is the same as the allelic frequency (males only have one copy of the X chromosome, XY). Since females have 2 copies of the X chromosome (XX) and the deficient phenotype is mostly expressed in the homozygous/compound heterozygous mutated genotype, the frequency of the phenotypic disorder is the square root of the allelic frequency. However, female heterozygotes with one mutated allele can present with a range of phenotypes from no symptoms through a severe deficiency; frequency of "intermediate" phenotypes (not deficient but not normal either) in females can be as high as the frequency of deficiency in males (106).

The NIH Genetic Testing Registry (GTR) displays genetic tests that are available for the *G6PD* gene. Molecular genetic testing can be used to confirm the diagnosis of G6PD deficiency in males and testing may also be used to screen females with a family history of G6PD to see if they are carriers. In females, G6PD deficiency occurs mostly in homozygous and compound heterozygous individuals (who have inherited 2 copies of *G6PD* deficiency alleles); in heterozygous individuals (one normal *G6PD* allele and one deficiency *G6PD* allele) skewed X chromosome inactivation of the functional allele (59) can result in deficient phenotypes. Therefore, genetic testing alone is insufficient to assess risk of hemolysis in heterozygous individuals.

In routine clinical practice, G6PD deficiency is diagnosed by measuring G6PD activity in red blood cells (62, 107). Two different types of enzyme activity tests are used, and they are classified as qualitative or quantitative. For some medications, such as tafenoquine, a specific enzymatic activity threshold is used to determine the safety of the medication and as such a quantitative test may be required for individuals with intermediate levels of enzyme activity based on the qualitative test (108). False results may be obtained immediately after a blood transfusion, because transfused cells are likely to have normal G6PD levels, and immediately after an episode of hemolysis, because young red blood cells have higher levels of G6PD. Therefore, when necessary, screening for G6PD enzymatic activity should be performed 2–3 months after a blood transfusion or hemolytic episode (5, 62). Diagnosis using qualitative test methods is less accurate for females with intermediate G6PD activity due to heterozygous *G6PD* alleles (109).

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

2022 Statement from the US Food and Drug Administration (FDA):

Hemolysis has been reported in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Monitor for hemolytic anemia as this can occur, particularly in association with other drugs that cause hemolysis.

Please review the complete therapeutic recommendations that are located here: (3)

[1] *The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labeled all formulations containing the generic drug. Certain terms, genes and genetic variants may be corrected in accordance to nomenclature standards, where necessary. We have given the full name of abbreviations, shown in square brackets, where necessary.*

Nomenclature for Selected *G6PD* Alleles

Common allele name / condition	Alternative names / condition	HGVS reference sequence		WHO Classification*	dbSNP reference identifier for allele location
		Coding	Protein		
G6PD B	WT	NM_001042351.3	NP_001035810.1	IV/ Normal	--
G6PD A+	p.Asn126Asp	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	IV/ Normal	rs1050828
G6PD Sao Borja	p.Asp113Asn	NM_001042351.3:c.337G>A	NP_001035810.1:p.Asp113Asn	IV/ Normal	rs5030870
G6PD A-	A-$^{202A/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient (B)	rs1050828 rs1050829
		NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met		
G6PD A-	A-$^{680T/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient	rs1050828 rs137852328
		NM_001042351.3:c.680G>T	NP_001035810.1:p.Arg227Leu		
G6PD A-	A-$^{968C/376G}$	NM_001042351.3:c.376A>G	NP_001035810.1:p.Asn126Asp	III/ Deficient (B)	rs1050828 rs76723693
		NM_001042351.3:c.968T>C	NP_001035810.1:p.Leu323Pro		
G6PD Bangkok	p.Lys275Asn	NM_001042351.3:c.202G>A	NP_001035810.1:p.Val68Met	III/ Deficient (A)	
G6PD Kalyan-Kerala	p.Glu317Lys	NM_001042351.3:c.949G>A	NP_001035810.1:p.Glu317Lys	III/ Deficient (U)	rs137852339
G6PD Orissa	p.Ala44Gly	NM_001042351.3:c.131C>G	NP_001035810.1:p.Ala44Gly	III/ Deficient (B)	rs78478128
GP6D Canton	p.Arg459Leu	NM_001042351.3:c.1376G>T	NP_001035810.1:p.Arg459Leu	II/ Deficient (B)	rs72554665
G6PD Chatham	p.Ala335Thr	NM_001042351.3:c.1003G>A	NP_001035810.1:p.Ala335Thr	II/ Deficient (B)	rs5030869
G6PD Mediterranean	p.Ser188Phe	NM_001042351.3:c.563C>T	NP_001035810.1:pSer188Phe	II/ Deficient (A)	rs5030868
G6PD Viangchan	p.Val291Met	NM_001042351.3:c.871G>A	NP_001035810.1:p.Val291Met	II/ Deficient (B)	rs137852327
G6PD Villeurbanne	p.Thr334del	NM_001042351.3:c.1000_1002delACC	NP_001035810.1:pThr334del	I/Deficient with CNSHA	

Additional allele information available from PharmGKB and CPIC's G6PD Allele Definition Table (revised 2018).
Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society.
* WHO classifications based on (110), classification of these alleles under the updated WHO categories are taken from work described in (111) and the data deposited at (112). Please note that not all alleles have an updated classification at the time of writing.
WHO - World Health Organization, PharmGKB - Pharmacogenomics Knowledgebase, CPIC - Clinical Pharmacogenetics Implementation Consortium, CNSHA - chronic non-spherocytic hemolytic anemia, G6PD - glucose-6-phosphate dehydrogenase

Acknowledgments

The author would like to thank Ashutosh M. Shukla, MD, Professor in Medicine, Director of Advanced CKD and Home Dialysis Programs, University of Florida and Veterans Health Care System, Veterans Affairs, Gainesville, FL, USA; Slobodan Rendic, PhD, Independent Scientist, Zagreb, Croatia; Germana Bancone, PhD, Centre for Tropical Medicine and Global Health, University of Oxford, Oxford, UK; and an anonymous reviewer for their review of this Summary.

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