**Short Report: Glucose-6-Phosphate Dehydrogenase Deficiency A– Variant in Febrile Patients in Haiti**


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**Abstract.** Haiti is one of two remaining malaria-endemic countries in the Caribbean. To decrease malaria transmission in Haiti, primaquine was recently added to the malaria treatment public health policy. One limitation of primaquine is that, at certain doses, primaquine can cause hemolytic anemia in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In this study, we genotyped two mutations (A376G and G202A), which confer the most common G6PD variant in West African populations, G6PD A–. We estimated the frequency of G6PD A– in a sample of febrile patients enrolled in an on-going malaria study who represent a potential target population for a primaquine mass drug administration. We found that 33 of 168 individuals carried the G6PD A– allele (includes A– hemizygous males, A– homozygous or heterozygous females) and could experience toxicity if treated with primaquine. These data inform discussions on safe and effective primaquine dosing and future malaria elimination strategies for Haiti.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PDd) is a genetic red blood cell disorder caused by mutations in the G6PD gene on the X chromosome. The reduced G6PD enzyme concentration increases oxidative damage in red blood cells resulting in hemolysis. One of the more common G6PDd variants, G6PDd A–, has only 12% enzyme activity and reaches frequencies as high as 20% in West African populations. G6PDd is not usually life-threatening, except when G6PDd individuals are exposed to oxidizing medications such as primaquine, which increases the destruction of red blood cells, causing hemolytic anemia. Primaquine is an antimalarial drug that can be used as a gametocytocide in malaria elimination programs with the goal of limiting transmission of parasite gametocytes. Previous studies have shown that hemolytic anemia in response to primaquine treatment can occur in individuals carrying the G6PDd A– variant (for review, see White and others, 2012), most often observed in hemizygous males and homozygous females. In an ideal setting, malaria patients would be screened for G6PDd before they are prescribed primaquine. However, G6PDd screening is not always feasible in resource-limited regions as it requires specialized training and equipment. The World Health Organization (WHO) recently reviewed the literature on the safety and efficacy of single-dose primaquine and concluded that a primaquine dose of 0.25 mg/kg is safe and effective for treating malaria, regardless of G6PD status. However, WHO recommends that countries currently implementing primaquine treatment plans with the previously recommended dose of 0.75 mg to continue doing so until further information is available.

Haiti, one of two remaining malaria-endemic countries in the Caribbean in addition to the Dominican Republic, revised their antimalarial policy in 2012 to include primaquine with the standard chloroquine regimen, despite limited information on the prevalence of G6PDd in the population. It is likely that G6PDd is common in Haiti given the country's West African ancestry, but actual prevalence data are only now being generated. Furthermore, very limited data are available on the genetic mutations underlying G6PDd in the Haitian population. Here, we present the first genetic study on G6PDd in the Haitian population, in which we sequence a portion of the G6PD gene that contains the mutations conferring the G6PD A– variant and we use these data to estimate the frequency of G6PDd in a sample of Haitian individuals. Our sample is composed of febrile patients enrolled in a malaria study from two health center sites in the West Department of Haiti. Febrile patients are likely targets of a primaquine mass drug administration to either quickly reduce transmission in malaria outbreak scenarios or eliminate malaria in low transmission regions like Haiti. Baseline data on the frequency of the G6PD A– allele in a sample representing the most likely candidates for targeted mass drug administration can inform public health policy discussions on safe and effective primaquine dosing in Haiti.

We collected blood spot samples on filter paper from patients, 2 to 80 years of age, enrolled in a malaria study at two health center sites in Terre Noire and Leogane, Haiti as previously detailed. The study was approved by the Haiti Ethical Review Board, University of Florida’s IRB-01, and the Office of Research Protections, U.S. Army Medical Research and Materiel Command. Informed consent was obtained from all adult participants and from the parents or legal guardians of minors.

Samples were transported to the University of Florida Genetics Institute for genetic analysis. G6PD mutations were identified using the following protocol: DNA was extracted using a Qiagen QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and a portion of G6PD encompassing exons 3, 4, and 5 (partial) was amplified using the polymerase chain reaction (PCR) with primers 13125F9 and G6PD5R.10 Promega Hot Start Kit (Promega, Madison, WI) was used with a final reaction volume of 25 μL and the following components: 1 x Buffer, 1.5 mM MgCl2, 0.2 mM each dNTP, 0.5 μM each primer, 2.5 U Taq, and 2 μL DNA extract. The temperature cycling protocol was as follows: 95°C for 1 min, and then

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35 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 1 min followed by a final extension at 72°C for 10 min. Amplicons were sequenced using Big Dye Master mix and run on an ABI Genome Analyzer (Applied Biosystems, Foster City, CA). The DNA sequences were aligned to a reference sequence (National Center for Biotechnology Information (NCBI) Reference Sequence NC_000023.10) using Sequencer and scanned for G6PDdA– mutations A376G (rs1050829) and G202A (rs1050828), and other mutations.

The complete evaluation of mutations in the G6PD gene, which is located on X chromosome, requires accurate sex data; therefore, we determined the participants’ sex using the following genotype-based protocol: blood spots were rehydrated with 100 μL of Whatman FTA purification reagent (GE Healthcare, Little Chalfont, UK) and three times with 100 μL of water. After drying for an hour, punches were rehydrated with 2 μL of water and transferred to tubes for PCR. A portion of the amelogenin gene, which occurs on both X and Y chromosomes and has a deletion in the X chromosome of 6 base pairs (bp), was amplified using primers Amel-A and Amel-B. The PCR was performed in a 25 μL reaction mixture with a final concentration of 0.2 μM of each primer and 1× of the Apex master mix taq (Apex Bioresearch Products, North Liberty, IA), which will have final concentrations of 75 mM Tris-HCl pH8.5, 20 mM (NH4)2SO4, 15 mM MgCl2, 0.1% Tween 20, 0.2 mM dNTPs, and 0.025 U DNA polymerase. The PCR cycling protocol was carried out as in Mannucci and others. The PCR product was loaded onto a 4% high resolution agarose gel and separated by electrophoresis for 3 hours at 90V, and UV illumination was used for visualization. Sex was assigned based on the number of bands seen; females show one band (106 bp) and males show two bands (106 and 112 bp).

In total, we genotyped 168 individuals (54 males and 114 females) from the Leogane (N = 56) and the Terre Noire (N = 112) sites. Of the 168 individuals genotyped for the G6PDd A– alleles (i.e., both 376G and 202A mutations), eight were A– hemizygous males, two were A– homozygous females, and 23 were A– heterozygous females (Table 1). The remaining 88 females and 47 males did not carry the G6PDd A– allele and have the A– heterozygous females caused by X-inactivation in females, which is the process in which one of the X chromosomes is inactivated randomly in each cell. Because of the random inactivation of either the normal or abnormal X chromosome in any given cell in heterozygous individuals, the phenotype of G6PD A– heterozygous females could range from normal to deficient, as would their risk for hemolytic anemia in response to primaquine.

The frequency of the G6PDd A– variant in Haiti can be seen as a baseline estimate of the level G6PDd in Haiti’s population. In reality, the actual estimate is likely even higher, as more than 180 G6PDd mutations have been identified worldwide. Although the G6PDd A– variant makes up the vast majority of G6PDd cases in most West African populations, there are other G6PDd mutations that do reach polymorphic levels in some populations. Further studies should investigate the distribution of the A– variant in other parts of Haiti and include full G6PD gene sequence data with G6PDd phenotype data, to obtain a more complete picture of the genetic background of G6PDd in Haiti, as the severity of hemolytic anemia after primaquine exposure varies based on G6PDd genotypes. Additionally, more studies should follow response to primaquine treatment in G6PDd A– individuals in Haiti to confirm that hemolytic anemia is not a high risk at the new recommended lower dose. Still, we show that the G6PDd A– variant is prevalent in our sample. Therefore, the public health policy for malaria treatment should enforce a lower primaquine dose for current malaria treatment.

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<th>Table 1</th>
<th>Frequency of G6PDd A– and non-A– individuals at two health center sites</th>
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<td>G6PD variant</td>
<td>Genotypes</td>
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<tr>
<td>Males</td>
<td>A– hemizygote</td>
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<td>Non A– hemizygote</td>
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<td>Total male</td>
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<td>Non A– homozygote</td>
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recommendations or future mass drug administration programs based on the high prevalence of G6PD A– in Haiti and the WHO recommendation for lower primaquine dosing.

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